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Vol. III

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CONTENTS

CHAPTER 1

Plants used as a traditional biopesticide

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Abstract

Application of chemical pesticides leads to the development of resistance in insects, destruction of beneficial microorganisms and increase in residual problem which can be a threat to human health and environment. So, development of eco-friendly plant based pesticide needs to be done for the pest management. Keeping this in view, an attempt has been made to enumerate the plants used traditionally as a biopesticide through field survey using Passport Data Form in different landscapes of Odisha state, India. Results revealed that 11 plant species is recorded belong to 11 genus and 5 families. These plants are used to treat seed, seedlings and crops in different regions of Odisha, India.

Keywords: Biopesticides, Eco-friendly, Pest managements, Plants

1.1. INTRODUCTION

In developed and developing countries, agriculture plays an important role not only fulfilling the food requirements but also improving the economy of the country. To get more product, farmers use chemical pesticides which may have bad impacts on environment (Kandpal 2014). Biopesticides are the naturally occurring compounds or agents that obtain from the animals, plants and micro-organisms. Biopesticides are eco-friendly and host specific (Kumar *et al*., 2021). Basing on the nature and origin, biopesticides are various type such as botanical, growth promoters, predators and pheromones (Semeniue *et al*., 2017). Due to some antimicrobial agents and bioactive compounds, plants and microorganisms are the major source of biopesticides (Nefzi *et al*., 2016). Different plant families have different types of bioactive compounds (Lengai and Muthorni 2018). Mainly, biopesticides are of three types. Firstly, microbial pesticides which is important for plant disease managements. It contains a microorganism which can control a various kind of pests. They can be used to control mosquitoes and black flies (Kalra and Khanuja 2007). Secondly, plant incorporated protectants which is a substance derived from the genetic

material of a plant and thirdly biochemical pesticides which occur naturally from plant extracts, fatty acid, pheromones and control pest by non-toxin mechanism. It includes the substance that act as growth regulators or repel or attract insects (Kumar *et al.*, 2021). As biopesticides are eco-friendly, efficacy and sustainable, it attracts special interest and one of the promising alternatives to manage environmental pollution. It also gains interest in growing organic food (Bailey *et al.*, 2010). Most of the countries minimize the use of chemical pesticides and promote the use of biopesticides (Kumar and Singh 2014). Pest control is a major concern for human, plant health and productivity. People are in a searching to discover agents for pest control alternative to synthetic pesticide. Biopesticides is the alternate option as it is cost effective, safer and readily available (Gupta and Dikshit 2010).

Insecticides derived from the microorganisms get a special chance from developing countries to do research which could be helpful to develop biopesticides to protect crops (Nathan *et al.*, 2006). Biopesticides don't affect air and water quality. Microorganism as well as plant products are used which have no risk to human health (Goettel*et al*., 2001). Biopesticides can be derived from some bacteria like *Bacillus thuringiensis*, some fungi, viruses, protozoa and some beneficial nematodes which are used in field crop and garden use. Microbes and their metabolomic products are identified as biopesticides (EPA 2013).

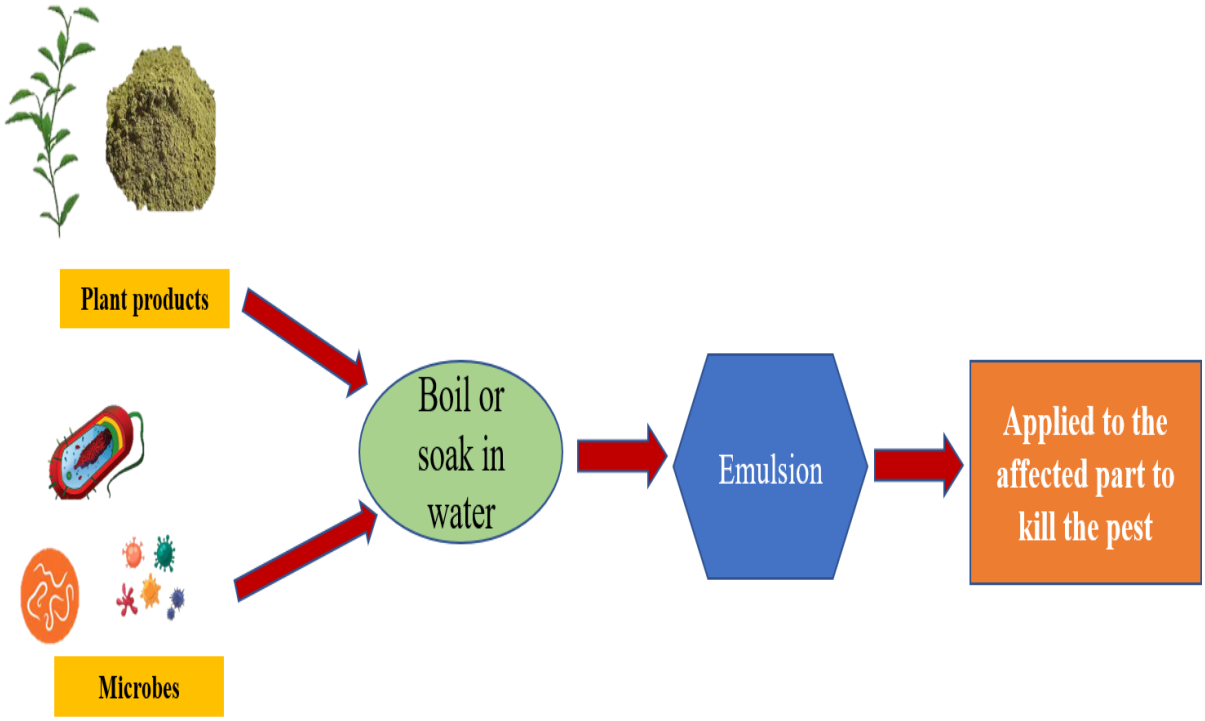

Figure 1. Preparation of biopesticide

The general preparation method was boiling the fresh plant material in water or grinding the material then soaking in the water. The extracts are made to emulsion and applied on the plant to kill the pest (Figure 1) (Nanyingi *et al*., 2008; Muthee *et al*., 2011). There are also some

plants derived pesticides called botanical biopesticides which have the ability to kill or sterilized the insects, to control weed or to promote the plant growth. About 6000 plant species are identified having insecticidal activities (Nawaz *et al*., 2016). The botanical biopesticides are used traditionally since the ancient time. The products of plant like neem and tobacco have been used as insecticides (Koul 2012). To protect crops and store product from the insect pest the botanical pesticides are used. They are volatile in nature and have low environmental risk than synthetic pesticides (Nawaz *et al*., 2016). Botanical pesticides are slow acting crop protectants. Keeping the importance of natural biopesticides, an attempt has been made to enumerate the plants used as biopesticide by different rural & tribal groups of Odisha, India.

1.2. METHODOLOGY

A survey was made during August 2020 to June 2021 in selected districts of Odisha and collected the plant species as per standard method (Kumar *et al.*, 2017).

1.3. RESULTS & DISCUSSION

Survey results revealed that about 11 plant species belong to 11 genus and 5 families are frequently used as a biopesticide by rural and tribal people of Odisha (Plate 1; Table 1). Among the enumerated species, *Azadirachta indica, Millettia pinnata* and *Vitex negundo* are observed as a common plants used by the local community as a biopesticide and insecticide. The local community use the dry leaves of *Vitex negundo* to store the pulses and grains. Leaf extracts of *Azadirachta indica* is used against the pest in bulbils of *Dioscorea* species. The stem of *Cleistanthus collinus* is used to kill the pest of lowland paddy. Details are listed in Table 1.

Table 1: List of plants used as biopesticide

Botanical name	**Family**	**Host(s)**
Azadirachta indica	Meliaceae	*Solanum tuberosum*
Euphorbia hirta	Euphorbiaceae	*Arachis hypogea*
Curcuma longa	Zingiberaceae	*Brassica oleraceae, Solanum lycopersicum*
Allium sativum	Amaryllidaceae	*Oryza sativa Gossypium hirsutum*
Cinnamomum verum	Lauraceae	*Zea mays*
Jatropha curcas	Euphorbiaceae	*Zea mays, Triticum aestivum*
Zingiber officinale	Zingiberaceae	*Solanum lycopersium, Mangifera indica*
Millettia pinnata	Fabaceae	*Seedlings of Forest Tree*
Vitex negundo	Lamiaceae	*Seedlings of Forest Tree*

Melia azedarach	Meliaceae	*Seedlings of Forest Tree*
Cleistanthus collinus	Phyllanthaceae	*Oryza sativa*

Researchers also studied the plant based biopesticides in different parts of country. The powder made from the leaves, bark, fruits and seeds of Neem can be used as insecticides (Kale *et al*., 2020). Baido *et al*., (2012); Vinodhini and Malaikazhandan (2011) reported that Neem is used as a bopesticide against the pest in cultivation of *Solanum tuberosum.* Dar *et al*., (2014) reported that *Cinnamomum verum* extract is useful in *Zea mays* cultivation. During the survey and reading the literature, authors noted that a good herbal biopesticides should be strong repellent and need other qualities too (Figure 2).

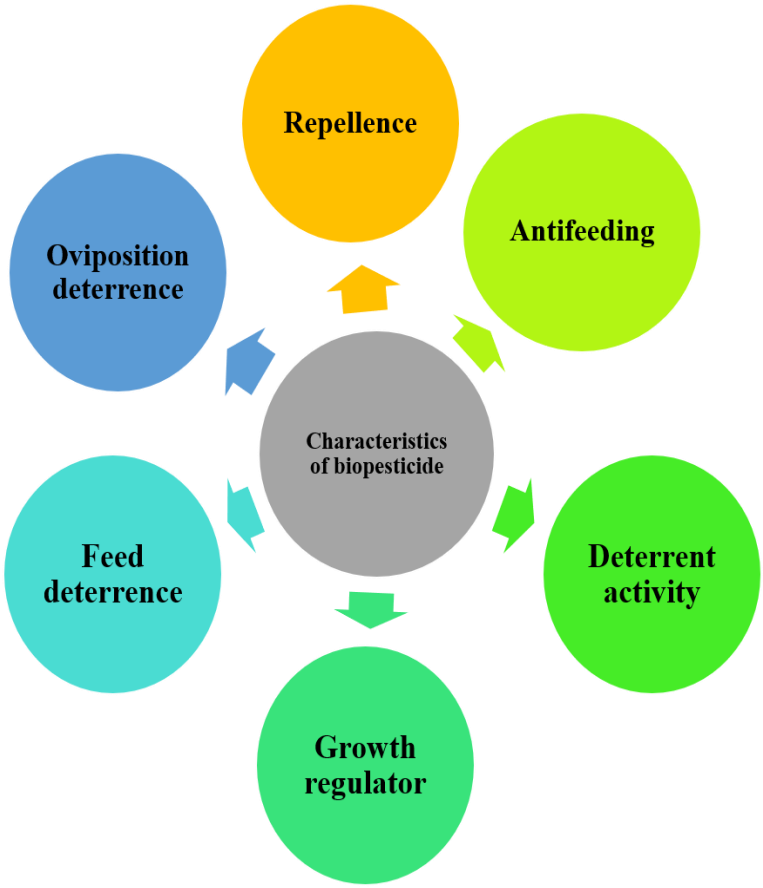

Figure 2: Characteristics of biopesticides

Plate 1: Some common plants used as biopesticide, 1) *Cleistanthus collinus*, 2) *Azadirachta indica*, 3) *Millettia pinnata*, 4) Flowers of *Azadirachta indica* and traditional method of making herbal biopesticide by the Santhal community at Mayurbhanj, Odisha

1.4. CONCLUSION

The bioactive compounds present in the plants are known to have the pesticidal effects. The biopesticides derived from the plants are eco-friendly, safe and beneficial for the environment. It does not harm the soil, water or air quality of the environment. Hence, plant derived pesticides should be used for pest control. The present study highlights the importance & traditional uses of local plants as a biopesticide. It gives a base line data for the formulation of herbal biopesticide which could be safe for environment and human health care.

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CHAPTER 2

Treatment of bovine mastitis by using ethnoveterinary herbal medicine

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Abstract

Mastitis is the one of the most common diseases in dairy cattle worldwide causing great economical loss. Mastitis is mainly of bacterial origin. Staphylococcus aureus and Escherichia coli are two most common organisms causing this disease. As the vast use of antibiotics to treat this disease can have harmful impact on human health by consumption of dairy products and also can give rise to antibiotic resistant strains of bacteria, ethnoveterinary medicine can really be considered as a better approach to treat it. Therefore, Ricinus communis, Asparagus racemosus, Terminalia bellirica and Piper betle are taken as per traditional knowledge. The inhibitory effects of these were tested against S.aureus and E.coli by using broth dilution method and agar disc diffusion method. So the promising results of these experiments could be utilized as a natural antibiotic against mastitis and also it has no or less adverse effect and cost effective.

Keywords: Antibacterial activity, Bovine mastitis, Ethno-veterinary medicine

2.1. INTRODUCTION

Chronicled proof proposes that cows have been milked since at least 3100 BC and bovine mastitis has probably existed since that time. Mastitis is the most frequent malady of dairy cows and has all around perceived as hindering consequences for animal prosperity and dairy ranch

profitability (Ruegg 2017). Bovine mastitis is defined as the inflammation of the mammary gland'. It is caused by a diverse group of organisms such as bacteria, mycoplasma, yeasts and algae. 137 different organisms have been identified as a cause of this disease. However ,in most of the cases it is of bacterial origin. Only five species of microbes (*Escherichia coli, Streptococcus uberis, Staphylococcus aureus, S. dysgalactiae* and *S. agalactiae*) represent practically 80% of all analyze. Mastitis pathogens have been classically classified into either ‘contagious' or ‘environmental'. Basically, the contagious pathogens are the organisms adapted to survive within the host, mainly within the mammary gland. They are capable of developing sub-clinical infections, which can typically be detected as an elevation in the somatic cell count of milk from the affected quarter, it usually spreads from cow to cow at or around the time of milking. Conversely, the environmental pathogens are best depicted as opportunistic invaders of the mammary gland, not adapted to survive within the host , they usually `invade', multiply, lead to a host immune response and are quickly eliminated. The major contagious pathogens are *S. aureus*, *S. dysgalactiae* and *S.agalactiae* and the major environmental pathogens are *Enterobacteriaceae*, particularly *E. coli* and *S. uberis* (Bradley 2002).

In clinical mastitis one or more of the following symptoms were observed : primary sign of inflammation in one or more of an udder’s quarters, symptoms of systemic reaction such as fever, depression, disturbed appetite, and abnormal physical character of milk such as clot formation, discoloration, alterations in viscosity, aberrant smell, or presence of blood. In subclinical mastitis there is absence of observable clinical signs, the presumptive diagnosis was done based on laboratory diagnostic tests of milk samples including California Mastitis Test (CMT) and SCC (Somatic Cell Count). Cattle with positive CMT or those having SCC >200,000 cells/mL but lacking clinical signs were considered as affected with subclinical mastitis (Ashker *et al*. 2015). Mastitis is one of the most economically important diseases of dairy cattle, representing 38 % of the absolute direct expenses of the common production diseases. It is hard to evaluate the losses associated with clinical mastitis, which arise from the costs of treatment, culling, death and decreased milk production. Mastitis has likewise been distinguished as the most common cause of death in adult dairy cows. It is even more difficult to evaluate the losses related with sub-clinical mastitis, which emerge as a after-effect of treatment, decreased milk yield and constituent quality and an increase in the risk of culling. Apart from the costs outlined

above, both clinical and sub-clinical mastitis have adverse affect in subsequent fertility. Besides the financial implications of mastitis, public health ought to not be neglected. The broad utilization of antibiotics in the treatment and control of mastitis has possible consequences for human health as an increased risk of antibiotic resistant strains of bacteria emerging that may then enter the food chain (Bradley 2002). Therefore, from the current point of view of the developing countries, synthetic drugs are costly and sometimes incapable for curing diseases as well as have lethal adverse impacts. Thus, there is an earnest need to investigate new antibacterial components with diverse chemical structures and novel mode of actions because of the increase in the occurrence of new and reappearing pathogenic diseases to supplant those which have lost their effectiveness. Moreover, we know our traditional and folkloric medicine has been utilizing since long and aromatic plants to increase the shelf life of foods as they inhibit the growth of bacteria and yeasts.

Several studies have reported that numerous herbs have varying degree of antimicrobial activities. So, the natural medicinal plants may be used as new antibacterial agents (Akter *et al.* 2014). So there are a number of studies going on focusing on alternative therapies in different areas of the health sector. When it comes to Ethnoveterinary medicine, several studies have shown the antibacterial activity of natural plant extracts and some of their compounds in regards to isolates obtained from mastitis cases. To prevent and treat mastitis, several breeders and veterinarians have used phytotherapy. Herbal solutions or medicinal ointments are commonly used for local use, also green or dry plants are given orally for treatment. *S. aureus* is one of the major causative organisms in case of mastitis in cattle. The use of phytotherapeutic medication has given adequate results in case of mastitis in cattle (Peixoto *et al.* 2015). Therefore, the main objective of this study is to examine antimicrobial activities of the four different plant extracts on major mastitis causing organism *S.aureus* and *E.coli* by means of the disc diffusion method.

2.2. METHODOLOGY

2.2.1. Field survey and data collection

The data are collected from the field survey conducted with local communities of study area during January 2020 to March 2020 .The study area includes two villages of Khordha district and one village of Bhadrak district and one village of Jajpur district (Plate 1-2). The standard

participatory rural appraisal method (Cunningham 2001) was followed for sampling and data collection to include the indigenous knowledge. This method includes semi-structured interviews and discussions with local cattle breeders. Opinions of them were recorded through questionnaire mainly regarding the symptoms during mastitis, duration of transmission from one infected to another, the total expenditure in treatment and the medicinal plants and other raw materials utilized to treat this disease traditionally and also the methods of preparation and these dosages used.

2.2.2. Collection of plant parts

According to the field survey and literature review 4 plants are selected that are used by local breeders rationally to treat mastitis. Fresh leaves of *Piper betle* (local name-pana, family-Piperaceae) were collected from local market of Saheed nagar, Bhubaneswar. Roots of *Asparagus racemosus* (local name-satavari, family- Asparagaceae) and fruits of *Terminalia bellirica* (local name-bahada, Family- Combretaceae) were collected from "Tribal Fair" of bhubaneswar. Fruits of *Ricinus communis* (local name-Jada, Family- Euphorbiaceae) were collected from near Saheed Nagar area (Plate 3). All are collected after proper identification. The experimental plant species were identified following Flora's Books (Haines 1925; Saxena and Brahmam 1995)

2.2.3. Preparation of plant extracts

Leaves, roots and fruits were air dried and then powdered in the grinder after drying, and powdered leaves were used for the preparation of extracts. To obtain the plant extract, Soxhlet method and percolation were followed (Tiwari *et al*. 2011). The powdered material of the experimental plant was kept in thimble and extraction was carried out using the Soxhlet apparatus. The residues were collected and left for air drying and dried crude extracts were stored in refrigerator for further experimental works.

2.2.4. Phytochemical assay

Phyto-chemical analysis were carried out on different extracts of different plant parts using standard procedure to identify the bioactive compounds (Harborne 1973;Trease and Evans 1989;

Tiwari *et al*. 2011). For phytochemical assay, 2 g of each powder were soaked in 20 ml of n-hexane , distilled water, methanol (1:10) respectively and stored in refrigerator for 24 hours for the secondary metabolites to get dissolved. It was filtered in Whatman's filter paper just before testing.

Test for tannin

2 ml of plant extract was added with 5 drops of 10 % lead acetate. A light yellow precipitate confirms the presence of tannin.

Test for phenolic compounds

2 ml of extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenolic compound.

Test for saponin

0.5 ml of extract was added with 2 ml distilled water and shaken vigorously. The stable persistent froth indicates the presence of saponin.

2.5. Antibacterial activity

Microbial Strain

There are many causative organisms responsible for mastitis as have been cited by different researchers. After analyzing works of different researchers, two of the chief causal organisms responsible for mastitis gram positive *Staphylococcus aureus* and gram negative *Escherichia coli* had been selected. The extracts of experimental plant parts were screened for antibacterial activity against them.

Disc Diffusion Assay

Antibacterial activity was done using slight modification of standard method of Disc Diffusion assay (Amanda *et al*. 2012**)**. Antibacterial activity using disc diffusion assay was done using the 6 mm of disc prepared from Whattman filter Paper (Amanda *et al*. 2012). Each extract was dissolved in dimethyl sulfoxide. 6 mm of discs were kept in the drug for 12hours before placing

to the agar plates. The zones of growth inhibition around the discs were measured after 18 to 24 hours of incubation at 37°C for bacteria. The sensitivities of the microbial species to the plant extracts were determined by measuring the sizes of inhibitory zones (including the diameter of disc) on the agar surface around the discs, and values less than 8 mm were considered as not active against microorganisms. Triplicates were maintained and the experiment was repeated thrice. For each replication the readings (zone of inhibition) were taken and the mean values were recorded. Mean was performed to calculate taking triplicate values of zone of inhibition(mm) of samples using Excel, Microsoft Corporation-2010,US.

2.3. RESULTS

During the survey 38 people of between 20 to 70 years age group were approached for the present study to get the information about the ethnoveterinary plants and practices [Table 4-5]. It was noted that good proportion, approximately 60 % of the informants, are female [Table 6]. Data was collected from different veterinarians regarding the drugs used for mastitis in cattle listed in Table 7. The standard antibiotic for antimicrobial assay can be selected by analysing the data represented on the Table 7.

Based on the information collected from the study area and literature reviewed, an attempt was made to justify the rationale behind the claims and to identify the presence of bioactive components in the selected plant extracts through qualitative tests (Plate 4). The phytochemical screening of selected plant extracts revealed the presence of the secondary metabolites as listed in Table 8. Tannin was present in both the aqueous and methanolic extract of all the four plant extract. Saponin is absent in methanolic extract of all 4 plants. Phenolic compound was present on both aqueous and methanolic extract of all 3 plants (*P.betle, R.communis, T.bellirica*) except *A.racemosus*.

Based on the findings on the presence of secondary metabolites and bioactive compounds on the selected plants, further experiments were designed to study possible antimicrobial activities of these plant extracts on *Staphylococcus aureus* and *E.coli*. The result of antibacterial activity of methanolic extract of selected plant parts were presented in Figure 1. The zone of inhibition produced by the reference antibiotic Penicillin against *staphylococcus aureus* and *Escherichia*

coli was also presented. The results of disc diffusion assay showed fair antibacterial activity when compared with standard antibiotic.

2.4. DISCUSSION

Bovine mastitis is one of the most common diseases occurring in dairy cattle. It is also regarded as one of the costly dairy cattle diseases as it hinders both quality and quantity of the milk production. To find out the herbal treatment for bovine mastitis the data was collected from 38 milk men and women. The data regarding the symptoms and treatment of mastitis were studied. Out of 38 people, 14 people provided the information regarding mastitis in cattle. All the informants observed swelling of udder in cattle as the common symptom for mastitis. Most of them noticed small clots in milk and very low milk production. As per the information usually only one animal that got infected first, then transmitted to other. Few mentioned the watery condition of milk and appearance of blood during milking. Temperature of cattle seemed to be a little high during this period. As females did most of the work regarding cattle care, they were the first to notice sick animal. They mostly provided the first-aid to the cattle for common diseases. All of the informants (except the tribal people of Godibari) depended on allopathic medication for the treatment of mastitis. No information regarding the use of medicinal plants against bovine mastitis were found from the study area. As for the tribal informants, mastitis had not been observed in their cattle as they did not perform milking. The tribal folks seemed to depend on local plants and herbs to treat some diseases, but no information for herbal treatment of mastitis was recorded. So studying from the research papers, two herbal treatments for mastitis had been selected. Firstly, 250 gm of crushed roots of *Asparagus racemosus* are mixed with about 100 g of fruits of *Trigonella foenum-graecum, Foeniculum vulgare, Terminalia chebula, Terminalia bellirica, Piper nigrum, Elettaria cardamomum* , flower buds of *Eugenia caryophyllu* and 200 g of bulb of *Allium cepa* homogenized in water adding little jaggery were administrated orally for the treatment of mastitis (Kumar *et al*. 2013). Secondly, form a paste of 10 betel leaves (*Piper betle*) with 100 g of castor oil (*Ricinus communis*) ,three parts were administrated orally and one part was applied on infected udder for the treatment of mastitis (Agar Aas paas Pashu Daktar Na Ho. Book). So from the study, root extract of *Asparagus racemosus*, fruit extract of *Terminalia bellirica*, leaf extract of *Piper betle*, fruit extract of *Ricinus communis* were selected for further phytochemical assay and antimicrobial activity in-vitro to check the potential of these extracts

against mastitis causing pathogens, *S.aureus* and *E.coli*. The phytochemical screening of the plant extracts showed the presence of tannin, saponin and phenolic compounds (Table-5). Tannin was present in both the aqueous and methanolic extract of *Piper betle, Ricinus communis, Asparagus racemosus, Terminalia bellirica.* Only the aqueous extract of *Asparagus racemosus, Terminalia bellirica* showed the presence saponin. Saponin is absent in methanolic extract of all 4 plants. Phenolic compound was present on both aqueous and methanolic extract of all 3 plants (*Piper betle, Ricinus communis, Terminalia bellirica*) except *Asparagus racemosus*. Presence of high content of saponins and tannins in plant extracts could be considered as the basis for its antimicrobial activity as saponin and tannin rich plants have profound antimicrobial property. Tannins are the important chemical groups of compounds with constitutive structural function present in almost every part of the plants including leaves, fruits, roots, bark and wood. Tannins have been reported for disruption of the cytoplasmic membrane, interruption in the proton motive force, active transport, coagulation of cell substances and electron flow. This can be a reason that almost all the four plant extracts having tannin content were able to inhibit all the tested organisms (Ali *et al*. 2018). Phenolic compounds were identified as one of the major bioactive compounds used as antimicrobial agents. They can cause redox reactions at the plasma membrane and sequester electrons from the respiration process. Phenolics can also cause localized disintegration and irregularity in the outer membrane that led to the leaking of cytoplasm. It is therefore rational that phenolic compounds have been found *in-vitro* to be effective antimicrobial substances against a wide range of microorganisms (Ghimire *et al*. 2017). Methanolic extract of all four plants used in this study showed promising antimicrobial activity. Each extract found to be active against the tested microorganisms. All four plant extracts showed antimicrobial activity against both the Gram-positive bacteria (*S.aureus*) and Gram-negative bacteria (*E.coli*) using disc diffusion assay. Other researchers also did some work on experimental plant parts against *S.aureus* and *E.coli* (Akter *et al*. 2014; Uddin *et al*. 2012; Rampadarath *et al*. 2016; Parveena *et al*. 2018). All the four plants showed antimicrobial activity against *S.aureus* and *E.coli*, which are the chief mastitis causing organism as per study. The extract of *P.betel* and *R.communis* proved to have antibacterial activity against tested pathogen(in-vitro), so their use against mastitis can be applicable. The application of *A.racemosus* and *T.bellirica* extract can be productive in mastitis as for their better antibacterial performance against the mastitis causing pathogens.

2.5. CONCLUSION

The present study concluded that the methanolic extract of *P.betel, R.communis* and *A.racemosus, T.bellirica* have promising antibacterial effect against *S.aureus* and *E.coli*. These extracts also exhibited presence of various secondary metabolites such as tannins, saponins and phenolic compounds. Therefore their use in the treatment of mastitis seems to have potential results. These also provide an effective eco-friendly control. The antimicrobial results justified the traditional use and practice of these plants in the treatment of mastitis. So taking into consideration of all the findings it can be mentioned that all the four plants can contribute major role in drug research for mastitis. In future, the present study can be extended by using these two treatments *in-vivo* in dairy cattle having mastitis to examine their effectiveness. Also the antibacterial activity of these selected plants can be evaluated using different solvent system other than methanol to check for better performances. Other parts of the selected plants can also be investigated in future to check their antibacterial activity.

Plate 1: Field survey for collection of information from Bhadrak & Jajpur districts, 1) Discussion with a female cattle breeder of Sathibankuda village of Bhadrak District regarding mastitis, 2) Discussion with a male cattle

breeder of Gunthunipada village of Jajpur district regarding mastitis, 3) A cow named Nali suffering from mastitis in Gunthunipada village, 4) A cow named Sasa just recovered from mastitis in Gunthunipada village

Plate 2: Field survey for collection of information from Khordha distrct, 4) Discussion with a male cattle breeder regarding mastitis in kantabada village, 5) Discussion with tribal people of Godibadi village regarding ethnoveterinary medicinal plants and their practices, 6) A tribal man showing a fruit of *Semecarpus anacardium* (common name-valia) that has been used to treat foot rot disease of cattle, 7) Discussion with Veterinarian Dr. Jaimini Mahapatra regarding treatment of mastitis in cattle

Plate 3: Collection of Plant parts for experimental works, Fruits of *Ricinus communis* , Roots of *Asparagus racemosus* (top, left to right) & Fruits of *Terminalia bellirica* , Leave of *Piper betle* (bottom , left to right)

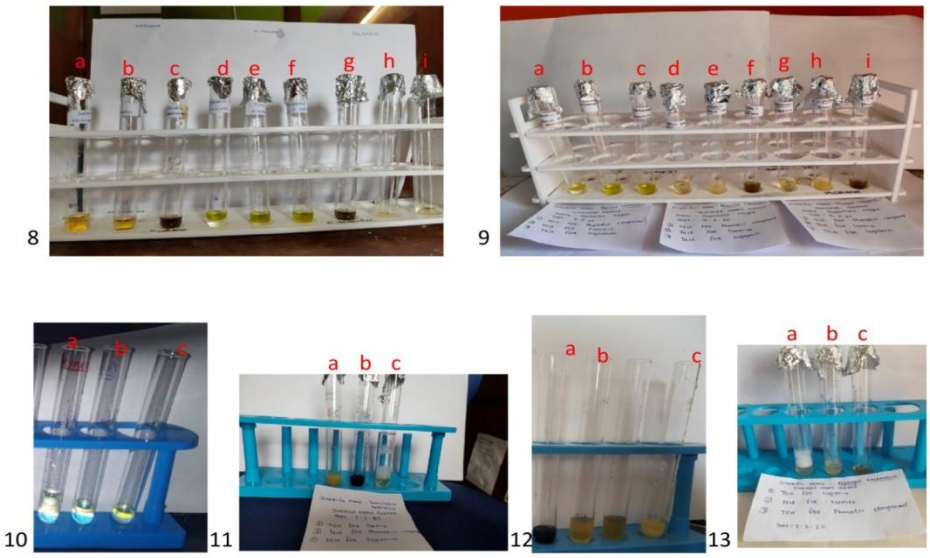

Plate 4: Phytochemical screening of selected plant parts, 8. a,b,c phytochemical results of methanolic extract of *P.betel*, 8.d,e,f phytochemical results of n-hexane extract of *P.betel*, 8.g,h,i phytochemical results of aqueous extract of *P.betel*, 9.a,b,c phytochemical results of n-hexane extract of *R.communis*, 9.d,e,f phytochemical results of aqueous extract of *R.communis*., 9.g,h,i phytochemical results of methanolic extract of *R.communis*, 10.a,b,c phytochemical results of n-hexane extract of *T.bellirica*, 11.a,b,c phytochemical results of aqueous extract of

T.bellirica, 12.a,b,c phytochemical results of methanolic extract of *T.bellirica*, 13. Phytochemical results of aqueous extract of *A. racemosus*

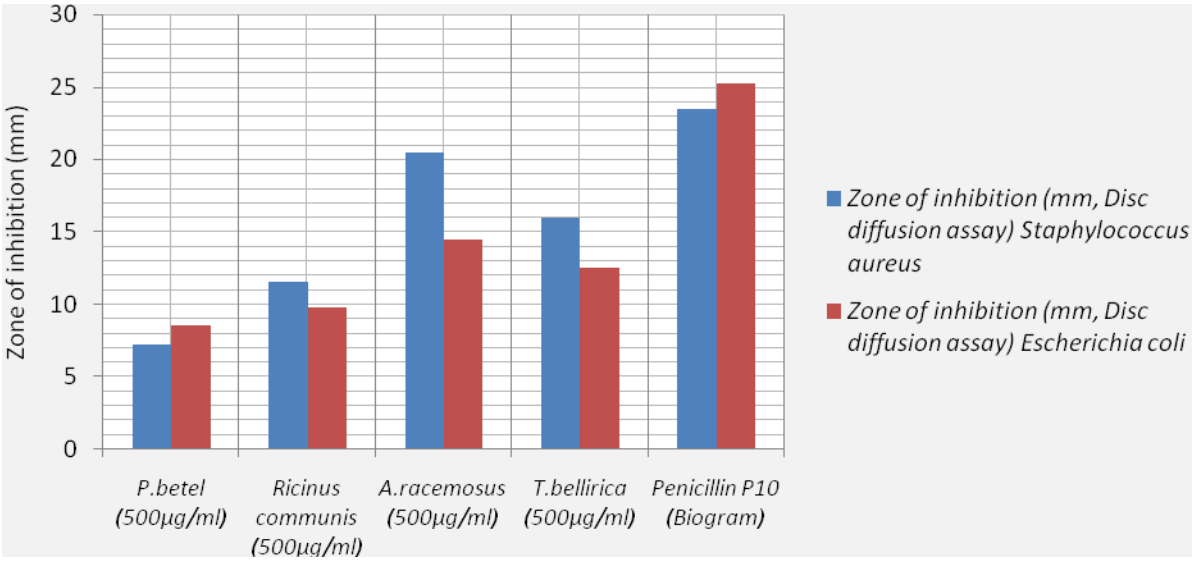

Figure 1: Antibacterial activity of selected plant parts

Table 1: Pathogens responsible for mastitis

Pathogen(s)	**Symptoms**	**Source**
Staphylococcus aureus , *Streptococcus agalactiae* , *Streptococcus dysgalactiae* , *Streptococcus uberis* , *Escherichia coli*	sub-clinical mastitis - an elevation in the somatic cell count in the milk from the affected quarter. clinical mastitis –swelling ,hardness, redness of udder,watery appearance , flakes, clots or pus in milk	Bradley (2002)
Streptococcus agalactiae	Cause contagious mastitis, infection is usually slow, clots appear in the fore milk and increasing in amount, followed by gradual induration of the quarter, which may progress until milk secretion ceases.	Plastridge (1958)
Negative micrococci (*M. pyogenes*)	Mainly Subclinical mastitis , Seldom clinical mastitis, presence in the udder may cause a mild degree of irritation, mild or moderate intensity lesions	Plastridge (1958)
Corynebacteria pyogenes	causes severe acute mastitis	Plastridge (1958)
Pseudomonas aeruginosa	Infection may persist in a given quarter for several months, with	Plastridge (1958)

	occasional attacks of clinical mastitis.	
Streptococci dysgalactiaa , *Streptococci uberis* , *Pasteurella multocida*, *Mycobacterium tuberoculosis*	Infection may persist in a given quarter for several months.	Plastridge (1958)
Cryptococcus neoformans	Swelling of udder, decreased milk production.	Plastridge (1958)
Staphylococcus aureus	Cause Clinical mastitis-cardinal signs of inflammation in one or more of an udder's quarters, signs of systemic reaction such as fever, depression, disturbed appetite, and abnormal physical character of milk such as clot formation, discoloration, alterations in viscosity, aberrant smell, or presence of blood. Subclinical mastitis-Cows with positive CMT or those having SCC >200,000 cells/mL but lacking clinical signs	Ashker *et al.,* (2015)
S. chromogenes , *Enterococcus faecium* , *Enterococcus faecalis*, *Macrococcus caseolyt* , *Enterobacteriaceae* sp. , *Bacillus* sp. , *Proteus* sp. , *Lysinibacillus fusiformis* , *Pseudomonas* sp. , *Escherichia coli* , *Kocuria* sp. , *Corynebacterium* sp. , *Enterococcus* sp. , *Aerococcus* sp. , *Vagococcus* sp , *Jeotgalicoccus* sp. , *Microbacterium oxydans* , *Microbacterium* sp	Both clinical and subclinical mastitis	Ashker *et al.,* (2015)
E. coli	often causes clinical mastitis, with clear signs of inflammation in the affected gland that range from mild to severe	Lavon *et al.,* (2019)
Staphylococcus aureus , *Streptococcus agalactiae* , Coliforms , streptococci , enterococci (major causative pathogens)	Cause clinical disease, with changes in milk composition, an increase in somatic cell counts and even death	Heringstad *et al.,* (2000)
Staphylococci, *Corynebacterium bovis*	moderate inflammation in mammary gland and slightly increased SCC	Heringstad *et al.,* (2000)

(minor causative pathogen)	and rarely lead to changes in milk composition, greatly reduced milk yield or clinical mastitis	
Corynebacterium bovis (minor causative pathogen)	No effect on milk yield, fat, protein, casein, and total solids but increased SCC and decreased lactose and milk solids non-fat content.	Gonclaves *et al.*, (2016)

Table 2: Drugs used against mastitis

Drugs	**Against**	**Source**
Ampicillin , Penicillin G, Streptomycin, Gentamycin , Erythromycin, Ciprofloxacine Oxytetracycline Trimethoprim/Sulphamethoxazole , Enrofloxacin, Doxycyclin .	*Staphylococcus aureus*	Ismail (2017)
Ampicillin, Ceftiofur, Cephalothin, Erythromycin, Oxacillin, Penicillin, Penicillin/Novobiocin, Pirlimycin, Sulfadimethoxine, Tetracycline.	*Staphylococcus aureus* , coagulase-negative *staphylococci*	Pol and Ruegg (2007)
Amoxicillin + Clavulanic acid Ceftiofur Cefalexin Ciprofloxacin Enrofloxacin Streptomycin Gentamicin Marbofloxacin Neomycin Oxacillin Penicillin Tetracycline Trimethoprim + Sulfamethoxazole	*Staphylococcus hyicus* *S. epidermidis* *S. intermedius* *Corynebacterium* spp.	Alba *et al.*, (2019)
Gentamycin Kanamycin	Pathogen not specified ,commonly for mastitis	Doehring and Sundrum (2019)

Neomycin Streptomycin Cefacetrile Cefalexin Cefazolin Cefoperazone Ceftiofur Cefquinome Danofloxacin Enrofloxacin Marbofloxacin Erythromycin Tylosin Lincosamides Lincomycin Pirlimycin Amoxicillin Ampicillin Benzylpenicillin Cloxacillin Oxacillin Penethamate Trimethoprim Sulfadimidin Sulfadoxin Oxytetracyline		
Benzylpenicillin Ampicillin, Amoxicillin, amoxicillin clavulanate, cloxacillin, kanamycin, oxytetracycline, doxycycline, cephoperazone, cephalonium, Lincomycin	*Staphylococcus aureus*	Moroni et al.(2006)
Ketoprofen	Coagulase negative *staphylococci*, Culture negative *streptococci*	Latosinski *et al.*, (2020)
Ceftizoxime	*Staphylococcus aureus*	Buragohain *et al.*, (2019)
Amoxicillin/clavulanate potassium Cefalexin Cefquinome Ceftiofur Clindamycin Daptomycin Enrofloxacin Oxacillin	*Staphylococcus aureus,* *Non aureus staphylococci* *Streptococcus* species	Cheng *et al.*, (2019)

Penicillin Tetracycline Vancomycin		
Amoxicillin/clavulanate potassium Cefquinome Ceftiofur Enrofloxacin Imipenem Kanamycin Polymyxin B Tetracycline	*Klebsiella* Species *Escherichia coli*	Cheng *et al.,* (2019)

Table 3: Ethnoveterinary herbal medicines used against mastitis

Plant	Pathogen	Source
Acacia nilotica (bark and leaves), *Aloe arborescens* (leaves), *Crassula multicava* (whole plant),*Tetradenia riparia* (flowers and leaves),	*Staphylococcus aureus* , *Streptococcus agalactiae* , *Streptococcus uberis* , *Staphylococcus chromogenes* , *Staphylococcus epidermidis* , *Klebsiella pneumonia* , *Pseudomonas aeruginosa,* *Proteus mirabilis* , *Proteus vulgaris* , *Enterobacter aerogenes*	Sserunkuma *et al.,* (2017)
Achyranthes aspera (root), *Croton macrostachys* (leaves), *Nicotiana tabacum* (leaves) , *Ziziphus spina-christi* (leaves) , *Ficus caria* (leaves)	*Staphylococcus aureus* , *Streptococcus agalactiae* , *Streptococcus dysgalactiae*	Kalayou *et al.,* (2012)
Fumaria indica *Adiantum capillus*	*Staphylococcus aureus* , *Escherichia coli* , *Streptococcus agalacti*ae , *Klebsiella pneumonia.*	Mushtaq *et al.,* (2017)
Withania somnifera (root), *Asparagus racemosus (root),* *Emblica officinalis (foot),* *Ocimum sanctum (leaves and seed),* *Tinospora cordifolia (seed),* *Tribulus terrestris (stem) ,*	Pathogen not specified, used against mastitis	Mushtaq *et al.,* (2017)

Nigella sativa (seed)		
Symphythum officinale, Sambucus nigra, Mentha sp. *Ocimum basilicum, Parapiptadenia rigida, Cuphea carthagenensis, Salmonella cholleraesuis, Alternanthera brasiliana, Achillea millefolium, Baccharis trimera Solidago chilensis*	*Staphylococcus aureus, Salmonella choleraesuis*	Mushtaq *et al.,* (2017)
Alternanthera brasiliana (aerial parts), *Achillea millefolium* (aerial parts), *Baccharis trimera* (aerial parts with flower), *Solidago chilensis* (aerial parts),	*Staphylococcus aureus*	Avancini *et al.,* (2008)
Symphythum officinal (leaves), *Sambucus nigra* (leaves), *Mentha* sp. (aerial parts with flower), *Ocimum basilicum* (aerial parts), *Parapiptadenia rigid* (bark), *Cuphea carthagenensis* (aerial parts),	*Staphylococcus aureus* , *Salmonella choleraesuis*	Avancini *et al.,* (2008)
Spathodea camanulata (leaves)	*Streptococcus agalactiae Streptococcus uberis, Escherichia coli, Coagulase positive Staphylococcus aureus* .	Das *et al.,* (2017)
Tridax procumbens (leaves)	Coagulase positive *Staphylococcus aureus*	Das *et al.,* (2017)
Allium sativum (bulb), *Bunium persicum* (seeds), *Oryza sativa* (seeds), *Triticum aestivum* (seeds/fruits)	*Staphylococcus aureus* , *Escherichia coli, Klebsiella pneumonia.*	Amber *et al.,* (2018)
Allium cepa (bulb), *Asparagus racemosus* (root) , *Mesua ferrea* (fruit)	Pathogen not specified used traditionally for mastitis	Kumar and Bharati (2013)
Piper betel (leaves) *Ricinus communis* (seed oil)	Pathogen not specified used traditionally for mastitis	Sahu (2019)

Table 4: Details of informants and collections sites

Informants	**Collection Site**	**Information Regarding Mastitis**
Tilottama Palai	Sathibankuda, Bhadrak	No

Sulekha Palai	Sathibankuda, Bhadrak	No
Khulana Palai	Sathibankuda, Bhadrak	No
Shrimanta Dalai	Sathibankuda, Bhadrak	Yes
Kanaklata Mahakud	Sathibankuda, Bhadrak	No
Pramila Sethy	Sathibankuda, Bhadrak	No
Satyabhama Nayak	Sathibankuda, Bhadrak	No
Pitambar Barik	Sathibankuda, Bhadrak	No
Sri.Muralidhara Nath	Sathibankuda, Bhadrak	No
Susanta Nayak	Sathibankuda, Bhadrak	No
Nirakar Barik	Sathibankuda, Bhadrak	Yes
Sri. Prabhakar Barik	Sathibankuda, Bhadrak	Yes
Sabita Barik	Sathibankuda, Bhadrak	No
Nirupama Parida	Sathibankuda, Bhadrak	No
Shashikala Mahakud	Sathibankuda, Bhadrak	No
Kaberi Mahakud	Sathibankuda, Bhadrak	No
Saraswati Parida	Sathibankuda, Bhadrak	No
Bhagabati Das	Gunthunipada, Jajpur	Yes
Sri.Radhakrushna Das	Gunthunipada, Jajpur	No
Sunil Kumar Das	Gunthunipada,Jajpur	Yes
Manarama Das	Gunthunipada, Jajpur	Yes
Niranjan Das	Gunthunipada, Jajpur	Yes
Rabinarayana Barik	Gunthunipada, Jajpur	No
Kalpana Das	Gunthunipada, Jajpur	No
Malati Palai	Gunthunipada,Jajpur	No
Bidulata Das	Gunthunipada, Jajpur	Yes
Sri.Abhaya Kumar Das	Gunthunipada, Jajpur	No
Babucharan Das	Gunthunipada,Jajpur	Yes
Achutyananda Das	Gunthunipada, Jajpur	Yes
Prasanta Kumar Das	Gunthunipada, Jajpur	Yes
Nityananda Das	Gunthunipada, Jajpur	Yes
Swarnalata Das	Gunthunipada,Jajpur	No
Pramila Das	Gunthunipada, Jajpur	No
Hrudananda Das	Gunthunipada, Jajpur	Yes
Champu Bansingh	Godibari, Khordha	No
Haria Bansingh	Godibari, Khordha	No
Bisu Thiria	Godibari, Khordha	No
Narahari Behera	Kantabada, Khordha	Yes

Table 5: Ethnovertinary information collected from field

Location	Mastitis	Other Diseases	Herbal Use	Allopathic Medicine Use
Sathibankuda,Bhadrak	Yes	Cowpox & Footrot	No	Yes
Gunthunipada,Jajpur	Yes	Footrot	No	Yes
Godibari,Khordha	No	Lumpy Skin Disease (LSD), Footrot , Actinomycosis, Dysentery	Valia Fruits (*Semecarpus anacardium*) To Treat Foot Rot Disease, Leaves of	No

			Pergularia daemia With Salt To Treat Eye Infection.	
Kantabada, Khordha	Yes	Footrot , LSD, Eye Infection	No	Yes

Table 6: The gender of informants in present study

Number of Informants	Village(s)	Number of Male Informants	Number of Female Informants	Number of Informants Regarding Mastitis
17	Sathibankuda,Bhadrak	6	11	3
17	Gunthunipada,Bhadrak	7	10	10
3	Godibadi,Khordha	2	1	0
1	Kantabada, Khordha	1	0	0

Table 7: The medicines/ bioactive compounds used against mastitis

Drugs	First hand information	Types	Sources
Enrofloxacin	From survey (Dr. Jaimini Mahapatra)	Fluoroquinolone	Trouchon *et al.* (2016)
Marbofloxacin	From survey (Dr. Jaimini Mahapatra)	Fluoroquinolone	Spreng *et al.* (1995)
Ceftiofer sodium	From survey (Dr. Jaimini Mahapatra)	Cephalosporin	Yancey *et al.* (1987)
Pendistrin SH	From survey (Dr. Niranjan Sahu)	Penicillin G, Streptomycin sulphate , Sulphamerazine, Hydrocortisone in Plastobase	Jadhav *et al.* (2010), Pyorala (2009)
Mastiwok	From survey (Dr. Niranjan Sahu)	Cefoperazone sodium	Cagnardi *et al.* (2010)

Table 8: Bioactive compounds present in selected experimental plant parts

Plant Name	Plant Parts	Solvent	Bioactive compounds		
			Phenolic compounds	Tannin	Saponin
Piper betle	Leaf	N-Hexane	-	-	-
		Aqueous	+	+	-
		Methanol	+	+	-
Ricinus communis	Fruit	N-Hexane	-	-	-
		Aqueous	+	+	-
		Methanol	+	+	-
Asparagus racemosus	Root	N-Hexane	-	-	-
		Aqueous	-	+	+
		Methanol	-	+	-

Terminalia bellirica	Fruit	N-Hexane	-	-	-
		Aqueous	+	+	+
		Methanol	+	+	-

[+ sign represents Presence and – sign represents Absence]

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CHAPTER 3

Lentibulariaceae of North-East India: an ecological indicator medicinal herb

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Abstract

Lentibulariaceae is known as family of carnivorous plants. They indicate the ecological parameters of the landscapes. About 40 species of Lentibulariaceae are reported from India. Among them, most are endemic and unique which is found in the North-Eastern Regions (NER) and Western Ghats of India. 10 species of Lentibulariaceae is enumerated and their importance are discussed here. They possess medicinal values and most of the species are used in respiratory problems. The relation of this species to the landscapes of study areas are established and gathered their importance to screen future novel bioactive compounds to fight against the diseases & disorders including the ecological aspects to understand the impacts of climate change.

Keywords: Lentibulariaceae, North-Eastern India, *Utricularia*, Abiotic factors, Ecological parameters

3.1. INTRODUCTION

The Lentibulariaceae or Bladderworts family are generally inhabited in freshwater wetlands, wet soils as terrestrial or aquatic species, waterlogged places and unhealthy ponds. Polluted water bodies, area having industrial sewage, chemicals runoff from agricultural practices etc., all are responsible for the decreasing aquatic

insectivorous plants. Main cause of decreasing the plants population is due to anthropogenic activities. Due to wrongly as common one and the disappearance of these insectivorous are being confirmed by confrontation with Silesian Flora of Schube (1903). Difference in the ecological habitats their function and structural features differs. Threatened species need to preserve or introduced in respective adequate areas (Predota 1986). Bladderwort family has more than 230 species (Parnell 2005; Fleischmann 2012a), approximately 233 species (http://www.theplantlist.org/). In India, a total of 44 insectivorous plants having 40 species of Lentibulariaceae and about 21 insectivorous plants from Northeast India containing four genera and 16 species of *Utricularia* (Table 1) has been reported (Albert *et al.*, 1992; Ellison and Gotellli 2001; Fleischmann 2012b; Rice 2006). *Utricularia* is an annual or perennial aquatic or perennial plant. Small organisms like insect larva, aquatic worms water flees etc. are captured by its sensitive bristles or hairs called bladder mouth. Traps ranges from 0.2 mm to 12 mm (Taylor 1989). With the water current the insects get contact with those hairs, it moves into the bladder and door is closed when it fills with water. The insect is being digest later when the enzyme is secreted. Carnivorous plants trap the insect to get amino acids from the prey (Hermann and Heckert 2007).

3.2. MEDICINAL VALUES OF LENTIBULARIACEAE AND OTHER CARNIVOROUS PLANTS

The landscapes of India enjoy rich plant biodiversity which provide a suitable platform to search new drug for various diseases. The Lentibulariaceae family possesses many traditional uses and as well modern clinical drug developments. Some reports claims that *Utricularia* is used for burns, kidney stone treatments, helps in weight lost maintenance, stimulate gallbladder secretions, respiratory problems etc. The secondary metabolites of *Pinguicula* species have very few

pharmacological uses in the past (Baffray *et al.*, 1985; Juniper *et al.*, 1989), some flavonoid like apigenin, 6-hydroxyluteolin, hypolaetin, isocutallarin, leutolin, scutellarin and malvidin-glucoside (Jay and Gannet 1973, 1974) and carotenoids (Neamtu and Bodea 1972). Leaves of the plant are also utilized as food items (Loyd 1995 1996). *Pinguicula vulgaris* used for milk curdling in Norway (Alm 2005). *Drosera burmannii* to cure blood dysentery (Mitra and Mukherjee 2010; Singh *et al.*, 2011; Vaidyanathan *et al.*, 2013). *Utricularia* is reportedly nutritious, mildly astringent, and diuretic. *Utricularia aurea* shows antitumor activity (Choosawad *et al*., 2005) and used to cure malarial diseases (Deka and Devi 2015). *Utricularia reticulata* used against urinary problems.

3.3. DESCRIPTIONS OF LENTIBULARIACEAE OF NER

3.3.1. *Utricularia aurea*

Description: Submerged floating herbs; rhizoids usually absent, if present always 4, up to 10 x 0.6 cm, verticillate at base of raceme, inflated, fusiform, covered with foliar like segments; stolons up to 1 m long, c 2.5 mm thick, glabrous, floating below the surface of water, branched. Foliar organs up to 6 cm long, lanceate when spread; primary segments 3-5 per node, semi-verticillate; secondary segments 2 from a point; ultimate segments capillary, terete, setulose; foliar scales rarely present, up to 13 mm across, dissected, setulose along margins. Traps 1-5 mm across, ovoid to obovoid, at axils of foliar organs and on secondary foliar segments, stalked; mouth lateral, oblique; appendages usually 2 or more in number, capillary, simple or branched, often with a ring of setae around mouth. Racemes up to 25 cm long, c 3 mm thick, up to 10- flowered; scales rarely present, similar to bracts; bracts 1.5-2 x 1.3-2 mm, basifixed, ovate to suborbicular, 1-nerved, truncate at base, acute to acuminate at apex; bracteoles absent; flowers up to 12 mm long;

pedicels 7-18 mm long, recurved and distally thickened in fruit. Calyx-lobes subequal, ovate to oblong, fleshy, papillose at base, recurved or spread in fruit; upper lobe c 3 x 2 mm (up to 7 x 5 mm in fruit), obtuse at apex; lower lobe c 2.5 x 2.2 mm (up to 7 x 4 mm in fruit), rounded to denticulate at apex. Corolla bright yellow; upper lip c 5 x 4 mm, ovate, hairy at base, obtuse to retuse at apex; lower lip c 6 x 9 mm, more or less obovate, hairy in throat, bigibbous at base, truncate, undulate at apex; spur more or less equal to lower lip in length, constricted at middle, papillose, glandular hairy at base, obtuse at apex. Stamens c 2 mm long; filaments curved, dilated above, papillose; anther thecae confluent. Pistil c 2 mm long, papillose; ovary subglobose; style thick; stigma 2-lipped, lower lip hairy, margins ciliate, upper lip obsolete. Capsules up to 7 mm across, globose with a long beak, circum scissile; placenta up to 5 mm long, ovoid. Seeds 0.8-1 mm across, polygonal, margin winged, hilum central; testa reticulate, cells more or less isodiametric (Plate 1). Fl. & Fr.: August-April with a peak during October-February; flowering late in eastern parts of peninsula. Habitat: In stagnant or slow running waters from sea-level to high altitudes. The stolons and foliar organs grow thick in ponds near coastal areas. Chromosomes: n = 21 (Siddiqui 1959; Subramanyam and Kamble 1968). Pollen: 17-19-colporate, 29 x 39 µ and 20-22-colporate, 29 x 38 µ (as *U. flexuosa*) (Huynh 1968); 13- 15-colporate, 30 x 39 µ (Taylor 1989). Distribution: India - Distributed almost throughout the country. Sri Lanka, India to Japan and Australia.

3.3. 2. *Utricularia bifida*

Description: Herbs; rhizoids up to 15 mm long, capillary, branches up to 1 mm long, papillose; stolons up to 3 cm long, filiform, branched. Foliar organs up to 10 x 0.5 mm, linear, on stolons, 1-nerved, rounded at apex. Traps c 1 mm across, globose, on vegetative organs; stalk thickened distally; mouth basal; appendages 2,

subulate, simple. Racemes 2.5-18 cm long, erect, 1-8- flowered; scales 1-1.5 mm long, basifixed, ovate, acute at apex, nerves 1 or 5, or absent; bracts 1.2-2.5 mm long, basifixed, ovate to linear-lanceate, 1- or 3- nerved, acute at apex; bracteoles 0.7-1.5 mm long, subulate; flowers 5-13 mm long; pedicels 2-5 x 1-1.5 mm, broadly winged, erect in anthesis, recurved in fruit. Calyx-lobes subequal, ovate; upper lobe 2-4 x 2-3 mm (3-6 x 2.5-4.5 mm in fruit), rounded at apex; lower lobe 2-3 x 2-3 mm (3-5.5 x 2.5- 4.5 mm in fruit), rounded and rarely bi- tridentate at apex. Corolla yellow; upper lip 2-6 x 1-2 mm, linear-oblong, rounded at apex; lower lip 2.5-6 mm across, orbicular to obovate, hairy in throat, gibbous at base, rounded at apex; spur 3-6 mm long, subulate, descending, acute at apex. Stamens c 1.5 mm long; filaments strap shaped, 1-nerved; anther thecae distinct. Pistil c 1.5 mm long; ovary ovoid; style short, distinct; stigma 2-lipped, lower lip slightly reflexed, upper lip represented by a semi-orbicular projection. Capsules 2-3 x 1.5-2.5 mm, ovoid, slightly compressed, wall uniformly membranous; placenta c 1.5 mm across, more or less globose, stalked. Seeds 0.25-0.4 mm long, ovoid, ellipsoid to obovoid, numerous; hilum lateral; testa reticulate, cells large, elongate, striated within. Fl. & Fr.: July-December. Habitat: In wet and marshy areas near perennial water sources, falls, streams, lakes and in rice fields from sea-level to 1500 m. Pollen: 3- or 4-colporate, 26-28 x 34-35 µ (Thanikaimoni 1966); 3-colporate, 24 x 30 µ (Taylor 1989). Distribution: India - Throughout the country except Northwest India. Sri Lanka to Japan and South to North Australia. Notes: *Utricularia bifida* is allied to *U. recta* among Indian species, but can be easily distinguished by its recurved fruiting pedicel, rounded apices of calyx-lobes, uniformly membranous capsule wall and presence of striations within the testa cells. This species is used in Indian medicine for urinary disorders.

3.3. 3. *Utricularia caerulea*

Description: Herbs; rhizoids up to 3 cm long, glandular, rarely branched; stolons up to 2 cm long, 0.1-0.3 mm thick, sparsely glandular, branches hyaline. Foliar organs 4-8 x 1-1.3 mm, spathulate, rosulate, 1- 6 per node on stolons, 1-nerved, rounded to obtuse at apex. Traps 1-2 mm long, ovoid, on vegetative organs; stalk short; mouth terminal with a rim of glandular hairs; upper lip extended into a beak-shaped appendage, glandular, Racemes 3.5-40 cm long, 0.5-1 mm thick, simple or rarely branched, terete or flattened, glabrous; flowers clustered at apex or lax; scales 1.5-4 x 0.7-1 mm, medifixed, elliptic to rhomboid, 1-nerved; bracts 1.5-5 mm long, medifixed, elliptic to rhomboid, sparsely papillose without, 1-nerved; bracteoles 1-2 mm long, linear, basisolute, papillose; flowers up to 6 mm long; pedicels up to 2 x 0.4 mm, erect, terete, often recurved in fruit, papillose. Calyx-lobes subequal, hooded, papillose; upper lobe 2-2.5 x 1.5-2.5 mm, broadly ovate to orbicular, obtuse or rarely acute at apex. Corolla purple, pink, rose, blue, violet, white or cream-coloured, papillose; upper lip 2-4 x 1.5- 1.8 mm, oblong to rarely deltoid, constricted at middle with two horn like projections on ventral surface, ciliate at lower margins, truncate, notched or emarginate at apex; lower lip 1.5-5 x 2-4.5 mm, semi-orbicular to broadly ovate, yellow in throat, gibbous at base, rounded or shallowly 3-lobed at apex; spur 3-5 mm long, longer than lower lip, horizontally projected, often curved upwards, acute or notched at apex. Stamens c 1 mm long; filaments strap-shaped, curved, 1-nerved; anther thecae distinct. Pistil c 1 mm long; ovary ovoid, attached to upper calyx-lobe at base; style short; stigma 2-lipped, lower semi-orbicular and hairy, upper filiform and glabrous. Capsules 1.5-2 mm across, subglobose to obliquely ovoid, papillose, dehisce vertically by a ventral slit; placenta c 1 mm across, subglobose, pitted. Seeds 0.2-0.3 mm long, ovoid, ellipsoid to obovoid; hilum terminal; testa reticulate, cells elongate. Fl. & Fr.:

August-December along West Coast, Northeast India and West Bengal; August-April in East Coast and Central India with a peak during December-February. Habitat: On wet or marshy open sandy soil, mud and gravelly areas, under both seasonal and perennial conditions; from sea level upwards. Chromosomes: n = 20 (Subramanyam and Kamble 1968). Pollen: 3- or 4-colporate, 32-34 x 20-22 µ and 32-35 x 27-29 µ (*U. nivea*) (Thanikaimoni 1966) 3-colporate, 19 x 32 µ (Taylor 1989). Distribution: India - Distributed in Eastern and Northeastern, Central and Southern states: Uttar Pradesh, Bihar, West Bengal, Meghalaya, Odisha, Madhya Pradesh, Gujarat, Goa & Dadra, Maharashtra, Andhra Pradesh, Tamil Nadu, Karnataka and Kerala. Madagascar, Sri Lanka, India to Japan, Malesia and Australia. Notes: *Utricularia caerulea* was much discussed regarding its taxonomy, nomenclature and typification. Oliv. (l.c.) and Clarke (I.c.) applied the name to the Koenig's specimen in LINN (= *U. graminifolia* Vahl).

3.3.4. *Utricularia exoleta*

Description: Herbs; rhizoids usually absent, if present up to 5 cm long, c 1 mm thick at base, fusiform, tapering towards apex, branches botryform; stolons up to 20 cm long, c 0.2 mm thick, profusely branched. Foliar organs up to 1 cm long, simple or 1-3 times dichotomously divided from base or at a short distance from base; ultimate leaf segments few, slightly compressed or terete, sparsely setulose along margins. Traps up to 1.5 mm across, obliquely ovoid, replacing foliar segments or rarely on rhizoids; stalk evenly thickened; mouth lateral, oblique; appendages usually 2- or rarely more. Racemes 4- 15 cm long, erect, solitary or fasciculate, glabrous, 1-3- flowered; scales absent or rarely 1-2, c 0.6 x 1 mm, basifixed, obovate to semiorbicular, truncate or slightly 3-lobed at apex; bracts 0.8-1 x 1-1.3 mm, transversely oblong, 5-nerved, truncate or denticulate at apex; flowers up to 7 mm long; pedicels 2-8 mm long, terete, erect or suberect. Calyx-

lobes subequal, obovate to ovate, rounded or truncate at apex; upper lobe c 1.4 x 1.3 mm (c 3 x 3 mm in fruit); lower c 1.3 x 1.3 mm (c 2.5 x 2.5 mm in fruit). Corolla yellow; upper lip c 3 x 3 mm, orbicular to ovate, truncate or rounded at apex; lower lip c 2.7 x 2.5 mm, orbicular to broadly ovate, bigibbous at base, rounded, truncate or rarely 3-lobed at apex; spur as long as lower lip, conical, glandular within, obtuse or notched at apex. Stamens c 1 mm long; filaments flat, curved; anther thecae distinct. Pistil c 1 mm long; ovary globose; style small; stigma 2-lipped, lower lip larger and semi-orbicular, upper lip obsolete or denticulate. Capsules c 3 mm across, globose, bivalvate; placenta c 2 mm across, globose, glabrous, pitted. Seeds c 1 mm wide, lenticular, with a broad, irregular corky wing; hilum prominent; testa cells arranged radially. Fl. & Fr: January-May with a peak in February and March. Habitat: Submerged floating in shallow water and in swamps or rooted in mud; from sea level to 1700 m, often growing with *U. aurea*. Pollen: 11-12 colporate, 40-45 x 30-33 µ or 28 x 45 µ (Thanikaimoni 1989). Distribution: India - Distributed throughout the country including Andaman & Nicobar Islands. Tropical Africa, to Japan and Northern Australia. Notes: *Utricularia khasiana*. According to the authors, the plant had never set flowering during their many years of observation. This is the only aquatic bladderwort recorded from Andaman & Nicobar Islands. Taylor (1989), however, includes *U. exoleta* R. Br. in *U. gibba* L. - complex.

3.3. 5. *Utricularia furcellata*

Description: Herbs; rhizoids up to 2 cm long, simple or sparsely branched, glandular; stolons absent. Foliar organs with pseudo petioles up to 12 mm long, rosulate, absent from vegetative organs; expanded portion 1-5 x 1.5-6 mm, orbicular or suborbicular, veins dichotomous. Traps 1-1.5 mm across, on rhizoids, obliquely ovoid, glandular, stalked; mouth lateral; appendages of radiating

multicellular hairs from expanded lip. Racemes up to 9 cm long, often glandular at base, 1-5-flowered; bracts c 0.8 mm long, medifixed, lanceate, constricted near base, truncate at base, acute at apex; bracteoles c 0.8 mm long, similar to bracts but slightly curved; flowers c 5 mm long; pedicels c 4 mm long, terete, erect in flower, spread in fruit. Calyx-lobes unequal, papillose; upper lobe 1.5-2 x 2.3-3.5 mm, 5-nerved, emarginate at apex; lower lobe c 1 mm long, oblong, rounded or dentate at apex, nerves absent. Corolla white or pink, papillose, yellow in throat; upper lips c 1.2 x 2 mm, deltoid, 2-nerved, emarginate at apex; lower lip c 2.5 x 3.5 mm, 4-lobed, hairy in throat, slightly raised at base; spur c 3 mm long, glandular within, acute at apex. Stamens c 0.8 mm long; filaments curved; anther thecae distinct. Pistil c 1 mm long; ovary ovoid, papillose; style distinct; stigma 2-lipped, lower lip recurved, upper lip obsolete or represented by a small projection. Capsules c 2 mm across, obliquely ovoid, attached to upper calyx-lobe at base, strongly keeled on ventral surface, dehisce longitudinally on ventral side; placenta c. 0.7 mm across, subglobose. Seeds c 0.5 mm long, ovoid to ellipsoid, attached tangently to placenta, glochidiate; hilum lateral. Fl. & Fr.: August-November. Habitat: Along dripping rocks and moss-covered tree trunks above 1500 m. Pollen: 4-colporate, 40-45 x 48-50 µ (Thanikaimoni 1966); 3-4-colporate, 30 x 35 µ (Taylor 1989). Distribution: Endemic to Khasia hills and mountains of Sikkim and Darjeeling. Notes: *Utricularia furcellata* Oliv. is closely related to *U. striatula* Sm.

3.3. 6. *Utricularia multicaulis*

Description: Herbs. Foliar organs more or less orbicular with a pseudopetiole. Racemes up to 5 cm long, tufted, erect, glabrous, 1 -3-flowered; bracts c 1 mm long, medifixed, elliptic-lanceate, truncate to trifid at base, acute at apex; bracteoles up to 1 mm long, medifixed, elliptic-lanceate, truncate to bifid at base, acute at apex; flowers c 3 mm long; pedicels c 5 mm long, erect in anthesis,

recurved in fruiting. Calyx-lobes unequal; upper lobe c 1.5 x 2 mm, suborbicular, emarginate at apex; lower 1 x 0.7 mm, obovate to oblong, emarginate to minutely denticulate at apex. Corolla fleshcoloured; upper lip c 1 mm long, semi-orbicular, entire to emarginate at apex; lower c 2 mm across, 3-4-lobed, rarely 6-lobed, yellow in throat; spur 2 mm long, conical to cylindrical, straight or slightly curved, obtuse at apex. Stamens not seen. Pilstil c 1 mm long; ovary ovoid; style short, thick; stigma 2-lipped. Capsules c 1.5 mm across, subglobose, dehisce ventrally by a vertical slit; placenta connecting base and apex of capsule. Seeds up to 0.8 mm long, ovoid, appendicular at one end; hilum subterminal; testa echinate in definite rows. Fl. & Fr.: July-August. Habitat: Distributed in hill ranges between 1800 and 4800 m, as an epiphyte or terrestrial. Pollen: 3- or 4-colporate, 25 x 31 µ (Thanikaimoni 1966); 3- or 4-colporate, 26 x 35 µ (Huynh 1968); 3- or 4-colporate, 25 x 33-36 µ (Taylor 1989). Distribution: India - Only in Sikkim. Eastern Himalayas extending to China and Myanmar. Notes: *Utricularia kumaonensis* Oliv. is sometimes confused with *U. multicaulis* Oliv. But the seeds of *U. multicaulis* have prominent hilum and appendages confined to only one end of the seed, whereas in *U. kumaonensis* the hilum is not prominent and the appendages are distributed on both ends of seeds. P. Taylor (in Herb. Kew) has chosen J.D. Hooker s.n. "1" Sikkim as the lectotype of this species.

3.3. 7. *Utricularia recta*

Description: Herbs; rhizoids up to 1.5 cm long, thick, glandular, branches up to 3 mm long, papillose; stolons up to 8 cm long, sparsely branched. Foliar organs up to 5 cm long, linear, gland-dotted, 1-nerved, rounded or obtuse at apex. Traps c 1 mm across, subglobose, slightly compressed; often a columnar appendage presents on stalk; mouth basal; appendages 2, subulate, simple, glandular, gland-tipped. Racemes up to 20 cm long, erect, terete, each up to 10- flowered; scales 1-2 mm

long, basifixed, lanceate to ovate-deltoid, 1-nerved, acute to acuminate at apex; bracteoles shorter than bracts, subulate to linear, 1- nerved; flowers up to 15 mm long; pedicels 3-6 mm long, erect, winged. Calyx-lobes unequal; upper lobe c 3.2 x 2.7 mm (c 5 x 4 mm in fruit), broadly ovate, acuminate at apex, acuminate to caudate at apex in fruit; lower lobe c 3 x 1.5 mm (c 5 x 3 mm in fruit), oblong to oblanceate, or obovoid, bidentate at apex. Corolla yellow, purple streaked; upper lip up to 4 mm long, oblong, constricted at middle, crested across on ventral surface, ciliate at lower margin, obtuse at apex; lower lip up to 6 mm across, suborbicular to ovate, hairy in throat, gibbous at base, rounded to truncate at apex; spur up to 5 mm long, curved and acute at apex. Stamens c 1 mm long; filaments twisted; anther thecae distinct. Pistil c 1.2 mm long; ovary ovoid; style short; stigma 2-lipped. Capsules c 3 x 2.2 mm, ovoid, dehisce by a marginally thickened vertical slit; placenta c 2 mm long, ovoid. Seeds subglobose to obovoid; hilum terminal, prominent; testa reticulate, cells oblong. Fl. & Fr.: August-November. Habitat: Terrestrial herb in wet or marshy places in open ground up to an elevation of 3,300 m. Chromosomes: n = 7 (Subramanyam and Kamble 1968 - as *U. scandens*, the specimen from Shillong alone). Pollen: 4-colporate, 21 x 25 µ (Taylor 1989). Distribution: India - Endemic of Khasi hills and Himalayas: recorded from Uttar Pradesh, Sikkim, Arunachal Pradesh, Assam and Meghalaya. Nepal, Bhutan, India & Southwest China. Notes: *Utricularia recta* is allied to *U. scandens* but differs in its erect inflorescence and unequal calyxlobes in fruit. The specific epithet "recta" is based on its rather stiffy erect inflorescence.

3.3. 8. *Utricularia scandens*

Description: Herbs; rhizoids up to 1.5 cm long, branches up to 1 mm, papillose; stolons up to 3 cm long, filiform, profusely branched. Foliar organs up to 15 x 1 mm, linear, 1-nerved, acute or rounded at apex. Traps c 1 mm across, more or less

globose; stalk glandular, often columnar growth present near base; mouth basal; appendages 2, simple, subulate. Racemes up to 25 cm long, twining, rarely erect in smaller ones, glabrous, 1-9-flowered with sterile bracts present in between fertile ones; scales 0.7-1.5 mm long, basifixed, ovate to lanceate, acute to acuminate at apex, 1-nerved, rarely nerves absent; bracts 1-1.5 mm long, basifixed, broadly ovate, 1-nerved, acuminate to caudate at apex; bracteoles 0.3-1.4 mm long, linear to lanceolate, 1-nerved, rarely nerves absent; flowers 5-12 mm long; pedicels 1-5 mm long, erect, winged. Calyx-lobes 2-3 x 1.1 - 3 mm (3-5 x 2-4 mm in fruit), ovate; upper lobe acute to acuminate at apex; lower lobe bi- or tridentate at apex. Corolla yellow; upper lip 2-3 mm long, obovate to oblong, constricted near middle, a crest running across at middle, obtuse to emarginate at apex; lower lip 3-6.5 x 2.5- 3 mm, more or less obovate, hairy in throat, gibbous at base, rounded or shallowly emarginate at apex; spur 2-6 mm long, subulate or rarely conical, acute and curved at apex. Stamens c 1 mm long; filaments flat, twisted; anther thecae distinct. Pistil c 1 mm long; ovary ovoid; stigma bilipped, lower lip oblong and hairy, upper lip semiorbicular and glabrous. Capsules c 2.5 x 1.5 mm, oblongoid to ovoid, dorsiventrally compressed, dehisced margin thickened; placenta c 1.5 x 1 mm, compressed, oblong, shallowly pitted. Seeds 0.2-0.35 mm long, ovoid to ellipsoid; hilum prominent; testa reticulate, cells elongated. Fl. & Fr.: Throughout the year with a peak during September to December. Habitat: Along wet and marshy places, twining among themselves or on other plants; from sea-level to 2,600 m. Chromosomes: n = 6 (Subramanyam and Kamble 1968). Pollen: 3- or 4-colporate, 22-23 x 28-30 µ (Thanikaimoni 1966); 3- or 4-colporate, 20 x 25 µ (Huynh 1968); 3-colporate, 25 x 30 µ (Taylor 1989). Distribution: India - in Uttar Pradesh, Bihar, West Bengal, Meghalaya, Odisha, Madhya Pradesh, Andhra Pradesh, Tamil Nadu, Karnataka and Kerala. Africa to Northern Australia through

India and Malesia. Notes: *Utricularia scandens* Benj. is easily recognized in the field by its combination of characters like twining racemes, yellow flowers and presence of sterile bracts in between fertile ones.

3.3. 9. *Utricularia subulata*

Description: Herbs; rhizoids up to 1 cm long, tapering towards apex, branches papillose; stolons up to 1 cm long, glandular, branched. Foliar organs up to 1 cm long, linear, 1-nerved. Traps up to 0.5 mm across, ovoid; mouth lateral; appendages 2, filiform, branched. Racemes up to 8 cm long, zigzag, terete, papillose at lower half, 1-3-flowered; scales up to 1 x 0.4 mm, medifixed, linear-elliptic, papillose, ciliate along margins, acuminate at apex; bracts c 0.5 mm across, peltate, broadly ellipsoid to orbicular, smooth; flowers 5-10 mm long; pedicels 1-2 mm long, terete. Calyx-lobes subequal, ovate, obtuse to acuminate at apex, nerves prominent; upper lobe c 1.3 x 0.7 mm, lower c 1 x 1 mm. Corolla yellow; upper lip c 2.5 mm long, ovate to orbicular, obtuse to rounded at apex; lower lip c 3 mm across, 3-lobed, bigibbous at base, obtuse to truncate at apices of lobes; spur up to 4 mm long, glandular within, obtuse at apex. Stamens c 0.6 mm long; ovary obovoid; stigma bilipped, upper lip obsolete, lower orbicular and ciliate. Capsules c 1 mm across, subglobose, opens by a ventral pore with lid; placenta c 0.4 mm across, subglobose. Seeds c. 0.2 mm long, obovoid; testa reticulate, cells elongated. Fl. & Fr.: August. Habitat: On open moist sandy soil over rocks and stones. Chromosomes: n= 15 (Kondo 1972). Pollen: 7-9-colporate, 23 x 20 µ (Huynh, 1968); 8-9-colporate, 19 x 23 µ & 8-colporate, 30 x 20 µ (Taylor 1989). Distribution: India - Known from a single collection from Meghalaya. Pantropical. Notes: *Utricularia subulata* is characterized by its branched appendages of traps, medifixed scales, peltate bracts, absence of bracteoles, prominently nerved calyx-lobes, and operculate capsule. Taylor (1977) stated "-it is curious that the species

(*U. subulata*), which is widespread and abundant in the New World, Africa, Madagascar, should never have been found in India. There can be no doubt that the Malesian plant is the same as the African & American ones".

3.3. 10. *Utricularia striatula*

Description: Herbs; rhizoids up to 2.5 cm long, simple, few or altogether absent; stolons up to 9 cm long, simple, rarely branched, glandular. Foliar organs with pseudo petiole up to 15 mm long, orbicular to obovate, rosulate at scape base, scattered on stolons, expanded portion up to 5 mm across, veins dichotomously branched. Traps up to 1.5 mm across, globose to obliquely ovoid; mouth lateral; appendages glandular hairy, on expanded, divergent upper lip. Racemes up to 18 mm loong, erect, often zigzag, glabrous, up to 10-flowered; scales if present 1-1.5 mm long, medifixed; bracts 0.6-2 mm long, medifixed, constricted at middle, limbs unequal; bracteoles 0.7-1.5 mm long, medifixed, limbs unequal; flowers up to 10 mm long; pedicels 1.5-7 mm long, filiform, spreading in anthesis, spreading or recurved in fruit, papillose atleast in fruiting. Calyxlobes highly unequal, papillose; upper lobe 1.2-4 x 2-4 mm, obovate to obcordate, emarginate, truncate or obtuse at apex; lower lobe 0.6-2 x 0.4-2 mm, oblong to ovate, truncate, notched or rounded at apex. Corolla pink, lilac, violet, white to variously tinged, often yellow-blotched near base; upper lip 0.6-2 mm across, semi-orbicular to deltoid, bidentate, truncate or irregular at apex; lower lip 3-7 x 3-10 mm, 3- or 5-lobed, throat hairy, base raised or flat, rounded or truncate at apices of lobes; spur 1-6 mm long, cylindrical or conical, rarely reduced to a mound, obtuse, acute or retuse at apex. Stamens up to 1.2 mm long; filaments strap-shaped, curved; anther thecae subdistinct. Pistil up to 1.2 mm long; ovary obliquely ovoid, attached to base of upper calyx-lobe; style distinct; stigma 2-lipped, lower lip oblong to semiorbicular, upper lip obsolete. Capsules 1.5-3.5 mm long, obliquely ovoid, attached to upper calyx-lobe, keeled

on ventral side, dehisce by a longitudinal ventral slit; placenta c 1 x 0.6 mm, flask-shaped. Seeds 0.3-0.4 mm long, clavate, cylindrical or oblongoid, attached radially to placentum, glochidiate. Fl. & Fr.: July-December (-February). Habitat: Epiphytic or terrestrial; found on wet moss covered tree trunks, dripping rocks in shade, vertical face of wet rocks and less commonly on moss covered stone walls; from 150 m upwards, mostly in higher altitudes. Pollen: 3-coIporate, 18-20 x 27-29 µ (Thanikaimoni 1966); 3-colporate, 16 x 27 µ (Huynh 1968); 3- colporate, 20 x 25 µ (Taylor 1989). Distribution: India - Almost throughout the country. Tropical Africa to China and Malesia through Sri Lanka and India. Notes: The allied species of *Utricularia striatula* are distributed in the Himalayas, Khasi hills and Malesia; *U. furcellata*, perhaps is the closest ally. The other species - *U. kumaonensis*, *U. brachiata* and *U. multicaulis* have comose seeds in contrast with glochidiate seeds of *U. striatula*. The size and structure of corolla very much in this species. The lower lip of corolla is either 5- or 3- lobed, the lobes are often obscure and the size always exceeds the size of calyx-lobes except in few extreme cases. The spur is completely reduced forming a saccate structure, in few specimens collected from Lonavla, Maharashtra, and these plants cannot be treated distinct due to imperceptible gradation. The occurrence of cleistogamous flowers is high in this species. According to Killian (Taylor 1964) the cleistogamous flowers are seen in the plants which receive sunlight for a period of less than one hour per day. It was observed during this study that such plants are abundant on vertical side of rocks facing north where the sunlight is comparatively less, and on tree trunks where it never falls directly.

3.4. TRAP TYPES & MECHANISMS

Carnivorous plants show different modes of trapping mechanism. Active trap happens when their prey performs motions, for example, *Drosera* (sundew) leaf

blades to get prey. In Passive type trap, the plant moves their trapping parts such as Snap trap (rapid leaves movement), Suction trap (capture insects or microorganism) and Lobster pot trap (force the prey being trap). The suction traps of *Utricularia* (Lentibulariaceae) are well known moving structure. Among Lentibulariaceae, three genera have different modes of trapping like Genlisea (corkscrew plants) have sub-terrestrial eel-traps (Darwin 1875; Lloyd 1942; Fleischmann 2012a), *Pinguicula* (butterworts) possesses sticky leaves (Darwin 1875; Lloyd 1942; Heslop-Harrison 1971) and *Utricularia* (bladderworts) capture and digest small insects and microorganism (Darwin 1875; Lloyd 1942; Guisande *et al.* 2007)**.** Different modes of trapping leads to know the ecology and its evolution with its trapping mechanism (Gibson and Waller 2009). Lentibulariaceae family of *Utricularia* captures preys like Tardigrada, Nematoda, Gastropoda, Acaridae, Rotifera, Ciliata, Crustaceae etc. Small fishes (Moseley 1884; Gudger 1947). Diatoms in *U. volubilis* (Płachno *et al.*, 2014). Other microorganisms like algae, bacteria and protozoa are found inside the bladder leads to complexity food-web relationships with the plants (Hegner 1926; Schumacher 1960; Peroutka *et al.*, 2008; Koller-Peroutka *et al.*, 2015).

3.5. BIOCHEMISTRY OF TRAPPING ORGANISM

Insectivorous plants get nutrients by trapping insects and microorganism. The plants survived by getting nitrogen from insects. Inorganic to organic form of nitrogen converted to each other. Along with pathogenic fungi like endophytes (*Beauveria* and *Metarhizium*) they shows symbiotic relationship (Behie *et al.*, 2013). The inner surface having slippery wax traps and digest the insects. The crystals mixture of aliphatic compounds and triacontanal 43% responsible for crystal formation (Riedel *et al.*, 2003). The digestive zone mainly responsible for digestion, absorption and transport of the insect-derived nitrogen compounds

(Owen *et al*., 1999). Wax crystals of different percentage contains alkanes, aldehydes, primary alcohols, free fatty acids, esters and triterpenoids. The pH of the fluid released had shown from pH 5.0 down to 3.0 (Heslop-Harrison and Knox 1971). According to Heslop-Harrison and Knox (1971); Heslop-Harrison (1975); and Heslop-Harrison and Heslop-Harrison (1981), the enzymes detected in the digestive glands of *Pinguicula* were acid phosphatase, amylase, esterase, leucine amylopeptidase, peroxidase, protease and ribonuclease. *Drosera* species may contain polysaccharides (Gowda *et al.*, 1983).

3.6. ECOLOGICAL ASPECTS

The insectivorous plant present in the nutrient poor areas mainly aim to get organic nitrogen and phosphorous. Various ecological aspects play a major role in their habit of various habitat. The presence of Lentibulariaceae in different habitat is due to their difference in ecological demographic, climatic, edaphic factor, genetic diversity, and may be due to difference in their pollination patterns. The urgent need of conservation of these family is much needed as many anthropogenic activities endangered the plant kingdom. The growth of *U. vulgaris* is affected by the temperature and humus acid (Kosiba 2004). The mode of insect trapping to its mucilage is high but less number of insects present if the plant lives in a sunny, rocky and dry area. But the plant lives in a shaded and wet area, the presence of preys is high but mode of trapping is reduced due to less mucilage on (Legendre 2000). Selectivity in trapping by *U. uliginosa* (Jobson and Morris 2001).

3.7. CONCLUSION

The insectivorous plants of Lentibulariaceae family shows unique morphological feature. There is need of proper monitoring of these plants especially in North Eastern India since it is a very rich biodiversity in all the places. Different

ecological aspects result into the difference in their population inventory and its specific adaptations to the present environment. The traditional values known should be monitored well and need for application through modern medical world.

Plate 1: Collection and plant parts of collected *Utricularia aurea* (Field work, flower parts and fruit)

Table 1: Diversity of Carnivorous species under Lentibulariaceae

Order	**Family**	**Genus**	**World**	**India**	**North-Eastern**
Lamiales	Bybliddaceae	Byblis	7		
	Lentibulariaceae	Pinguicula	94	1	1
		Genlisea	27		
		Lentibulariaceae	234	39	16
	Total		362	40	17

Table 2: Species of Lentibularaceae reported from North-Eastern India

Plant species	**Flowering periods**
Utricularia aurea	October-February
Utricularia bifida	July – December
Utricularia caerulea	August – December
Utricularia exoleta	January – May
Utricularia furcellata	August – November
Utricularia multicaulis	July – August
Utricularia recta	August – November
Utricularia scandens	September – December
Utricularia subulata	August
Utricularia striatula	July – December

Table 3: Most common associate floral species of Lentibulariaceae

Name	**Family**
Althernanthera philoxeroides	Amaranthaceae
Pistia stratiotes	Araceae

Hydrilla verticillata	Hydrocharitaceae
Lindernia procumbens	Linderniaceae
Brachiaria mutica	Poaceae
Eichhornia crassipes	Pontederiaceae
Azolla pinnata	Salviniaceae
Salvinia cucullata	Salviniaceae

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CHAPTER 4

Effect of covid-19 on trade of medicinal and aromatic plants in Uttarakhand, India

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Abstract

Since time immemorial human has been dependent on plants for the curing of various ailments and diseases. In the time of COVID-19 pandemic, suddenly the demand for medicinal plants has increased. This has proven the direct impact of COVID-19 pandemic in the trade of medicinal and aromatic plants in state of Uttarakhand. The present study was designed to understand the current trends in the marketing and trade of Medicinal & Aromatic Plants (MAPs) in the selected areas of Uttarakhand (Pauri & Haridwar) state to identify the fluctuations in prices, demand and supply of MAPs especially during COVID-19 pandemic. A door-to-door questionnaire survey on the price of medicinal and aromatic plants was conducted during frequent visits from February 2021 to April 2021 at different herbal shops in selected areas of Uttarakhand, India. Trading data of around 40 medicinal and aromatic plants has been recorded and documented in the present study along with their prices, source of collection and their demand and supply chain.

Keywords: Pandemic, questionnaire, trade, cultivation, biodiversity

4.1. INTRODUCTION

India is one among the twelve mega diversity countries, commanding 7% of the world's biodiversity and supporting 16% of the major forest types, varying from alpine pastures in the Himalayas to temperate, subtropical, tropical forests, and mangroves in the coastal areas. The industrial demand for the medicinal plants resources has been rise due to the global buoyancy in the herbal sector engaged in production of herbal products during the unexpected and challenging pandemic of COVID-19. In India nearly 9,500 registered herbal industries and a multitude of unregistered cottage-level herbal units depend upon the continuous supply of

medicinal plants for manufacture of herbal medical formulations based on Indian Systems of Medicine. In addition to the industrial consumption, significant quantities of medicinal resources are consumed in the country under its traditional health care practices at the household level, by traditional healers and by practitioners of Indian Systems of Medicine. MAPs are produced and offered in a wide variety of products, from crude materials to processed and packaged products like pharmaceuticals, herbal medicines, teas, cosmetics, sweets, dietary supplements, varnishes and insecticides (Lange 1997). Whereas, more than 6,000 higher plant species are estimated to be used in the codified and folk healthcare traditions in the country, the quantum of their consumption has remained a matter of great interest. About 70,000 plant species are used in folk medicine worldwide (Farnsworth and Soejarto1991). It was noticed that there have been drastic increase in the sales of tulsi, brahmi, ashwagandha and lemongrass in some parts of Uttarakhand in this pandemic. The nursery owners are reporting that their sale is not only restricted to India but also there is huge demand of medicinal and aromatic plants from foreign countries also.

4.2. METHODOLOGY

The market study of trade patterns for medicinal plant products was conducted with particular reference to plants in selected areas of Uttarakhand (Pauri & Haridwar) during COVID-19. To analyze the trade in medicinal plants, representatives of diverse stakeholder were interviewed using Passport Data Form (Annexure 1; Plate 1). Cultivars/gatherers of medicinal plants, farmers, local dealers and the ayurvedic pharmacies were selected for interview. Respondents were also asked about the quantity of each species collected from the wild and about their annual income earned from the sale of targeted plants and returns to the work invested and the express incurred on them. For the profiling of medicinal plants in Uttarakhand, literature survey was done and listed in Table 1 & 2.

4.2.1. PRESENTATION OF DATA

Systematic enumeration of the plants products is arranged alphabetically. The families, vernacular names, description of the voucher specimen, use of the plants etc. are mentioned.

Plate 1. 1-2) Survey with local traders; 3) Nursery of medicinal plants; 4) Ashwagandha (*Withania somnifera*); 5) Tejpatta (*Cinnamomum tamala*): 6) Kalmegh (*Andrographis paniculata*)

4.3. RESULTS

The study was conducted in the selected areas of Uttarakhand state. The information was collected by visiting and on the basis of interviews with owners of herbal shops and the employees of government agencies. The survey identified four key stakeholders'groups in the medicinal plant trade; Farmers, traditional herbal medicine. The marketing of medicinal plants in the study area is largely informal, dominated by simple technologies and the survey interactions driven primarily by economics. Details are listed in Table 3.

Table 1. The profile of medicinal plants in Uttarakhand

Total number of species	964
Number of families	158
Number of tree species	160
Number of shrub species	190

Number of herb species	614
Use of plant species in curing number of ailments	135

Source-Kala (2005)

Table 2. Profile of medicinal families in Uttarakhand

Family	**Number of Species**
Asteraceae	87
Fabaceae	58
Lamiaceae	49
Liliaceae	29
Apiaceae	28
Euphorbiaceae	26
Ranunculaceae	26
Orchidaceae	23
Gentianaceae	18
Poaceae	18
Rubiaceae	18
Rosaceae	30
Verbenaceae	18

Source Kala (2005)

Table 3. Market price of raw materials of some important medicinal and aromatic plants available with traders in Garhwal and Kumaon Region of Uttarakhand traded mostly during Covid-19

Plant Name	**Trade name**	**Price INR/kg**	**Marketable part (DRY)**
Aconitum heterophyllum	Atiskadwa	6000	Tuber
Allium stracheyi	Faran	700	Leaves
Amomum subulatum	Badi elaichi	1000	Fruits
Andrographis paniculata	Kalmegh	80	Herbage
Angelica glauca	Gnadrayan	150	Roots
Asparagus racemosus White Yellow	Satavari	 150 450	Roots
Centella asiatica	Brahmi	190	Whole plant
Cinnamomum tamala	Tejpat	60 1400 180	Leaves Oil Bark
Coleus barbatus	Patharchur	80	Roots
Cymbopogon flexuosus	Lemon grass	1500	Oil

Dactylorhiza hatagirea	Salampanja	9000	Tuber
Emblica officinalis	Amla	55	Fruits
Gloriosa superba	Kalihari	250	Roots
Lavandula officinalis	Lavendar	1500 35000	Flower Oil
Mentha arvensis	Mentha	100 2000	Herbage Oil
Nardostachys jatamansi	Jatamansi	1200	Roots
Ocimum sanctum	Tulsi	85 10000	Leaves Oil
Paris polyphylla	Satuwa	3000	Roots
Picrorhiza kurroa	Kutki	1200	Stolon/roots
Piper longum	Pipali	750	Fruits
Podophyllum hexandrum	Bankakri	600	Roots
Rauwolfia serpentina	Sarpagandha	800	Roots
Rosa damascena	Gulab	300	Leaves
Rubia cordifolia	Manjisth	250	Roots
Saussurea costus	Kuth	250	Rhizome/roots
Swertia chirayita	Chirayita	450	Whole plant
Terminalia bellirica	Bahera	30	Fruits
Terminalia chebula	Harad	25	Fruits
Valeriana jatamansi	Tagar	450	Rhizome/roots
Withania somnifera	Ashwagandha	250	Roots

4.4. DISCUSSION

The trade of medicinal plants varies across the state. The fluctuation in trade of medicinal plants is in same trend as reported by Kala CP (2015). NMPB also reported that the trade of four medicinal plants viz Ashwagandha, Kalmegh, Giloy and Tulsi has shown drastic increase in market values due to immense use during the period of Covid-19 pandemic and their trade is increased particularly the trade of Ashwagandha and Kalmegh as they have immunomodulatory and hepatoprotective activity (http://tradestat.commerce.gov.in).

4.5. CONCLUSION

Medicinal plants represent an important asset to the livelihoods of many people in developing countries and especially in this Covid-19 pandemic, the need of medicinal and aromatic plants has increased drastically. In Uttarakhand, where the livelihood of many people is dependent on cultivation of medicinal and aromatic plants, the trade of medicinal and aromatic plants has increased drastically. The

present study shows market strategies and trends of medicinal and aromatic plants in trade at Uttarakhand in general. The trade seems to be fluctuated at increasing rate due to Covid-19. The trade of immunomodulatory medicinal herbs such as Tulsi, Ashwagandha, Giloy, Brahmi, Aonla, Dugdhi, Turmeric and Fenugreek have drastically increased. This has benefitted the farmers, stakeholders, nursery owners and cultivars a lot. The price of five medicinal plants viz *Acorus calamus, Andrographis paniculata, Asparagus racemosus, Bacopa monnieri and Berberis aristata* have fluctuated a lot during the last five years.

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Annexure I

MARKET SURVEY QUESTIONNAIRE

General information

Respondent's Name:

Age: Occupation: Education:

Address: Gender: Annual income:

Location: Date:

Involvement category:

(1) Trader

(2) Collector

(3) Farmer

1) Are you engaged in Medicinal Plant trade? If yes, for how many Years?.....
2) Are you engaged in Medicinal and Aromatic Plant cultivation?
3) Have the trend of Medicinal plant cultivation and marketing increased or decreased in the Coivd-19 pandemic?
4) Increasing b) Decreasing c) Stable/None of the above
5) Are you able to sell your medicinal plants products easily?
   a) Yes/very easily
   b) No/very difficult
   c) Little bit difficulties
6) What are the problems in selling your Medicinal Plant products?
   a) Low price per kg
   b) Low demand
   c) Policies
   d) Social
   e) Lack of buyers
7) According to you, how the problems of marketing can be resolved?
8) Means of supply: [grow] [buy] [collect from wild]? If you collect from the wild, please mention where?.....
9) How many quantities (Kg) of total Medicinal Plants products?
10) Are there any substitutes for the most traded/used species of medicinal plants?
11) To whom and where do you sell your Medicinal plant products?
    a) Agents
    b) Middlemen
    c) Businessman
    d) Wholesaler
    e) Mandi
12) In your opinion, what are the prospects /potentialities of Medicinal plants
    a) More income is comparison to agriculture crops
    b) Increase productivity of soils
    c) Utilize the abandoned and uncultivated lands
    d) Decrease the use of allopathic medicines
13) Which Medicinal plants are mostly cultivated and marketed in your area?
14) The sale of which medicinal plant have increased drastically in Covid-19?
15) The information regarding the uses of medicinal plant products in the religious, cultural activities from the owners of Ayurvedic Pharmacies and people associated with various rituals were collected and documented.

CHAPTER 5

A review on *Murraya koenigii* (l.) Spreng. A potent medicinal plant

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Abstract

Several plant extracts have been reported to offer superior therapeutic benefits than traditional medicinal therapy. In the account of AYUSH, all traditional methods of medicine are included. The Ayurvedic, Yoga, Unani, Siddha, and Homeopathy systems of medicine were included in the AYUSH department. Murraya koenigii (L.) Spreng. is a member of the Rutaceae family and is commonly employed in the Ayurvedic system of medicine as a medicinally important herb of Indian provenance. Antioxidant, antidiabetic, anti-inflammatory, anticancer, and neuroprotective properties are among them. In this review paper is described about the therapeutic value of Murraya koenigii.

Keywords: Antioxidant, Ayurvedic, *Murraya koenigii,* Siddha, Traditional

5.1. INTRODUCTION

Murraya koenigii is a species of the Rutaceae family and growing in every area of the Tropical region up to a height of 1500 to 1655 meters above sea level (Gupta and Prakash 2009; Jain *et al.*, 2012). Curry Leaf in English, Mitha Neem in Hindi, Karuveppilei in Tamilnadu, and Surabhinimba in Sanskrit are some of its other vernacular names (Trimen 1900). It has been utilized as medicine for curing ailments for a long time, and people in both developed and developing countries have realized that plants play an important role in human life and India is considered to be the biodiversity of medicinal plants (Mandal *et al.*, 2021; Mallick and Mahalick 2020). *M. koenigii* has been demonstrated to be a natural healing

herb (Singh *et al.*, 2014). The many therapeutic and preventive distinctive features of this plant have been well known to humans since time immemorial (Mallick and Mahalick 2020). *M. koenigii* has been utilized in Indian cooking over millennia and has a wide range of applications in traditional medicine and often known as curry leaf or Karipatta in Indian dialects, is a member of the Rutaceae family, which has over 150 genera and 1600 species (Satyavati *et al.*, 1987). P-gurjunene, P-caryophyllene, P-element, and O-phellandrene are the most essential chemical components responsible for its distinct unique aroma. This leaf is commonly used as a condiment and lends a unique flavor to any cuisine. But the modest curry leaf is capable of much more than just flavor. Curry leaves are high in carbohydrates, fibre, calcium, phoshorous, irons, and vitamins like vitamin C, vitamin A, vitamin B, and vitamin E, which help your heart function better, fight infections, and energize your hair and skin. Carbazole alkaloids are abundant in the plant (Kumar *et al*., 1999).

5.2. BOTANICAL DESCRIPTION

Shrubs or trees, to 4 m tall. Leaves 17-31-foliolate; leaflet blades ovate, 2-5 × 0.5-2 cm, base obtuse to rounded and oblique, margin entire or crenulate. Inflorescence's terminal, paniculate, many flowered. Flowers 5-merous, ellipsoid in bud. Sepals ovate, less than 1 mm. Petals white, oblanceolate to oblong, 5-7 mm. Stamens 10. Stigma capitate. Fruit bluish black, ovoid to oblong, 1-1.5 cm, 1- or 2-seeded. Seed coat membranous (Saxena and Brahmam 1994).

Flowering: March-April; fruiting: July-August (Saxena and Brahmam 1994)

5.3. ETHNOBOTANICAL USES OF *MURRAYA KOENIGII*

M. koenigii is a plant which has various important uses in the field of traditional system of medicine. Based on ethnomedicine and other uses, It is used as

anantidysentric, stimulant and management of diabetes. Some other uses are mentioned in Table 1.

Figure 1: Morphology of *Murraya koenigii plant*

Table 1: Medicinal uses of *M. koenigii* plant parts

Parts used	Traditional Uses	Source(s)
Leaf	Soups, curries, fish and meat dishes, eggs dishes, traditional curry powder blends, seasoning, and ready-to-use other food preparations all use fresh leaves, dried leaf powder, and essential oil.	Chowdhury *et al.*, (2008)
	The essential oil extracted from leaves is widely used in the soap and cosmetics business, as well	Gupta and Nigamurraya

	as in aromatherapy.	(1970)
	Curry leaf oil with your usual body care cream or lotion helps to cure skin problems such as pimples, athlete's foot, ringworm, itches, acne, boils, and septic injuries and burns by applying it to the affected region.	Dasgupta *et al.*, (2003); Gupta and Sharma (2010)
	Using essential oils *M. koenigii* is utilised in cosmetics as a sun protection and anti-erythema agent.	Prakash and Natarajan (1974)
	M. koenigii essential oil is used in the soap and cosmetic sector for aromatherapy.	Chakraborty (1974); Chowdhury and Chakraborty (1969)
	Curry leaf extract aids in the reduction of white areas on the body and helps with pigmentation.	Anwer *et al.*, (1973)
	Curry leaves and essential oil are used both orally and externally for healthy, long, strong, and lustrous hair. A balanced diet with an equal percentage of vitamins, minerals, iron, and other nutrients is needed to keep hair healthy.	Debosree *et al.*, (2012), Bhattacharya and Chowdhury (1984)
	Curry leaves are cooked in coconut oil until they are reduced to a blanked residue, which is then used as a hair tonic to maintain natural hair tone and stimulate hair development.	Adebajo *et al.*, (2004); Harve and Kamath (2004)
	Curry leaf oil aids in muscle and tissue	Narsimha *et al.*,

	contraction.	(1968); Nagappan *et al*., (2012)
	Curry leaf oil's high vitamin A and calcium content is used to treat osteoporosis, calcium shortage, and cancer radiotherapy and chemotherapy treatments.	Srinivasan (2005)
Root	*M. koenigii* root juice can be used to treat kidney pain. It can be used to prevent hair from greying prematurely. The juice of root is good to cure kidney stone as well as cure kidney related problems.	Chowdhury and Chakraborty (1969)
Fruits	Fruits are considered as astringent.	Parrota (2001)
Branches	Branches are very popular for cleaning the teeth used as dantun.	The wealth of India (2003).

5.4. CONCLUSION

M. koenigii is a shrub of Rutaceae with therapeutic properties. Anti-diabetic, cholesterol-lowering, antibacterial, antiulcer, antioxidative, cytotoxic, anti-diarrhoea, anti-cancer, and phagocytic effect have all been reported. The accessible literature and extensive availability in India, this plant is an appealing choice for future pre-clinical and clinical studies for medicinal applications.

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CHAPTER 6

Antibacterial activity of selected medicinal plants against *Shigella flexneri*

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Abstract

S. flexneri which is a gram negative facultative anaerobes that belong to the family, Enterobacteriaceae. This bacteria may occur as the organism pass through the small intestine and causes disease like diarrhea and food poisoning. The important sources of medicinal plants like Psidium guajava, Oroxylum indicum and Pergularia daemia are noted for their antimicrobial activity against S.flexneri. Hence an attempt has been taken to gather the reported information and availability of this plant species in the urban areas of Bhubaneshwar. Survey was made during the January 2020 to March 2020 to locate the said species in the study areas. Results revealed that selected plant species are used to treat many disease and disorder in general and particular against diarrhea. The phytochemical screening and antibacterial activities for evaluation of their pharmacological potential was carried out. The results revealed that selected plant species are rich with diverse bioactive compounds and excellent MIC (Minimum Inhibitory Concentration) against S. flexneri. The results indicate that there is wide scope in isolation of active compounds and formulation of drugs against food poisoning and diarrhea.

Keywords: Antimicrobial activity, Medicinal plants, MIC, *Shigella flexneri*

6.1. INTRODUCTION

Shigella flexneri is a diarrhea causing gram negative, non spore forming, non motile, facultative anaerobic, rod shaped bacteria, enteric pathogen and together with three other *Shigella* species, *Shigella dysenteriae*, *Shigella sonnei*, and *Shigella boydii*, is responsible for about half of a million cases of shigellosis and commonly seen in developing countries (Schroeder and Hubert 2008). 10 to 100 micro-organisms are sufficient to cause disease results from contaminated food, poor hygiene, sanitation condition and direct person to person contact (Cabral 2010). Shigellosis can be characterized as a disease with over incidence in children ages 1-5. These are found in stomach, causes infection via bacterial penetration of the mucus membrane and then multiply in small intestine commonly known as colon (Smith 1988). The shigellosis diseases is manifested by 5-7 days of diarrhea, fever and abdominal pain, which is a result of the intestinal tissue destruction. These *Shigella* species are responsible for acute diarrhea, bloody diarrhea accompanied by fever and abdominal cramps. Classic dysentery is characterized by scant stools containing mucus, pubs and blood. Man is only the reservoir for *Shigella* species and sexual transmission has been observed in homosexual males. Shigellosis is responsible for rectal and colonic ulceration that do not develop beyond the lamina propria. *S.flexneri*'s effects include bacteremia, hemolytic uremic syndrome (HUS) and toxic mega colon (Mattock and Blocker 2017). *S.flexneri* develops actin based mortility enabling the pathogen to become efficient at cell-to-cell spread and host cell colonization. Infected cell became highly pro inflammatory and secrete IL-8(Interleukin-8). IL-8 attract neutrophills,chemokines and cytokines to the area damages the epithelial layer permeability and in turns advocates further *S.flexneri* invasion (Sansonetti *et al.*, 1999). Once initial invasion of *S.flexneri* occurs, the targeted epithelial cells require 45 minutes to 4 hours. to mount an inflammatory response. The highest incidence of *S.flexneri* infection is seen in children aged 1-4 (Shih *et al.*, 2015). In addition it also shows high incidence in the age 30-39 age group. Once contracted, 20% of *S. flexneri* infections require hospitalized. It can be prevented by good hygiene and in particular, people involved in food preparation and service must be disciplined in proper sanitation techniques and given adequate bathroom facilities (Taneja and Mewara 2016). For the above problems, medicinal plant species like *Psidium guajava*, *Oroxylum indicum* and *Pergularia daemia* which are belongs to

Myrtaceae, Bignoniaceae and Apocynaceae family respectively were selected to evaluate the antimicrobial activity for present study against *S.flexneri*.

6.2. Methodology

6.2.1. Ethnobotnical data collection on selected plant

The results presented here are based on the field work conducted with local communities of study area during January 2020 to February 2020. The methodological frameworks for the ethnobotnical study were as per the standard techniques of exploration. The standard participatory rural appraisal method was adapted for sampling and data collection to Incorporate the indigenous knowledge. Opinions of local people were taken regarding the uses of experimental plant species through questionnaires (Kumar *et al.*, 2017; Kumar *et al.*, 2018).

6.2.2. Preparation of plant extracts

Soxhlet method and percolation were adapted to obtain the plant extracts. The plant parts of experimental plant were collected and dried at room temperature under shade and were powdered after drying using mechanical devices. The powdered material of the experimental plant was kept in thimble and extraction was carried out using the Soxhlet apparatus.The residues were collected and left for air drying and dried crude extracts were in refrigerator for further experimental work (Kumar and Jena 2014).

Figure 1: powder of extracts of experimental plants

n-haxene extraction

5 g of the powder of each selected plant extracts are taken in different beakers and 50 ml of n-hexane was added to each extracts and then kept into refrigerator for minimum 24 hours.

Methanol extraction

5 g of the powder of each selected plant extracts are taken in different beakers and 50 ml of methanol was added to each extracts and then kept into refrigerator for minimum 24 hours.

Ethanol extraction

5 g of the powder of each selected plant extracts are taken in different beakers and 50 ml of ethanol was added to each extracts and then kept into refrigerator for minimum 24 hours.

Aqueous extraction

5 g of the powder of each selected plant extracts are taken in different beakers and 50 ml of distilled water was added to each extracts and then kept into refrigerator for minimum 24 hours.

5.3.Phytochemical Aasays

Phyto-chemical analysis were carried out on different extracts of different plant parts using standard procedure to identify the bioactive compounds.

Test for Saponin

0.5 g of dried powder was boiled in 15 ml of distilled water and filtered with Whattman 42 filter paper .5 ml of filtrate was mixed with 2 ml of normal distilled water and shaken vigorously. The stable persistent froth indicated the presence of saponins.

Test for Tannin

0.5 g of dried powder sample was boiled in 10 ml of distilled water and filtrated with Whattman 42 filter paper .2 ml of filtrate was taken in a test tube and 3-5

drops of 0.1 % ferric chloride solution were added .The brownish green or black coloration indicated the presence of tannins.

Test for Flavonoids

6 ml of dilute ammonium solution was added to a portion of the aquous filtrate of plant extract followed by addition of 8 ml concentrated sulphuric acid.A yellow coloration indicated the presence of flavonoids.

Test for Terpenoids

6 ml of extract was mixed in 2.5 ml of chloroform and then 3 ml of concerntrated sulphuric acid was added. A reddish-brown colouration of interface indicated the presence of terpenoids.

Test for Steroids

2 ml of plant extract was dissolved in 5 ml chloroform and then 5 ml of concentrated sulphuric acid was added. Formation of 2 phases (upper and lower yellow with green fluorescence) indicated the presence of steroids.

Test for Phenolic compounds

0.5 g of extract was treated with 3-5 drops of 1% ferric chloride solution. Formation of bluish black colouration indicated the presence of phenolic compounds.

Test for Reducing sugar

0.5 g of plant extract was dissolved with distilled water and filtered.The filtrate was boiled with 2 drops of fehling's solution A and B for 5 minutes.An orange-red precipitate was obtained indicated the presence of reducing sugar.

Evaluation of Antimicrobial activity

The extracts of experimental plant parts were screened for antimicrobial activity against one gram negative bacteria i.e *Shigella flexneri*. Antimicrobial activity was done using Broth dilution, Agar wall diffusion and Disc Diffusion assay (Kumar and Jena 2014).

Media used

Nutrient broth was used to maintain broth cultures. The constituents of the nutrient broth included 0.5 g NaCl, 0.5 g peptone and 0.3 g beef per 100 ml. an additional 1.5 of agar made up the nutrient agar medium.

Preparation of working slant

Stock culture were maintained at 4ºC on slant of semi-solid media containing 1.5% of agar-agar, 0.3% beef extract and 0.5% peptone. Active working cultures for experiments were prepared by transforming a loopful of culture mass from the stock. Slants were incubated for 24 h at 36±1.0ºC.

Broth preparation

Colonies of prepared slant was picked off using sterile loop and inoculated in sterile conditions in autoclaved cool liquid broth medium containing 0.3% of beef extract and 0.5% peptone. The broth was incubated for 24 hrs at 36±1.0ºC until there was visible growth indicated by turbidity standard.

Swabbing and Inoculation of drug

In Disc Diffusion Assay, swabbing was done using sterile swab. Then disc of respective aforesaid concentration were placed on media. Petri plates were incubated at 36± 1.0 ºC for 48 hrs. Zones of inhibition free microbial growth appeared around each disc in the form of clean rings which conformed the antibacterial activity of the respective samples. Those samples which did not have any inhibitory effect on the microbe did not form any clear ring. In this way, the antimicrobial activity of the sample was conformed. Triplicates were maintained and the experiments was repeated thrice. For each replication the readings (zone of inhibition) were taken and the mean values were recorded.

Data Analysis

Mean and SD (standard deviation) was performed to calculate taking triplicate values of zone of inhibition (nm for disc diffusion) of samples using Excel, Microsoft Corporation-2010,US.

6.3. RESULTS & DISCUSSION

Based on information collected about the medicinal values of these plants from study areas and literature, an attempt was made to justify the rationale behind the claims and to identify the presence of bioactive components in leaf and stem extracts through qualitative tests. The extracts of these selected plants were analyzed to know the presence of the metabolites in them. The antibacterial activity of *Psidium guazava, Oroxylum indicum* and *Pergularia daemia* extracts against *Shigella flexneri* was observed.

Table 1: Phytochemical Tests of selected experimental plant parts

Plant name	**Plant part**	**Solvent**	**Metabolites present**
Psidium guazava	Leaf	n-Hexane Ethanol Aqueous	Saponin, steroid, reducing sugar, phenolic compound
Pergularia daemia	Leaf	n-Hexane Ethanol Aqueous	Flavonoid, tannin, saponin, reducing sugar
Oroxylum indicum	Leaf	n-Hexane Ethanol Aqueous	Steroid, tannin, phenolic compound, reducing sugar

Table 2: Estimation of minimum inhibitory concentration (MIC) (µg/ml) of aqueous extract of selected experimental plants against MTCC 1457 (*Shigella flexneri)*

Extract(s)	**MTCC 1457**
Psidium guazava	500 µg/ml
Pergularia daemia	400 µg/ml
Oroxylum indicum	300 µg/ml
Inoculums control	Growth in all Concentration
Broth control	No growth

The phytochemical screening was done to examine the presence of the secondary metabolites such as saponin, terpenoids, steroids, tannin, flavonoids, phenolic compound and reducing sugar. Details of the results are listed in Table 1.

Figure 2: MIC of *Oroxylum indicum* against *S. flexneri*

It was observed that *Oroxylum indicum* showed excellent activity against MTCC 1457 followed by *Pergularia daemia* and *Psidium guazava* (Table 2; Figure 2). Use of natural medicines to cure various diseases has become an increasing trend. Herbal medicines have made significant contributions to modern medical practices. The polyherbal formulation contains three plant extracts *Psidium guajava*, *Oroxylum indicum,* and *Pergularia daemia.*The phytochemical screening revealed that they have diverse metabolites which are listed in the Table 1. The secondary metabolites present in most of the extracts were saponin, tannin, flavonoids, phenolic compound and reducing sugar. Traditionally saponins have been extensively used as detergents, as pesticides and mollucicides, in addition to their industrial wide range of pharmacological activities including expectorant, anti inflammatory, vasoprotective, hypocholestrolemic, immunomodulatory, hypoglycaemic, antifungal and antibacterial activities. Tannins are plant poly phenolic compounds and are called antimicrobial biomolecules. Tannins have good antimicrobial activities against several gram positive and gram negative bacteria. The results of broth dilution that shows the zone of inhibition of bacteria *Shigella flexneri* at different concentrations of the extracts of the formulated powders of the

3 plant extracts. the Results of MIC shows that there is no growth of the bacteria at different concentration of the formulation. The extracts of *Psidium guajava*, *Oroxylum indicum*, and *Pergularia daemia* inhibit the growth of *Shigella flexneri* with MIC of 500 µg/ml, 400 µg/ml and 300 µg/ml.

6.4. CONCLUSION

Diarrhea is a very common diseases among the rural and tribal people across the country. They use different parts of locally available plants to treat it. Most of the claims on plants are need to validate for further scientific works. The present study based on the validation the tribal claims on selected plants which is used to treat diarrhea to bring attentions towards scientific validation of tribal claims. The study highlights the importance of local plants and scientific validation to give base line data for advanced works on pharmacological activities of medicinal plants.

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CHAPTER 7

Medicinal parasitic plants of Odisha

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Abstract

Parasitic plants represent a peculiar model for the evolution of intra kingdom parasitism. They usually connect to the vasculature of the other flowering plants for water, nutrients and assimilate, that they need to complete their life cycle, by a specialized structure called haustoria. Till now, about 4500 species of parasitic plants have been explored throughout the world belonging to 280 genera and 20 families. In Odisha, a total of 19 species of parasitic plants belonging to 11 genera and 7 families are reported. The most common reported families are Santalaceae, Viscaceae, Loranthaceae, Lauraceae, Cuscutaceae, Scrophulariaceae etc. Parasitic plants have been traditionally used by the healers and many tribal communities. These plants are also used in various herbal formulations for the treatment of many diseases and disorders. The present study highlights the medicinal uses of unexplored parasitic plants of Odisha.

Keywords: Parasitic plants, Odisha, traditional therapeutic system

7.1. INTRODUCTION

Parasitic plants are taxonomically diverse group of plants which rely partially or completely on host plants for obtaining water, nutrients and assimilate to complete their life cycle, which they acquire by attaching to host roots or shoots using specialist structures known as haustoria. The site of attachment to the host classifies the parasite as either a root or shoot parasite (Press and Phoenix 2005; Musselman and Press 1995). Parasitic plants may also be classified as hemiparasites or holoparasites. On maturation hemiparasites contain chlorophyll, became photosynthetic and are able obtain water, with its dissolved nutrients, by connecting to the host xylem via the haustorium. Chlorophyll pigments are absent in holoparasites and are thus non-photosynthetic. They must rely totally on the contents of the host xylem and phloem (Nickrent and Musselman 2004). Parasitic

plants are found in all climatic zone in all continents of the world, excluding Antarctica (Eizenberg and Goldwasser 2018). They are distributed in 20 dicotyledonous families and in 280 genera (Nickrent 2007; Rubiales and Heide-Jorgensen 2011). They occur in many life forms, including annual and perennial herbs (Press and Phoenix 2005). Some parasitic species cause damage to the agricultural crops which is a serious problem for farmers in many parts of the world (Parker and Riches 1993; Musselman *et al.*, 2001). Although these plants are fatal to their host, they have long been known for their traditional medicine aspects (Kirana 1996; Chozin *et al.*, 1998). Chemicals content in these parasitic plants include alkaloids, flavonoids, saponins, phenolic compounds, steroids etc (Daniel *et al.*, 2012). In Odisha a total of 19 species of parasitic plants belonging to 11 genera and 7 families are reported. The most common reported families are Santalaceae, Viscaceae, Loranthaceae, Lauraceae, Cuscutaceae, Scrophulariaceae etc.

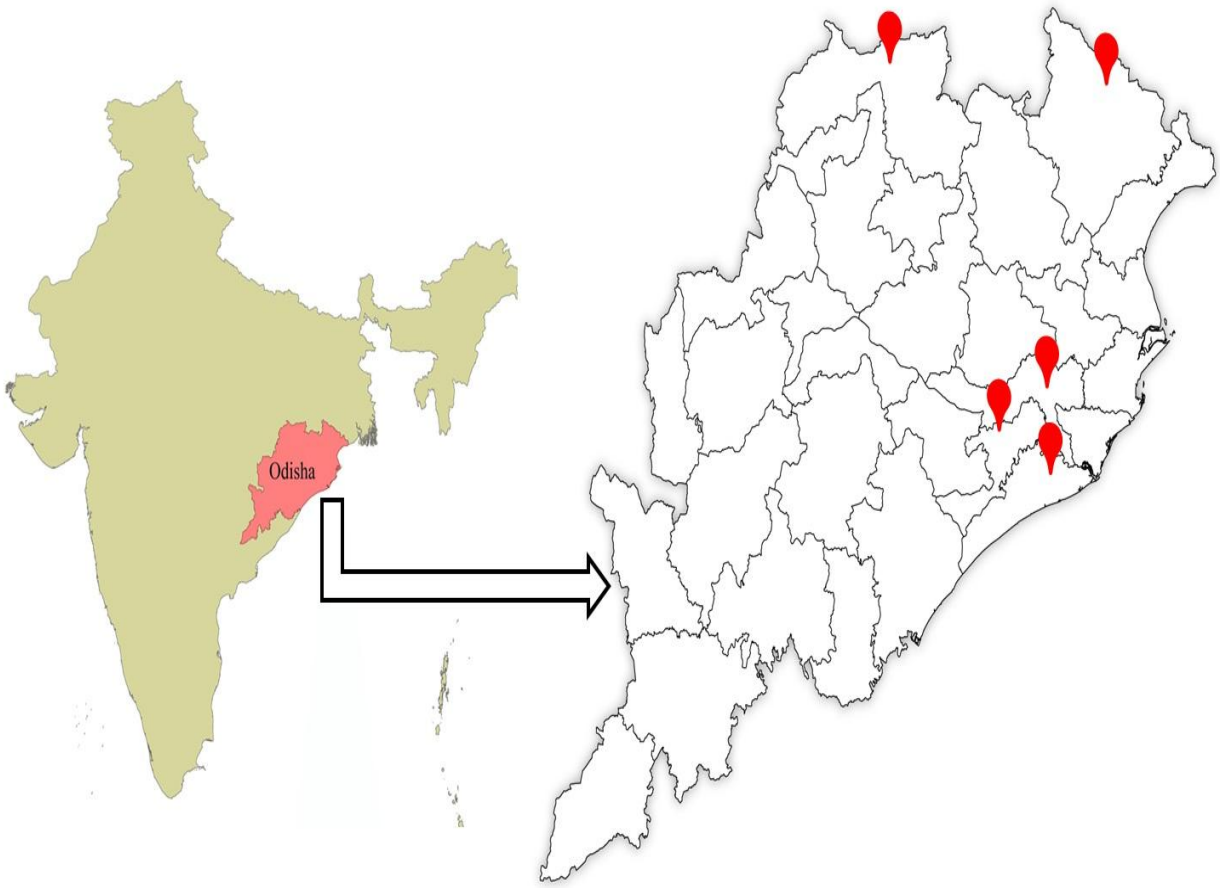

Figure 1: Geographical locations of study areas

These plants are used as traditional medicines and has less written evidences throughout the globe (Sahu *et al.*, 2018). Despite the profound effects that parasitic plants have on their host they still have many medicinal properties yet to explore.

7.2. MATERIALS AND METHODS

Six districts are (Puri, Khordha, Cuttack, Dhenkanal, Mayurbhanj and Sundargarh; Figure 1) selected for the present study during 2018-2021 and enumerated the commonly available parasitic plants. Literature and ehnobotanical survey (Plate 2) was carried out to gather the medicinal values of enumerated species (Haines 1925; Saxena and Brahman 1995; Sahu *et al.*, 2018). Passport Data Form, a semi-structured questionnaire was used to get the medicinal values of enumerated species from local communities, vaidyas and local healers (Mishra and Kumar 2021; Kumar *et al.*, 2017).

7.3. RESULTS

Traditional therapeutic systems are considered as the oldest forms of healthcare known to mankind on this earth. These herbal remedies have been evolved over the centuries within various communities and are still maintained as a great traditional knowledge. The survey revealed that about 13 parasitic plants are commonly used as a medicines belonging to 7 families (Table 1; Plate 1). It was observed that the enumerated plans are used to treat various diseases and disorders like cough, stomachache, kidney ailments, scabies, eczema, sores, urinary disorder, diuretic, diarrhea, swelling, muscle pain, heart disease, nervous disorder and intestinal parasites. Details are listed in table 1.

7.4. CONCLUSION

There are numbers of plant species are available in Odisha having sound ethnomedicinal values which are scientifically unexplored. Parasitic medicinal plants are not explored and need to document them with proper conservation aspects. The present study highlights the medicinal values of 13 medicinal parasitic plants used to cure different diseases and disorders. Further, scientific validation of tribal claims are needed to develop herbal formulation from them.

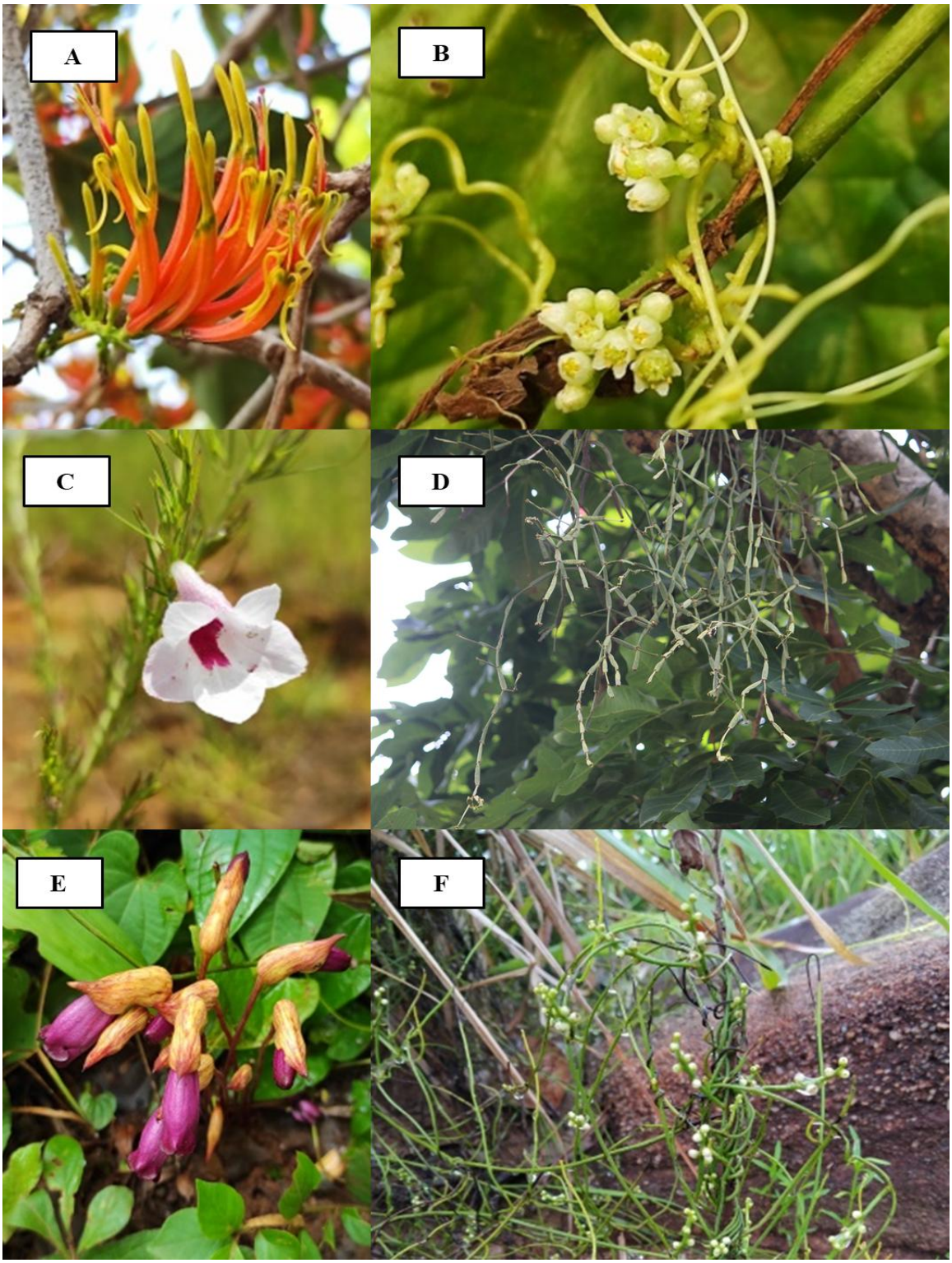

Plate 1: Some common parasitic plants of Odisha; A) *Dendrophthoe falcata;* B) *Cuscuta reflexa;* C) *Sopubia delphinifolia;* D) *Viscum articulatum;* E) *Aeginetia indica;* F) *Cassytha filiformis*

Plate 2: Ethno-medicinal survey on medicinal properties of parasitic plants in study areas

Table 1: Enumerated medicinal parasitic plants of study areas

Plant Name	Family	Parts use	Medicinal use (s)
Aeginetia indica	Orobanchaceae	Stem	Used to treat cough.
Balanophora polyandra	Balanophoraceae	Aerial part	Used to treat gastrointestinal problems.
Buchnera hispida	Orobanchaceae	Leaves	Paste of leaf powder is used to treat scabies and eczema.
Cassytha filiformis	Lauraceae	Whole plant	Juice of whole plant parts is used in kidney ailments.
Centranthera indica	Orobanchaceae	Flowers	Infusion of flowers are used to treat diuretic.
Cuscuta reflexa	Convolvulaceae	Whole plant	Juice of whole plant is used to treat urinary problems.
Dendrophthoe falcata	Loranthaceae	Flowers	Infusion of flowers are used in the treatment of diarrhoea.
Orobanche cernua	Orobanchaceae	Stem	Stem paste is used to relieve swelling.
Sopubia delphinifolia	Scrophulariaceae	Whole plant	Whole plant juice is used to heal sores.
Striga asiatica	Orobanchaceae	Flowers	Infusion of flowers are used to kill stomach parasites.
Viscum articulatum	Santalaceae	Leaves	Used to treat heart diseases.
Viscum monoicum	Santalaceae	Leaves	Used to treat nervous disorders.
Viscum orientale	Santalaceae	Fruit	Fruit paste is used in muscular pain.

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CHAPTER 8

Medicinal trees of Mayurbhanj, India

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Abstract

Mayurbhanj is an important district of Odisha, known for its dominant tribal population, vibrant culture and Similipal Biosphere Reserve. The ethnic communities of the state have their own rich traditional knowledge in the use of various tree species for treatment of different diseases and disorders. The local people have a long history of indigenous knowledge which is not adequately explored or documented. The present paper deals with the effectiveness of folk medicine for curative, remedial and medicinal uses of 45 tree species under 24 genera and 19 families. The present documentation of tree species and associated indigenous knowledge, can be used for developing management plans for conservation and sustainable uses in the study area.

Keywords: Ethno-botanical study, medicinal tree, Mayurbhanj, Odisha

8.1. INTRODUCTION

Medicinal plants are now becoming more widely used herbal products throughout the globe (Mikaili *et al.*, 2013). Undeveloped countries as well as developed countries, that is around 60-80% of the world population now prefer medicinal plants rather than synthetic drugs to maintain personal health condition and treating certain type of disease (Baul and Mohiuddin 2011; Mohammad 2019). These medicinal plants consider as a rich resources of secondary metabolites which can be used in drug development. In the development of human culture, medicinal plants played a key role throughout the world (Yudharaj *et al.*, 2016). About 12.5% of the 422000 plant species reported worldwide having diverse medicinal properties (Schippmann *et al.*, 2002; Baul and Mohiuddin 2011). Among these

species trees play a vital role in the formulation of herbal drugs. But now over-exploitation is a major threat to tree species. Tree assessment, an intensive research has been undertaken over the past five years to compile extinction risk information on the 58,497 tree species worldwide and found that 30 % of tree species that is about 4,585 are threatened with extinction, and at least 142 tree species are recorded as extinct in the wild (Newton 2008; BGCI 2021). Trees are not only an important source of wood for timber, but also provide non-timber forest products like medicines, fuel, fodder etc (Shah *et al.*, 2015). Each part of tree (wood, bark, roots, leaves, flowers, fruits or seeds) is fundamental to the well-being of millions of people and also for the extraction of compounds used for medicines. Lack of awareness about the potential uses of tree species, and particularly ignorance of the concerned authorities, have led to a decline in the population of this precious flora. So the documentation of tree taxa used as traditional medicines is urgent need, so that the knowledge can be preserved. Therefore, present ethno-botanical survey was made to fill the gap in indigenous knowledge related to the use(s) of tree species as a herbal medicines in Mayurbhanj district of Odisha, India.

8.2. MATERIALS AND METHOD

8.2.1. Study area

The district is known for its dominant tribal population. 62 tribes are notified as scheduled for Odisha state and about 45 are found in Mayurbhanj district like Santhal, Kandha, Gauda, Dehuri, Kolha, Munda, Ho etc. The district, for the most part, has red soil and belts of lateritic soil. The district has mostly northern tropical semi-evergreen species like Sal, Piasal, Sisu, Kurum, Kendu, Mahua, Asan etc. The climate of the district is characterized by oppressive summer and well distributed rainfall during monsoon months. December is usually the coldest month of the year when the mean daily minimum temperature is of the order of about 12° C (Census of India 1961; Dash *et al.*, 2016; Figure 1).

8.2.2. Data collection

Field work was carried out during August 2020 to August 202. Ethno-botanical information was collected through semi-structured interviews (Kumar *et al.*, 2017). Plants were identified with help of the Flora's books (Haines 1925; Saxena and Brahman 1995) and available literature (Kumar 2015). The information regarding

the uses of species was gathered from the villagers as well as traditional healers, particularly the old people (40-60 years) by direct interview. Literature search was also done to document the biological and pharmacological activities of the enumerated tree species.

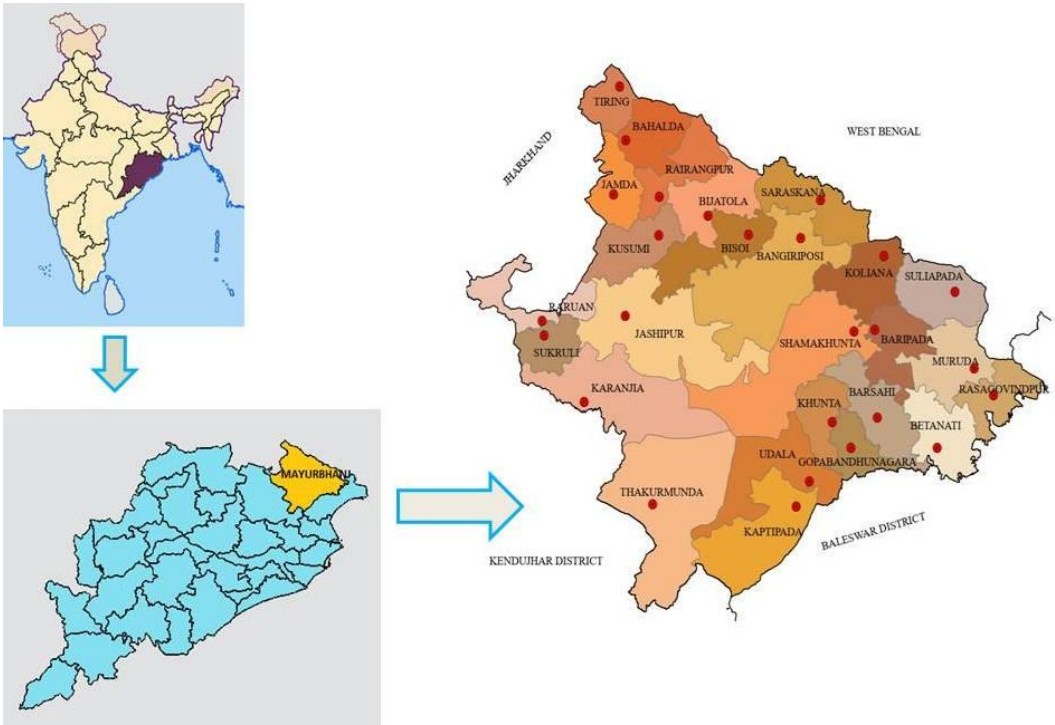

Figure 1: Geographical location of study areas

8.3. RESULTS

The results revealed that tree species like *Butea monosperma, Diospyrus melanoxylon, Dalbergia sissoo, Careya arborea, Madhuca longifolia, Cassia fistula, Oroxylum indicum, Teminalia chebula, Terminalia arjuna* and *Terminalia bellerica* are unique in their medicinal values and widely used by the local healers in the district. A total of 45 plants from 24 genera and 19 different families have been documented for their healing properties. These are the common ethno-medicinal tree species among the inhabitants of Mayurbhanj district because these plants are culturally important as they have long been using for generations and due to their rich bioactive constituents. The most frequent families are Fabaceae (7 species) followed by Combretaceae (6 species), Moraceae (5 species), Malvaceae (3 species), Ebenaceae (3 species), Bignoniaceae (3 species), Rubiaceae (3

species), Anacardiaceae (2 species), Lecythidaceae (2 species) and Sapotaceae (2 species). Rest of the families like Cornaceae, Meliaceae, phyllanthaceae, Ulmaceae, Annonaceae, Sapindaceae, Myrtaceae, Apocynaceae, Rhamnaceaeare represented with single plant species (Figure 2). The most widely utilized plant organs to prepare remedies were the bark (21), leaves (11), fruits (14), flowers (2), roots (1), seeds (1), latex (2) although branches, stems were also utilized in some remedies. It was observed that among all the parts, generally barks were mostly used during this survey work. The local people use to manage their primary health problems at home by using these different tree species found around them (Plate1). Details are listed and information provided in Table 1.

Table 1: Medicinal trees of Mayurbhanj, Odisha

Plant name	Family	Vernacular name	Parts use (s)	Used against
Alangium salvifolium	Cornaceae	Ankula	Leaves & fruit	Diarrhoea & dysentery
Albizia procera	Fabaceae	Dhalasiris	Bark	Rheumatism
Anogeissus latifolia	Combretaceae	Dhaura	Bark	Diarrhoea
Azadirachta indica	Meliaceae	Neem	Leaves , bark	Skin infections
Bombax ceiba	Malvaceae	Simili	Flower	Piles
Buchanania lanzan	Anacardiaceae	Chara	Bark	Dysentery
Butea monosperma	Fabaceae	Palash	Leaves	Boils & swelling
Careya arborea	Lecythidaceae	Kumbhi	Fruits	Cold & cough
Cassia fistula	Fabaceae	Sunari	Leaves	Malaria
Cleistanthus collinus	Phyllanthaceae	Karada	Leaves, roots and fruits	Gastrointestinal disorders
Couroupita guianensis	Lecythidaceae	Naga champa	Flower	Boost immunity
Dalbergia sissoo	Fabaceae	Sissoo	Bark	Blood diseases
Diospyros malabarica	Ebenaceae	Mankadakendu	Bark	Skin diseases
Diospyros melanoxylon	Ebenaceae	Kendu	Fruit	Stomach disorder
Diospyros	Ebenaceae	Kendu	Fruit	Cough

montana				
Dolichandrone spathacea	Bignoniaceae	Pannir	Bark	Gastrointestinal diseases
Ficus arnottiana	Moraceae	Paraspipal	Leaves	Skin diseases
Ficus benghalensis	Moraceae	Bara	Latex	Piles
Ficus hispida	Moraceae	Kagsha	Leaves	Diarrhoea
Ficus religiosa	Moraceae	Osta	Fruits	Asthma
Ficus semicordata	Moraceae	Bhuindimri	Bark	Dysentery
Haldina cordifolia	Rubiaceae	Kurum	Leaves & bark	Cholera
Holoptelea integrifolia	Ulmaceae	Churla	Bark	Scabies
Huberantha cerasoides	Annonaceae	Champati	Bark	Diabetes
Madhuca longifolia	Sapotaceae	Mahula	Bark	Skin diseases
Mimusops elengi	Sapotaceae	Baula	Leaves	Toothache
Mitragyna parvifolia	Rubiaceae	Gudikaima	Leaves	Jaundice
Morinda tinctoria	Rubiaceae	Achu	Leaf, fruit & bark	Diabetes
Oroxylum indicum	Bignoniaceae	Fanfana	Bark	Scabies & skin diseases
Pterocarpus marsupium	Fabaceae	Piasal	Bark	Diabetes
Millettia pinnata	Fabaceae	Karanja	Seed	Skin diseases
Saraca asoca	Fabaceae	Ashoka	Bark	Menorrhagia
Schleichera oleosa	Sapindaceae	Kusum	Bark	Dysentery
Semecarpus anacardium	Anacardiaceae	Bhalia	Fruits	Digestive problems
Sterculia colorata	Malvaceae	Pinj	Bark	Urine infection
Sterculia urens	Malvaceae	Genduli	Gum	Throat infection
Stereospermum chelonoides	Bignoniaceae	Pamphunia	Fruit	Leprosy
Syzygium cumini	Myrtaceae	Jamun	Fruits	Diabetes
Terminalia arjuna	Combretaceae	Arjun	Fruits	Improve blood circulation
Terminalia bellirica	Combretaceae	Bahada	Fruits	Cough

Terminalia chebula	Combretaceae	Harida	Fruits	Cough
Terminalia elliptica	Combretaceae	Asan	Bark	Diarrhoea
Terminalia phillyreifolia	Combretaceae	Phasi	Bark	Diarrhoea & dysentery
Wrightia antidysenterica	Apocynaceea	Kuruma	Bark	Digestive problems
Ziziphus marutiana	Rhamnaceae	Barakoli	Fruit	Stomach-ache

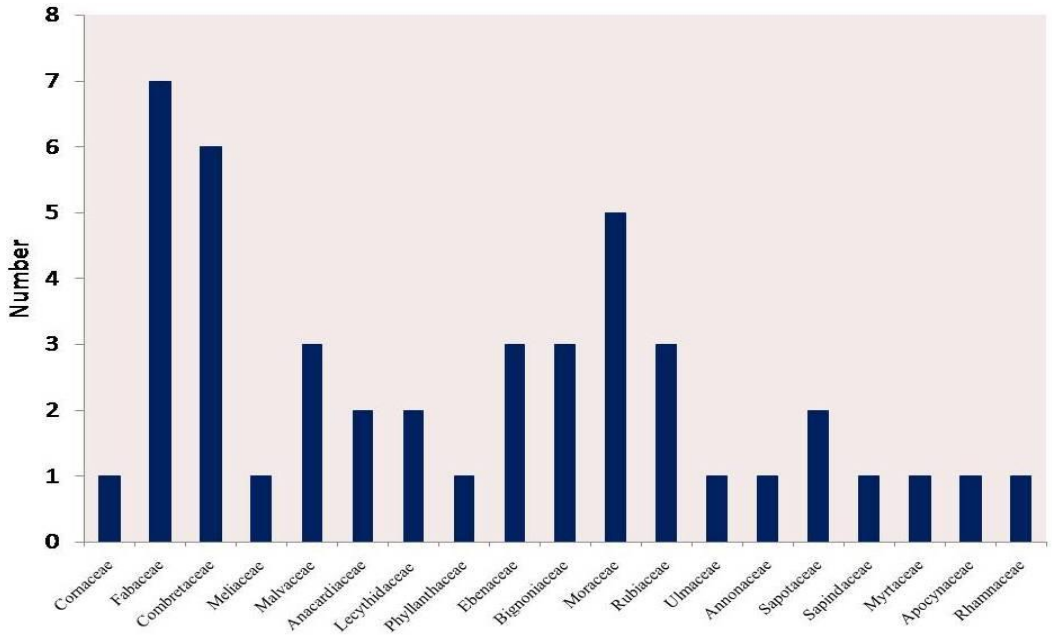

Figure 2: Diversity of medicinal tree

8.4. CONCLUSION

Conservation of traditional knowledge is very essential now, as loss has already been recorded due to multiple causes like development activities, population explosion, impact of tourism, deforestation, etc. whereas the primary cure of diseases is based upon deep observation of nature and their understanding of traditional knowledge of medical practices. Thus, conservation efforts as well as awareness programs are necessary for protecting the medicinal plants, especially the tree species and associated indigenous knowledge.

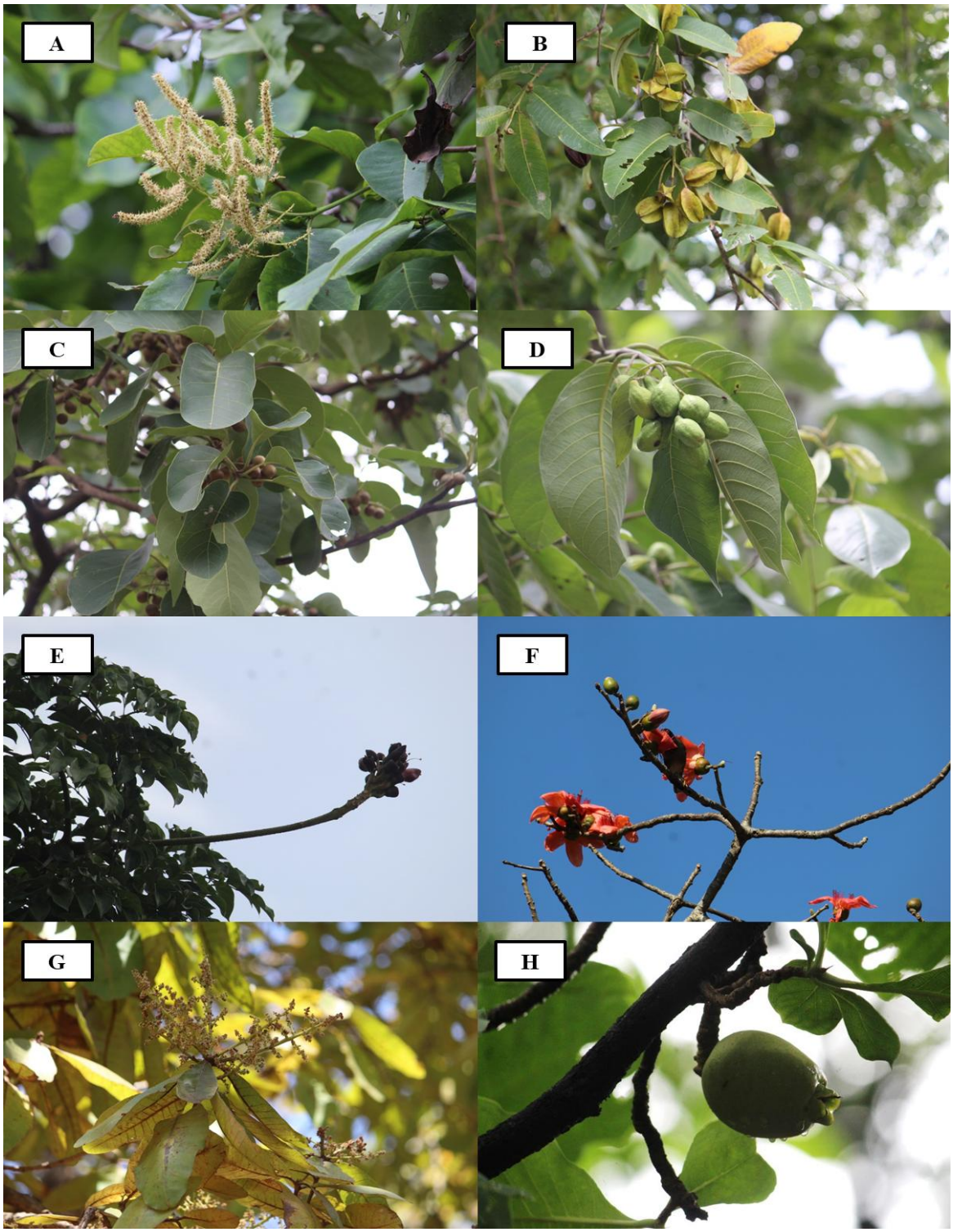

Plate 1: Some medicinal trees of Mayurbhanj, Odisha A) *Terminalia elliptica*, B) *Terminalia arjuna*, C) *Terminalia bellirica*, D) *Terminalia chebula*, E) *Oroxylum indicum*, F) *Bombax ceiba*, G) *Buchanania lanzan*, H) *Careya arborea*

The current ethno-botanical study provides the practical evidences about the uses of medicinal tree species among the inhabitants of Mayurbhanj district, Odisha. It also revealed that the medicinal trees of this region are a major source of herbal drugs for primary healthcare. These medicinal trees have the potential to replace the synthetic drugs which are restricted by their side effects.

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CHAPTER 9

Antibacterial activity of *Viscum articulatum*

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Abstract

Viscum articulatum is a parasitic herbaceous perennial plant mostly associated with deciduous trees. A number of parasitic plants remained unexplored just like various species of Viscum. It was deducted from various literature studies that phytochemical analysis of various Viscum species indicated the presence of antimicrobial properties. Hence, authors designed the present study to verify the antimicrobial potential of V. articulatum. For this, we followed phytochemical screening of various plant parts and antimicrobial activity was also checked as well by considering agar well diffusion assay, disc diffusion assay and broth diffusion assay. Along with this presence of secondary metabolites was also analysed by calculating Rf value from thin layer chromatography. From the result it was observed that V. articulatum is potentially rich in saponin, phenolics and tannins. Antibacterial screening showed Minimum Inhibitory Concentraction (MIC) at 100 mg/ml was 1.9 cm in agar well diffusion and MIC was found at 400 mg/ml in broth diffusion assay against Vibrio cholerae.

Keywords: Parasitic plants, Pharmacological values, Gram-negative bacteria

9.1. INTRODUCTION

Parasitic plants are the most fascinating group of plants due to their adaptability and coexistence with the host plant. They have the ability to survive on another host plant by obtaining all of its required nutrition via a haustorium from the host without donating any notable benefit [1]. There are about 4500 species of parasitic

plants including 280 genera which belong to 20 families all over the globe [2]. They comprise of nearly 1 % of the angiospermic plants. Common observable parasitic plant families are *Santalaceae*, *Viscaceae*, *Opiliaceae*, *Cuscutaceae*, *Lauraceae etc*. Almost half of the parasitic plant belongs to *Scrophulariaceae* (over 700 species) is followed by *Cuscutaceae*, *Viscaceae* and *Loranthaceae* [3]. India enjyos an eminent place with the presence of 132 species of plant which show parasitism. 18 species of parasitic plants are identified from Odisha [4]. The most common species in Odisha is *Cuscuta reflexa* Roxb. They use some specialized modified structures like haustorium, regume *etc.* The parasitic plant has the unique capacity to select the appropriate host plant for its growth and development [5]. Interspecific interaction, like the relationship between parasitic plant and host, is a common and indispensable phenomenon in the ecosystem. Bioactive compounds are the building block of nutrients that affect the organism both cytologically and physiologically. Tannin, lignin, acetone, flavonoids, phenolic compound *etc.* are most commonly found bioactive compounds. Bioactive compounds produced from *C. reflexa* Roxb. can provide effective antioxidant, antidiabetic, and anti-tyrosinase activities. *V. album* is one of the well-known medicinal plants and now a day it is well known for its anticancer activities. The phytochemical activity of parasitic plant is influence by the phytochemistry of the host plant [6]. Parasitic plants are a major source of edible food for many organisms. Various parts of the plant such as inflorescences, fruits, stems and flowers are utilised as a nutrient. *Viscum* is used to cure various diseases like pain, neuropharmacological disorders and various forms of tumours and cancers which are caused due to the free radicals. From the ancient time, *V. oriental* has been treated as a traditional medicine. It has been described in the literature to cure neuropharmacological disorders [7]. Other species of the genus *Viscum* like *V. album* is believed for

relaxing nervous system and control high blood pressure and heart attack as well. Notable use of *V. album* as medicine is for fever, measles and whooping cough. In Ireland and the United Kingdom, people use it for soothing the nerve and to simplify the heart function. It is also useful for epilepsy and hysteria. A mixture of *V. album* and *Cardamine pratensis* is applied for nervous afflictions [8]. Like other species of Viscum, *V. cruciatum* play a vital role in various types of diseases. It is applied against an antiseptic, emetic, purgative, anti-inflammatory, anti-arrhythmic, antispasmodic, anti-psychotic, anti-epileptic and useful to treat the liver and spleen enlargement [9]. The mixture of dried *V. verrucosum* powder and water is applied for the cure of swelling, intense burning sensation and difficulty in breathing. The paste of the whole plant is utilized against fractured heel bone, dislocation and cancerous wounds. The root of *V. articulatum* can cure leucoderma, constipation, and insomnia whereas leaves or twigs are effective for the treating respiratory ailments. Paste of the entire plant is the remedy for fever, ulcer, and blood diseases [10]. Keeping all the medicinal and therapeutic properties of various species of this parasitic plant, an attempt has been made to evaluate the antimicrobial potential of another species of *Viscum i.e., V. articulatum.*

9.2. MATERIALS AND METHODOLOGY

9.2.1. *Selection, identification and enumeration of selected experimental plant species from study areas*

The experimental plant *V. articulatum* was collected as per availability in Odisha. *V. articulatum* of the region were identified by using flora's books and published articles. The selected plant species were characterized using morphological characteristics followed by flora book and published articles [11].

9.2.2. *Collection of plant species*

The sample were collected and kept in poly bags tagged with the botanical name (*V. articulatum*) and stored out as per standard sampling procedure. Samples were preserved as voucher specimens and were deposited in the herbarium of Ambika Prasad Research Foundation, Bhubaneswar. The experimental plant species were properly washed and dried for further experiments.

9.2.3. *Preparation of plant extract*

The plant part of *V. articulatum* was collected and dried at room temperature under the shade and was powdered after drying using mechanical devices. The powdered material of *V. articulatum* was kept in thimble and extraction was carried out using the Soxhlet apparatus. The residues were collected and left for air drying and dried crude extract were stored in the refrigerator for further experimental work.

9.2.4. *Phytochemical assay*

Phytochemical analysis is a procedure to identify the bioactive compounds from the plant extract [12].

Test for Tannin

0.5 g of dried powder sample was boiled in 10 ml of distilled water and filtered with Whatman 42 filter paper. 2 ml of filtrate was taken in a test tube and 3 to 5 drops of 0.1% ferric chloride solutions were added. The brownish-green or blue-black colouration indicated the presence of tannins.

Test for Saponin

0.5 g of the dried powder was boiled in 15 ml of distilled water and filtered with Whatman 42 filter paper. 5 ml of filtrate was mixed with 2 ml of normal distil water and shaken vigorously. The stable persistent forth indicated the presence of saponins.

Test for Terpenoids

6 ml of extract was mixed in 2.5 ml of chloroform and then 3 ml of concentrated sulphuric acid was added. A reddish-brown colouration of interface indicated the presence of terpenoids.

Test for Phenolic compounds

0.5 g of plant extract was dissolved with 3 to 5 drops of 1 % ferric chloride solution. Formation of bluish-black colouration indicated the presence of phenolic compounds.

Test for Steroids

2 ml of plant extract was dissolved in 5 ml Chloroform and then 5 ml of concentrated sulphuric acid was added. Formation of upper red and lower yellow with green fluorescence indicated the presence of steroids.

9.2.5. *Antimicrobial activity*

Agar well diffusion assay

Agar well diffusion method was followed to test the antibacterial activity of extract of the *V. articulatum* against the five bacterial strains. Nutrient agar plates were prepared as per the manufacturer's instruction. 100 µl of nutrient broth cultures of the test microbes prepared a day before were poured over the plates uniformly and a lawn culture was prepared using sterile spreader in a laminar hood. Wells (6 mm) were made using sterile borer. The stock solution of samples was prepared in 100 % DMSO (Sigma) and twofold serial dilutions were made in the amount of 100 µl per well ranged from 0.5 to 2.0 mg/ml. 100 µl of samples were added by sterile syringes into the wells in the three above mentioned concentration and allowed to diffuse at room temperature for 2 hrs. Plates were incubated at 35±2˚C for 18 to 24

hrs. Triplicates the reading (diameter of zone of inhibition in cm) were taken and the mean ± SD values (diameter of zone of inhibition) were recorded [13].

Disc diffusion assay

Antibacterial activity using Disc diffusion assay was done using the 6 mm of disc prepared from Whatman filter paper. Each extract was dissolved in dimethyl sulfoxide. The sets of three dilutions (0.5, 1.0 and 2.0 mg/ml) of crude extracts and standard drugs were prepared. 6 mm of discs were kept in the drugs for 12 hrs before placing to the agar plates. The zones of growth inhibition around the disc were measured after 18 to 24 hrs of incubation at 37˚C for bacteria. The antimicrobial activity of the plant extract were determined by measuring the sizes of inhibitory zones (including the diameter of the disk) on the agar surface around the disk, and values less than 8 mm were considered as not active against microorganisms [14-16].

Media used

Nutrient broth was used to maintain broth cultures. The constituents of the nutrient broth included 0.5 g NaCl, 0.5 g peptone and 0.3 g beef per 100 ml. An additional 105 g of agar made up the nutrient agar medium [17].

Preparation of working slant

Stock culture was maintained at 4˚C on slants of semi-solid media containing 1.5 % of agar-agar, 0.3 % beef extract and 0.5% peptone. Active working cultures for experiments were prepared by transferring a loopful of culture mass from the stock. Slants were incubated for 24 hrs at 36± 1.0˚C.

Broth preparation

Colonies of prepared slants was picked off using the sterile loop and incubated in sterile condition in an autoclaved cool liquid broth medium containing 0.3 % of

beef extract and 0.5 % peptone. The broth was incubated for 24 hrs at 36± 1.0˚C until there was visible growth indicated by turbidity standard [18].

Swabbing & Inoculation of drugs

Swabbing with autoclaved cotton swab was done using broth strain on Petri plates. Well (6 mm) were made using sterile borer for Agar cup well method. The stock solution of samples was prepared in 100 % DSMO and two-fold serial dilution was made in the amount of 100 µl/well ranged from 0.5, 1.0, and 2.0 mg/ml. 100 microliter of sample was added by sterile syringes into the wells in three above mentioned concentration and allowed to diffuse at room temperature for 2 hrs. Only the solvent (DSMO) was poured into the well in another set of plates as part of the negative control. The positive control set consisted of standard antibiotics Kanamycin. For the disc diffusion assay, only swabbing was done using a sterile swab. Then discs of respective aforesaid concentration were placed on media. Both Petri plates (for Agar well diffusion & Disc diffusion method) were incubated at 36 ± 1.0˚C for 18 hrs. Zones of inhibition free microbial growth appeared around each well and disc in the form of clear rings which confirmed the antibacterial activity of the respective samples. Those samples which did not have any inhibitory effect on the microbe did not form any clear ring. In this way, the antibacterial activity of the samples was confirmed. Triplicates were maintained and the experiment was repeated thrice. For each replication, the reading (zone of inhibition) was taken and the mean values were recorded [19].

Data analysis

Mean and SD (standard deviation) was performed to calculate taking triplicate values of zones of inhibition (cm for agar well diffusion assay; mm for disc diffusion) of samples using Excel, Microsoft corporation-2010, US.

MIC using Broth Dilution assay

All the extracts of experimental plant parts were screened for their antibacterial activity. Antibacterial activity was assessed by MIC by serial dilution method. Selected colonies of aforesaid bacteria were picked off to a fresh isolation plate and incubated in corresponding tubes containing 5 ml of trypticase soy broth. The broth was incubated for 8±1 hrs at 35±2˚C until there was visible growth. Mc Farland No.5 standard and PBS (Phosphate buffer saline) were used to adjust the turbidity to get CFU/ml [16, 18].

Data interpretation

After the incubation, the tubes showing no visible growth after 8 hrs till 12 hrs were considered to be inhibitiory to bacteria which represent MIC values of a respective concentration. Inoculum's control showed visible growth due to no antimicrobial agents, whereas the broth control showed no growth due to the absence of bacteria. Triplicates were maintained and the experiment was repeated thrice, for each triplicates. The readings were noted.

9.2.6. *Thin-layer chromatography*

Chromatographic analysis was done using standard methods to evaluate the secondary metabolites [20].

Preparative TLC

Glass plate was cleaned with ethyl acetate then left for 15 minutes 3 g of silica gel was taken in a beaker and 15 ml of water was added. Then slurry was poured over the glass plate and was allowed to dry, then heated by hot plate for 10 minutes for activation of TLC plate.

Mobile phase

As per the methods, the mobile phases were prepared by taking chloroform and methanol ratio 1:9 and 9:1 to find out the Rf value. Mobile phases were taken as per polarity index in single, double and triple combining solvent system such as n-hexane, chloroform: Methanol, chloroform: Ethyl acetate: Formic acid (CEF) and Ethyl acetate: Methanol: Water (EMW).

Rf Values

Behaviour of an individual compound in TLC is characterised by Rf and is expressed as a decimal fraction. The Rf is calculated by dividing the distance the compound travelled from the original position by the distance the solvent travelled from the original position (the solvent front).

9.3. RESULTS & DISCUSSION

Present experiment emphasizes on the phytochemistry of plant and to explain the presence or absence of bioactive compounds through the quantitative test. The extract (aqueous, methane, hexane, acetone, and ethanol) of *V. articulatum* was scrutinized by qualitative test to know the presence of secondary metabolites like saponin, phenolics compound, tannin, terpenoid, and steroid.

Table 1: Phytochemical screening of plant extracts

Extract	**Phytochemicals**				
	Saponin	**Phenolic compounds**	**Tannin**	**Terpenoid**	**Steroid**
Aqueous	++	++	++	-ve	-ve
Methane	++	++	++	-ve	-ve
Hexane	-ve	-ve	-ve	-ve	-ve

Acetone	++	-ve	++	-ve	-ve
Ethanol	++	++	++	-ve	-ve

(++: present; -ve: absent)

It was clear from the above experimental data that the medicinally active bioactive compounds likes saponin and tannin were found in almost all extract except hexane extract but terpenoid and steroid was absent in all extract, phenolic compounds were present in aqueous, methane, and ethanol extract of *V. articulatum*. The overall result showed that *V. articulatum* is potentially a rich source of bioactive compound like Saponin, Phenolics compound and Tannin (Table 1).

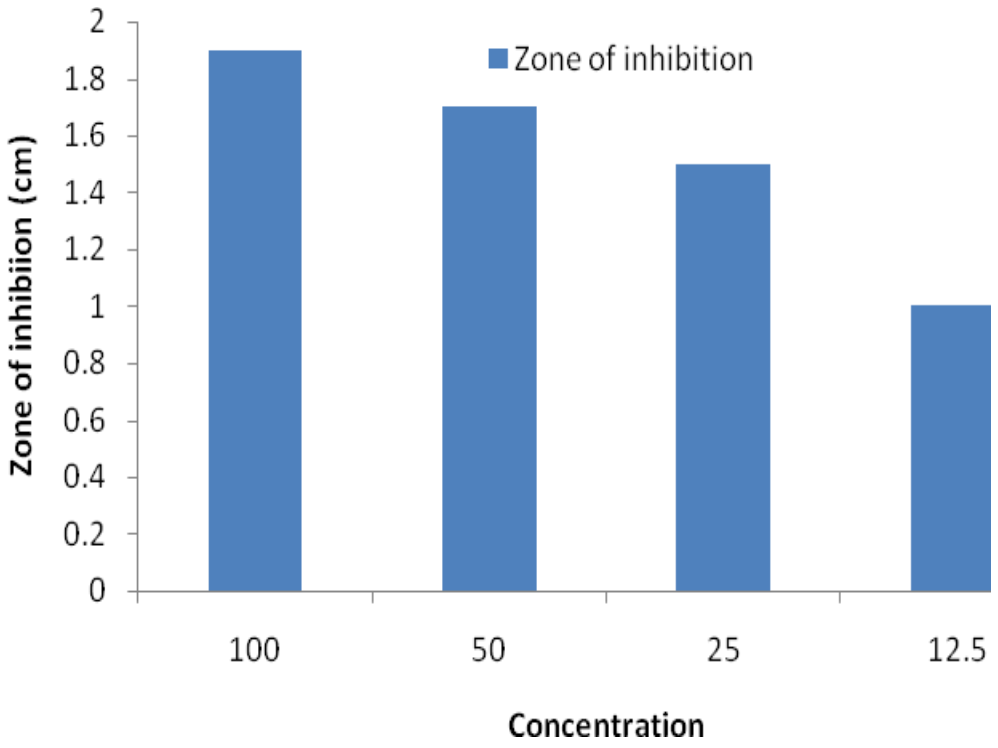

Figure 1: Antibacterial activity against *Vibrio cholera*

The results were observed according to the inhibition zones around each well due to the diffusion of antibacterial properties from the plant extract impregnated disc

into the surrounding medium. The experimental result of agar well diffusion assay showed that the maximum inhibition zone was obtained when the extract was applied without distilled water *i.e.,* 1.9 cm (Figure 1).

Table 2: Estimation of MIC

Concentration	***Vibrio cholera* (bacteria) growth**
Broth	No growth
Bacterial broth	Growth
100 mg/ml	Growth
200 mg/ml	Growth
300 mg/ml	Growth
400 mg/ml	MIC
500 mg/ml	No growth

The results were analysed in terms of inhibition zones around the hole because of the diffusion of antibacterial properties from the plant extract filled disc into the surrounding medium. The result table showed that the minimum inhibitory concentration (MIC) was found at 400 mg/ml. It revealed that bacterial growth found at 100 mg/ml, 200 mg/ml, 300 mg/ml and bacterial broth. There was no growth found in 500 mg/ml, aqueous and pure broth (Table 2).

Table 3: Rf values of aqueous extract of *V. articulatum*

Extract	**C:M**	**Rf1**	**Rf2**	**Rf3**
Aqueous extract (10µ)	1:9	0.75	0.625	0.725
	9:1	0.25	0	0

Aqueous extract (5µ)	1:9	0.35	0.1	0.1
	9:1	0.887	0.8	0.775

(C: Chloroform, M: Methanol)

The table showed that the Rf values were maximum in aqueous extract (5µl) of chloroform and methanol ratio 9:1 *i.e.*, 0.887, 0.8 and 0.775. The minimum Rf values were found in the aqueous extract (10 µl) of chloroform and methanol ratio 9:1 *i.e.*, 0.25, 0 and 0 (Table 3).

9.4. CONCLUSION

Parasitic plants are unexplored and known as weeds or useless but they are medicinally important having diverse bioactive compounds. The present study highlights the medicinal and pharmacological potential of *Viscum articulatum*. The phytochemical screening and ant-microbial activities revealed that it could be the raw material for the future formulation.

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CHAPTER 10

Antibacterial activities of *Acacia auriculiformis*

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Abstract

Acacia auriculiformis is a common tree species and an important source of fuel, furniture, natural dye, detergent, and medicine for various ailments. An attempt has taken to evaluate antimicrobial activity of fruit extracts. It was observed that, saponin is present in more amount in the aqueous fruit extract as compared to other metabolites. The fruit extracts showed the sound antibacterial activity against *Salmonella typhi*. Therefore, the fruit extract of *A. auriculiformis* can be forwarded to the development of antibacterial herbal drug against typhoid causing bacteria in the future.

Keywords: Antibacterial, Bioactive compounds, Pharmacological, Tree

10.1. INTRODUCTION

A. auriculiformis is an ornamental plant which is widely distributed tree species. It is rich with secondary metabolites like phenolic, tannins, saponin, and terpenoids and mainly used for pest control in agriculture [1]. It is a fast growing tree having drought tolerance capacity [2]. It is utilized as a raw material for the production of wood composite, pulp, paper, and furniture. It is used to cure diseases like wound, paracetamol intoxicated liver injury, alloxan induced type II diabetes, aches, sore eyes, rheumatism aborigines, inflammation malaria, skin disease like itching, allergy and rashes *etc*. It is also utilized as antidiabetic factor, anthelmintic factor, and antioxidant. Bark is used for free radical scavenging [3] and the bark of the stem is used for the preparation of ointment to cure the wound [4]. It is safe for vaginal epithelial use and inhibits the transmission of HIV as well as spermicidal activity. It is also used for the treatment of cancer and as an anti-depressant. The

bark of the plant traditionally used for the treatment of dysentery, diarrhea, and mouth wash. *A. auriculiformis* have a higher level of flavonoids and the heartwood extract showed high antifungal activity [5, 6]. Plant extract possess antimalarial, antimicrobial, anti-diabetic, spermicidal, cestocidal, central nervous system depressant, hepato-protective, wound healing, chemo preventive and antimutagenic activities. Plant species contain the compounds like galactose, arabinose, rhamnose, glucuronic acid, and methyl glucorunic acid [7]. It depends on root associated rhizobacteria which provide nitrogen [8]. Endo and ectomycorrhiza associated with *A. auriculiformis* result in plant growth promotion and salt stress improvement [9]. Green polymeric materials are also manufactured by the bark of the *A. auriculiformis* [10]. The bark extract of *A. auriculiformis* is used as a pesticide. As its bark produces much amount of tannin and natural dye it can be grown as a commercial plant for the coloration of the textiles, [11, 12]. Keeping all the pharmacological properties of *A. auriculiformis,* an attempt has been made to evaluate the antibacterial potential of fruits of *A. auriculiformis.*

10.2. MATERIALS AND METHODS

10.2.1. *Selection, identification and enumeration*

The experimental plants were identified by Dr . Sanjeet Kumar , APRF, India following flora's books [13, 14] and collected from the hilly side of Khandagiri, Khorda, Odisha[13, 15, 16].

10.2.2. *Collection of experimental plant species*

The sample was collected and kept in poly bags tagged with the botanical name [17, 18]. The information gathered from the interviews were compiled and analyzed. The experimental plant parts, their mode of consumption, preparation techniques, therapeutic uses, and palatability were noted.

10.2.3. *Ethnobotanical data collection*

The results presented here were based on the field work conducted with suburban and hill regions of Bhubaneswar, Odisha areas during the month of January to March in 2020. The methodological frameworks for the ethnobotanical study was done as per standard techniques of exploration and germplasm collection, [19, 20] qualitative and quantitative ethnobiological approaches in the field, interviews, elicitation methods, data collection, and further authentication. The standard participatory rural appraisal method was adopted for sampling and data collection to incorporate the indigenous knowledge. Opinions of tribal people and villagers were taken regarding the uses of experimental plant species through questionnaires. The informants were interview by showing the experimental plant parts and ethnobotanical data were recorded using passport data form. Further, group

discussions and cross questions among villagers were made for authentication of data on experimental plants (Figure 1).

10.2.4. *Preparation of plant extract*

The collected plant parts of *A. auriculiformis* were dried at room temperature under shade and powdered after drying using mechanical devices. The powdered material of *A. auriculiformis* was kept in a thimble and extraction was carried out using the Soxhlet apparatus. The residues were collected and left for air drying and dried crude extract were stored in a refrigerator for other experimental work.

10.2.5. *Phytochemical assays*

Phytochemical analysis is a procedure to identify the bioactive compounds from the plant extract [21, 22, 23].

Test for Saponin

0.5 g of the dried powder was boiled in 15 ml of distilled water and filtered with Whatman 42 filter paper. 5 ml of filtrate was mixed with 2 ml of normal distilled water and shaken vigorously. The stable persistent froth indicated the presence of saponin.

Test for Tannin

0.5 g of dried powder sample was boiled in 10 ml of distilled water and filtered with Whatman 42 filter paper. 2 ml of filtrate was taken in a test tube and 3 to 5 drops of 0.1 % ferric chloride solution were added. The brownish green or blue black coloration indicated the presence of tannins.

Test for Phenolic compounds

0.5 g of plant extract was treated with 3 to 5 drops of 1 % ferric chloride solution. The formation of bluish black coloration indicated the presence of phenolic compounds.

Test for Terpenoids

6 ml of extract was mixed in 2.5 ml of chloroform and then 3 ml of concentrated sulphuric acid was added. A reddish-brown coloration of the interface indicated the presence of terpenoids.

Test for Steroid

2 ml of plant extract was dissolved in 5ml chloroform and then 5ml of concentrated sulphuric acid was added. The formation of 2 phases (upper red lower yellow with green fluorescence) indicated the presence of steroids.

10.2.6. *Antibacterial activity*

The extracts of plant parts were screened for antibacterial activity against gram negative bacteria *Salmonella typhi* (MTCC 1252). MTCC (microbial type culture collection) bacterial strains were collected from the Institute of Microbial Technology (IMTECH), Chandigarh. Antibacterial activity was done using slight modification of standard methods of Agar well Diffusion assay [24], Disc Diffusion method [25, 26, 27] and Broth dilution assay [28].

Agar well diffusion assay

Agar well diffusion method [24] was followed to test the antibacterial activity of extracts of *A. auriculiformis* plant parts against one bacterial strain. Nutrient agar plates were prepared as per the manufacturer's instructions. Wells (6 mm) were made using a sterile borer. Stock solutions of samples were prepared in 100 % DMSO (Sigma). Triplicates were maintained and the experiment was repeated thrice. For each replicates the readings (diameter of zone of inhibition in cm) were taken and the mean±SD values (diameter of zone of inhibition) were calculated.

Disc Diffusion assay

Antibacterial activity using Disc diffusion assay was done using the 6 mm of disc prepared from Whatman filter paper [26]. 6 mm of discs were kept in the drugs for 12 hrs before placing them on the agar plates. The zones of growth inhibition around the discs were measured after 18 to 24 hrs of incubation at 37°C for bacteria.

Media used

Nutrient media was used to maintain broth cultures. The constituents of the nutrient broth included 0.5 g NaCl [29], 0.5 g peptone, and 0.3 g beef per 100 ml. An additional 1.5 g of agar made up the nutrient agar medium.

Preparation of working slant

Stock culture of MTCC 1252 are maintained at 4°C on slants of semi-solid media containing 1.5 % of agar, 0.3 % beef extract, and 0.5 % peptone. Active working cultures for experiments were prepared by transferring a loopful of culture mass from the stock. Slants were incubated for 24 hrs at 36±1.0°C.

Broth preparation

Colonies of prepared slant of MTCC 1252 were picked off using a sterile loop and inoculated in sterile conditions in autoclaved cool liquid broth medium containing 0.3 % of beef extract and 0.5 % peptone. The broth was incubated for 24 hrs at 36±1.0°C until there was visible growth indicated by turbidity standard.

Data analysis

Mean and standard deviation (SD) was performed to calculate taking triplicate values of zone of inhibition (cm for agar well diffusion assay; mm for disc diffusion) of samples using Excel, Microsoft Corporation-2010, US.

MIC using Broth Dilution assay

All the extracts of experimental plant parts were screened for their antibacterial activity.[28] Antibacterial activity was assessed by Minimum Inhibitory Concentration (MIC) by serial dilution method. The broth was incubated for 8±1 hrs at 35 ± 2° C until there was visible growth. Mc Farland no. 5 standard and phosphate buffer saline (PBS) were used to adjust the turbidity to get 10^5 CFU /ml.

Data Interpretation

After the incubation, the tubes showing no visible growth after 8 hrs till 12 hrs were considered to be inhibition of bacteria which represent MIC values of a respective concentration. Inoculums control showed visible growth due to no antimicrobial agents, whereas the broth control showed no growth due to the absence of bacteria. Triplicates were maintained and the experiment was repeated thrice, for each replicates. The readings were taken as aforesaid.

10.2.7. *Preparative TLC*

Glass plate was cleaned with ethyl acetate then left for 15 min. 3 g of silica gel was taken in a beaker and 15 ml water was added to it. Then slurry was poured over the glass plate and was allowed to dry, then heated by hot plate for 10 min for activation of TLC plate [30].

Mobile Phase

The mobile phases were taken as per polarity index in single, double, and triple combining solvent system such as n-hexane, chloroform: Ethyl acetate: Formic acid (CEF), and Ethyl acetate: Methanol: Water (EMW) [31].

RF values

The behavior of an individual compound in TLC is characterized by RF and is expressed as a decimal fraction. The RF is calculated by dividing the distance the compound travelled from the original position by the distance the solvent travelled from the original position (the solvent front).

10.3. RESULTS AND DISCUSSION

The phytochemical screening revealed the presence of diverse metabolites in fruits. From the analysis of results, it is found that secondary metabolites like tannin and phenolic compounds are present mostly in aqueous, methanol, ethanol, and acetone fruit extracts whereas saponin is present in the aqueous fruit extract only. But

presence of terpenoid and steroid is not found from any of the fruit extract. Hence the phytochemical screening of the experimental work resulted that the n-hexane extract does not show any result. The Aqueous extract of the plant fruit is show a better result than the other extract (Table 1). Traditionally this fruit is used as detergent, pesticides, and molluscicides for its natural foaming capacity due to the presence of saponin. A lot of pharmacological uses have been reported due to the presence of various kinds of secondary metabolites [32].

Table 1: Phytochemical screening of experimental plant parts

Plant extract (fruit)	**Saponin**	**Tanin**	**Phenolic compound**	**Terpenoid**	**Steroid**
Aqueous extract	+++	+++	+++	–	–
Methanol extract	–	+++	+++	–	–
Ethanol extract	–	+++	+++	–	–
n-hexane extract	–	–	–	–	–
Acetone extract	–	+++	+++	–	–

The results revealed that at more concentration (*i.e.* 500 mg/ml) the inhibitory activity against bacteria *Salmonella typhi* (MTCC 1252) is highest and at the lowest concentration (*i.e.* 100 mg/ml) the inhibitory activity against bacteria (MTCC 1252) is least (Table 2).

Table 2: Antibacterial activity using agar well diffusion assay (aqueous extract)

Concentration	**Zone of Inhibition**	**Strain**
		Salmonella typhi
500 mg/ml	1.7 cm	

400 mg/ml	1.6 cm	
300 mg/ml	1.5 cm	
200 mg/ml	1.4 cm	
100 mg/ml	1.3 cm	

The results showed that the bacteria (MTCC 1252) have no growth in aqueous fruit extract at concentration of 100 mg/ml and 200 mg/ml. The zone of inhibition of aqueous fruit extract against bacteria (MTCC 1252) showed that at concentration 300 mg/ml, 400 mg/ml and 500 mg/ml is high as compared to 100 mg/ml and 200 mg/ml. Hence bacteria grew efficiently in lower concentration (Table 3).

Table 3: Antibacterial activity using disc diffusion assay (aqueous extract)

Concentration	**Zone of Inhibition**	**Strain**
		Salmonella typhi
100 mg/ml	No. Inhibition	
200 mg/ml	No. Inhibition	
300 mg/ml	8.0 mm	
400 mg/ml	9.5 mm	
500 mg/ml	11.0 mm	

The results showed that the broth have no bacterial growth. The plant extract at 1000 mg/ml concentration showed MIC. The fruit extract at concentration 500 mg/ml, 400 mg/ml, 200 mg/ml and 100 mg/ml showed no growth of bacteria (**Table 4**).

Table 4: Estimation of MIC using broth dilution assay

Plant Extract (conc.)	**Bacteria (*Salmonella typhi*)**
Broth	No growth

Bacterial Broth	Growth
1000mg/ml	MIC(inhibit)
500mg/ml	No growth
400mg/ml	No growth
200mg/ml	No growth
100mg/ml	No growth

Aqueous extract was taken in two mobile phages for the analysis of TLC. Result revealed that in 5 µl and 10 µl, aqueous fruit extract of Chloroform: Methanol is 1:9 gives better result than 9:1 (Table 5).

Table 5: TLC studies of aqueous extract

C:M **1:9**	**C:M** **9:1**
(5µl) Rf_1=1 Rf_2=1 Rf_3=1	(5µl) Rf_1=0.15 Rf_2=1.31 Rf_3=0.16
(10µl) Rf_1=1 Rf_2=1 Rf_3=1	(10µl) Rf_1=1 Rf_2=1 Rf_3=1

Acetone fruit extract was taken for TLC in two phages. Result revealed that in 5 µl and 10 µl acetone fruit extract of C: M is 1:9 gives better result than 9:1 (Table 6).

Table 6: TLC studies of acetone extract

C:M **1:9**	**C:M** **9:1**
(5µl) Rf_1=1 Rf_2=1 Rf_3=1	(5µl) Rf_1=0.52 Rf_2=0.5 Rf_3=0.56
(10µl) Rf_1=1 Rf_2=1 Rf_3=1	(10µl) Rf_1=0.75 Rf_2=0.08 Rf_3=0.11

Methanol fruit extract was taken for TLC in two phases. Result revealed that in 5 µl and 10 µl methanol fruit extract of C: M is 1:9 gives better result than 9:1 (Table 7).

Table 7: TLC studies of methanol extract

C:M **1:9**	**C:M** **9:1**
(5µl) Rf_1=1 Rf_2=1 Rf_3=1	(5µl) Rf_1=0.15 Rf_2=0.12 Rf_3=0.17

Figure 1: Collection of *Acacia auriculiformis* fruits

10.4. CONCLUSION

A. auriculiformis wood is used as fuel and furniture, in the management of the ecosystem and biodiversity and for water conservation and prevention of soil erosion. The plant bark is more useful as folk medicine from ancient times. The plants extract is used for the treatment of various skin diseases. From the present study, it is concluded that the plant extract of *A. auriculiformis* has a number of bioactive compounds which showed antioxidant and antibacterial activities. Results also revealed that the fruits of *Acacia* can be used to wash clothes in the local area and also can be used to prepare detergent in the future because of the presence of secondary metabolite, saponin. Due to the presence of various secondary bioactive compounds, fruit extract of *A. auriculiformis* can be subjected to harmless antibacterial herbal drug discovery as well as utilizing its formulations against various diseases and disorders in the future.

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CHAPTER 11

Nutraceutical *Dioscorea* used by the Santhal community

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Abstract

Worldwide researchers are searching nutraceutical to combat the contemporary health problems. Tribal practices are major source for screening them. Santhal community was observed using the tuberous plants like Dioscorea species as food and medicinal agents. Keeping this in view, an attempt has been made to enumerate the species belonging to the genus Dioscorea used by the Santhal community of Odisha state. The Passport Data Form is used to gather the information from Mayurbhanj district of Odisha. The results revealed that 7 species of Dioscorea are used for nutraceutical purposes. The results, further recommended the value addition of enumerated species for developing livelihood option for the Santhal community of the state

Keywords: Livelihood option, Tuberous plants, Nutraceutical, Local community

11.1. INTRODUCTION

COVID-19 has brought attention towards the nutraceuticals. Researchers, biologists, intellectuals and local communities are searching nutraceutical agents having food and anti-microbial properties. The term "Nutraceutical" has been derived from the terms "nutrition" and "pharmaceutical". It applies to the products that are isolated from herbal products, dietary supplements, specific diets and processed foods (such as cereals, soups, and beverages) which can also be used as medicine apart from the nutritional purposes (Kalra 2003).The consumption of nutraceuticals has increased after COVID-19 but its consumption of nutraceutical is quite a tradition in different cultures of India in general (particularly in Odisha). Our ancestors used to consume food as per season and locality which use to

enhance food nutritional and medicinal values. The urban sprawl and modernization have also negatively reformed our traditional food habits. Keeping the importance of our traditional food habits, an attempt has been made to document the consumption practices of tuberous plants of genus *Dioscorea* by the Santhal community of Myurbhanj district of Odisha, India. Santhals are known educated community, usually involved in agricultural practices and in the collection of non-timber forest products from nearby forested areas (Kumar 2015). They have unique skills and their food habits are very unique and scientific too. They are one of the major community in Mayurbhanj district of Odisha state.

11.2. METHODOLOGY

The ethnobotanical survey was carried out during the months of August 2020 to April 2021 in different villages of Mayurbhanj district of Odisha state through semi-structured questionnaire. Also the reinvestigation has been done. The enumerated species of *Dioscorea* are identified by Dr. Sanjeet Kumar, Chief Executive Officer, Ambika Prasad Research Foundation, Odisha. Field Data Book number of enumerated species are obtained from Ambika Prasad Research Foundation, Odisha.

11.3. RESULTS AND DISCUSSION

During present investigation, results revealed that seven species of *Dioscorea* is consumed by the Santhal community of Mayurbhanj, Odisha. The enumerated species are *Dioscorea alata, Dioscorea bulbifera, Dioscorea hamiltonii, Dioscorea hispida, Dioscorea oppositifolia. Dioscorea puber*, and *Dioscorea walichii*. They are used as a supplement food having medicinal values. Beside used as a supplementary food, it was observed that *D. bulbifera's* tuber paste is used in skin infections; tuber of *D. hispida* is used as birth control agent; *D. hamiltonni* & *D. walichii* is used in gastrointestinal problems; *D. puber* tuber juice is used as tonic and tuber of *D. alata* is used as a cooling agent. Details are listed in Table 1.

11.3.1. Taxonomic enumeration

Dioscorea alata L. *(Desia aalu)*

Climber, stem twining to right with 4-winged above, large tubers, shallow or deep underground. Bulbils irregular, and large. Leaves mostly opposite, broadly ovate to cordate with about 9 costate. Fruits are winged and capsule.

Dioscorea bulbifera L. *(Pita aalu)*

Climber, stem twining to the left and smooth. Tubers are sub-globose in shape. Bulbils small with numerous eyes. Leaves alternate and rarely opposite. Quadrately oblong fruits with winged seeds.

Dioscorea hamiltonii Hook.f. *(Suta aalu)*

Climber, stem twining to the right and angled. Tubers are long-stalked. Bulbils are absent. Leaves are opposite or sub-opposite and sagittate base. Fruits are glabrous with winged seeds.

Dioscorea hispida Dennst. *(Korba aalu)*

Woody climber, stem twining to the left with prickles. Tubers irregular with root fibers. Leaves 3-foliolate. Fruits quadrately oblong with seeds winged at base.

Dioscorea oppositifolia L. *(Pani aalu)*

Climbers, stem twining to the right with purple stem when young. Tubers fusiform with narrow neck. Leaves are opposite with 3-5 nerved of which 3 unite in the tip. Glabrous capsule with orbicular seeds.

Dioscorea puber Bl. Enum. *(Kukai sanga)*

Woody tomentose climber, stem twining to the right. Tubers are cylindrical in shape. Bulbils are green or grey and axillary. Leaves are opposite and broadly ovate. Fruits are sub-cordate at top and bottom with winged seeds.

Dioscorea wallichii Hook. f. *(Meram tua sanga)*

Woody climber, stem twining to the left. Tubers are attached directly with the stem base. Bulbils are absent. Leaves are alternate. Fruits are broadly obovate with winged seeds.

Table 1: Nutraceutical *Dioscorea* species used by the Santhal Community

Local name	Scientific name	Parts used	Uses	Collection site(s)	Field Data Book Number
Pita aalu	*Dioscorea bulbifera*	Tuber	Tubers are cut into pieces and burned The burned roasted tubers are eaten as snacks.	Badampahar	DMO-01

Korba aalu	*Dioscorea hispida*	Tuber	Tubers are left overnight under running water and then do the successive boiling three times. The tubers are cooked as vegetables and consumed with main meal.	Sulaipat	DMO-02
Kukai Sanga	*Dioscorea puber*	Tuber	The tubers are washed and further cut into small pieces and dried in sunlight. The dried tubers are cooked and consumed as vegetables.	Sanuski	DMO-03
Suta aalu	*Dioscorea hamiltonii*	Tuber	The tubers are consumed raw as a snack.	Sulaipat	DMO-04
Mera tua sanga	*Dioscorea walichii*	Tuber	The tubers are consumed raw as a snack.	Jashipur	DMO-05
Pani aalu	*Dioscorea oppositifolia*	Tuber	The tubers are washed and do the successive boiling and cooked and consumed as vegetables.	Luhabadia	DMO-06
Desia aalu	*Dioscorea alata*	Tuber	The tubers are cooked as a vegetable and consumed with a main meal.	Ramchandarpur; Rairangpur; Luhabadia; Sulaipat	DMO-07

(DMO: Dioscorea of Mayurbhanj, Odisha)

Plate 1: *Dioscorea* species used by the Santhal community, A) Tuber of *Dioscorea bulbifera*; B) *Dioscorea walichii*; C) *Dioscorea hispida*; D) *Dioscorea oppositifolia*

Many researchers have reported about the traditional knowledge of Santhal community. In 2016, Jerath et al. documented the indigenous foods and their nutritive values used by the Santhal community of Jharkhand. They identified around 100 species of wild foods consumed by the community. In the year 2017, Gope et al. reported the indigenous knowledge components for sustainable development among the Santhal community. They also reported the use of medicinal plants– in different diseases and disorders by the Santhal community. Magesh and Rizvi (2017) have listed the indigenous knowledge of Santhal tribe of Dalma Wildlife Sanctuary, Jharkhand. They have documented nearby 30 medicinal plants used by Santhal in different healthcare problems. Nayak and Naik (2017) reported the indigenous knowledge on healthcare systems of Santhal community of

Mayurbhanj district of Odisha. Murmu et al. (2018) reported the traditional knowledge of Santhal community on weather forecast and biodiversity conservation. They find out that *Hierococcyx varius* and *Ploceus philippinus* plant species are protected by Santhals. According to community, flowering of *Mangifera indica* and some species of *Opuntia* also foretell the imminence of rainfall. Murmu et al. (2019) reported the traditional knowledge on wild edible plant species used by the Santhal community of Eastern Jharkhand and West Bengal. They have reported 49 species of wild edible plants consumed by the Santhal. Sachan et al. (2013) have documented the indigenous knowledge on wild mushrooms belongs to 8 genera and 6 families. Their study highlights the diversity and ethnomedicinal potential of some indigenous mushrooms from Similipal Biosphere Reserve Forest. In 2013, Mishra et al. documented 9 species of *Dioscorea* used by the tribal communities of Koraput, Odisha. They reported that the local communities consume frequently *D. oppositifolia, D. tomentosa, D. wallichi, D. hamiltonii* and *D. bulbifera*.

11.4. CONCLUSION

Currently, nutraceuticals are in high demand as they are safe and have both nutritional and therapeutic competence. However, they still remain unexplored and well documented. Our recent study revealed that tuberous plants like *Dioscorea* species used by the Santhal community of Odisha state are of nutraceutical importance. The seven species of *Dioscorea* are documented. The present study has brought up into the notice the role of plant species in the health care system. It has been observed that wild plant species are used by the Santhal community for the food as well as in the treatment of the some health problems. This indigenous knowledge of plants is very important. We should conserve this knowledge and plant species. Promoting this indigenous knowledge of ethnomedicine will help the Santhal community for their sustainable livelihood. So, the need of the hour is to preserve the biodiversity and judicious use of the natural resources. Present work documents the nutraceutical values of *Dioscorea* species and so, it is important to identify, protect, preserve and well document these wild edible plants which further add to the livelihood option for the Santhal community.

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CHAPTER 12

Analytical study of some medicinal plants and their uses of Chamba Block, Tehri Garhwal, Uttarakhand, India

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Abstract

Present investigation was carried out in Chamba block of district Tehri Garhwal to analyze the distribution (quantitative rate) and uses of some medicinal plants in the study area. Near about 60 important medicinal plant species, belonging to the 45 families have been observed. Utilization of identified plants as medicine by local inhabitants for the treatments of various types of diseases has also noticed. The number of herbaceous plants was higher among the identified plants including Gymnosperm. Medicinal plants have been used in healthcare since time immemorial. These are not only a major resource base for the traditional medicine and herbal industry but also provide livelihood and health security to a large segment of Indian population. Traditional systems of medicine continue to be widely practiced on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several synthetic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. Treatment with medicinal plants is considered very safe as there is no or minimal side effects. These remedies are coordinated with nature, which is the biggest advantage. The golden fact is that, use of herbal treatments is independent of any age groups and the sexes.

Keywords: Analytical, Ethno-medicine, Gymnosperm, population, treatments, herbaceous and drugs

12.1. INTRODUCTION

Uttarakhand is a part of north-western Himalaya, and still maintains a dense vegetation cover. It has been the reservoir of enormous natural resources of medicinal wealth, one of richest floristic zone and provides more than 300 species of medicinal plant in all over country (Gaur 1999).From prehistorically time, the Himalayan flora has been in use for various purposes including some scientific therapeutic uses. The old Indian literature such as Rig-Veda, Atherveda, Charka Sanhita, included various uses of plants of Himalaya region (Sharma *et al.*, 2011).

Plant parts are directly used as medicine by a majority of community people in all over world and have no side effect like allopathic medicine (Gangwar *et al.*, 2010). In hilly area of Garhwal Himalayas the medicinal plants are used by all section of the community, whether directly as folk remedies or the medicaments of the different indigenous system as well as in modern medicine (Alok 1991).The plant-based, traditional medicine system continues to play a significant role in health care, with about 80% of inhabitants relying mainly of traditional medicines for their primary health care (Owolabi *et al.*, 2007). Traditional knowledge on medicine since the time of Charak has led to the discovery of many important drug of modern age, (Uniyal *et al.*, 2002). Although researches have presented research papers from time to time about the medicinal properties of all the medicinal plants found in the Himalayan region. But the main aim of the present investigation is to obtain information about the medicinal properties of new species.

12.2. **METHODOLOGY**

Chamba, block, district Tehri Garhwal (Uttarakhand) was selected for the present investigation. 25 villages were selected. The information was collected with the help of questionnaire from the local peoples. Hundred questions were prepared and questions were asked to 10 people in each village. People in the age group of 35-60 years old (male and female) were selected for the authentic information. Various age groups of people were also interacted with to know the exact usefulness of observed medicinal plants. The focal point of the study was made in Badshahithaul, Tehri Garhwal and periodic field trips were made up to once a month during the flowering and fruiting season (Kumar 2017). Collected specimens were dried, pressed and identified properly with the help of available literature and monographs. Herbariums were submitted in the Tree Biology Laboratory, Department of Botany, H.N.B. Garhwal University, S.R.T. Campus Badshahithaul, Tehri Garhwal.

12.3. RESULTS AND DISCUSSION

All hill regions of Uttarakhand, are known as the sources of medicinal plants since ancient times. The medicinal plants are found almost everywhere in that places. Chamba block (Tehri Garhwal) is selected for the present investigation and it is also a hilly region of the Uttarakhand state. About 60 species of medicinal plants (29 herb, 13 shrub and 18 trees) have been observed from the study area, which are presented and discussed in table 1, 2 & 3. The plants pointed out by local

inhabitants were recorded, identified botanically with the help of flora of the Chakrata, Dehradun and Saharanpur (Kanjilal 1928) and finally matched with the Flora of District Garhwal: North West Himalaya (Gaur 1999) for accuracy.

Table: 1. Herb medicinal plants and their uses

Botanical name	**Family**	**Local name**	**Parts used**	**Medicinal Uses**
Ajuga parviflora	Lamiaceae	Bugle	Root, stem, leaves	Juice used as tonic to kidney problem, menstrual regulation, digestion, for viral and bacterial infection.
Amaranthus caudatus	Amaranthaceae	Marshu	Leaf, seed	Leaf juice is used for ulcers, diarrhea, and swollen mouth and throat. It also used to treat high cholesterol, rich source of minerals.
Amaranthus tricolor	Amaranthaceae	Pigweed	Leaves	Juice used in stomach disorder and cancers.
Bidens pilosa	Asteraceae	Kumra	Stem, Fruit	Fruit juice extract with honey used in cough and bronchitis, hepatitis, bacterial infections, inflammatory urinary tract infection.
Chenopodium album	Chenopodiaceae	Bethoo	Leaves	Anti-diarrheal, anti-inflammatory, useful in treating scurvy, intestinal disorder, painful limbs etc., rich source of vitamin C.
Colocasia esculenta	Araceae	Pindalu	Leaves, fruits	Leaf extract used various ailments such

				as asthma, arthritis, diarrhea, internal hemorrhage, neurological disorders and skin diseases.
Coriandrum sativum	Apiaceae	Dhania	Whole plant	Digestive disorders, stimulating appetite and relieving irritation, ointment to treat piles, rheumatism, menstrual disorders and painful joints.
Cynodon dactylon	Poaceae	Dhoob.	Whole plant	Juice used in fever, alleviate pain, wounds and cancerous sores, dropsy. Increase secretion and discharge of urine.
Datura stramonium	Solanaceae	Dhatura	Seeds	Powder used in asthma, gastrointestinal aches, abscesses, arthritis, headache, swellings and tumors.
Eupatorium adenophorum	Asteraceae	Kharna	Leaves	Leaf extract used in mosquito bites, itching lumps, athlete's foot, dermatitis, anti-inflammatory to stop bleeding.
Euphorbia helioscopia	Euphorbiaceae	Dudhi.	Leaves , root	Leaves, root used in bronchitis, asthma, colds, flu, skin irritations and snakebites. *Euphorbia*

				is also considered to boost breast milk production in lactating mothers.
Fumaria indica	Fumariaceae	Pit-papra	Root, leaves	Used in aches and pains, diarrhea, fever, influenza and liver complaint, blood purifier and skin diseases.
Girardinia diversifolia	Urticaceae	Kandali	Root, leaf	Roots juice used to treat constipation, headache and swollen joints.
Malva parviflora	Malvaceae	Soncheli	Leaves , seed	Leaves used as vegetable, seeds used in gonorrhea; leaf extract applied on cuts and wounds; roasted seeds chewed in throat irritation.
Mentha piperita	Lamiaceae	Peppermi nt	Stem, leaf	Juice and paste used in mouth-throat inflammation, cold-cough, digestive disorder, morning sickness, stomach cramps, reduce pain during menstrual cycle, muscle relaxer, headaches, nerve pain, toothaches, inflammation of the joints and general body

				aches.
Mirabilus jalapa	Nyctaginaceae	Dophriya	Root	Root paste used in mouth ulcers.
Ocimum tenuiflorum	Lamiaceae	JungliTulsi	Leaves	Used in fever, cold, cough, urinary troubles and vomiting.
Oenothera rosea	Onagraceae	Roseev	Seeds	Oil used in muscular pain and skin diseases.
Oxalis corniculata	Oxalidaceae	Khati-Buti	Leaf	Leaf powderis used with black piper to epilepsy patient.
Rumex hastatus	Polygonaceae	Amildu	Leaf, root	Leaf extract applied on wounds and cuts to check bleeding. Root is Ant rheumatic and used in skin disease also.
Rumex nepalensis	Polygonaceae	Nepal Dock	Leaf, root	Leaf extract applied to skin sores, fresh leaves rubbed on the affected part to relief from irritation caused by *Urtica* plant. Root is purgative, and applied to dislocated bones.
Saussurea heteromalla	Asteraceae	Murang	Leaves, Root	Leaf paste with mustard oil used to massage on Lecco derma and wounds. Root extract taken in fever and colic.
Solanum nigrum	Solanaceae	Makoi	Leaves, fruit	Fruits juice used in fever and alleviate pain, wounds and cancerous sores.

Stellaria media	Caryophyllaceae	Chickweed	Whole plant	Leaf juice used remedy to treat itchy in skin and pulmonary diseases.
Taraxacum officinale	Asteraceae	Dandelion.	Whole plant	Root and leaf extract used to treat infections, of liver.
Thalictrum foliolosum	Ranunculaceae	Kirmuli	Roots, leaves	Juice used in ophthalmic, colic and fever. Remedy for dyspepsia and ulcers, indigestion, toothache and for acute diseases.
Trigonella foenum	Fabaceae	Methi	Whole plant	Indigestion, sluggish liver, dysentery, diarrhea and dyspepsia.
Urtica dioica	Urticaceae	Kandali	Root, leaf	Root juice used during pregnancy due to its rich mineral value and vit. K, which guards against excessive bleeding, reduces nasal inflammation.
Viola reichenbachiana	Violaceae	Vanafsa	whole plants	Leaf extract or powder taken as diaphoretic useful in skin and blood diseases, flowers and leaves boiled with tea supposed to be good for fever and cough.

Table 2: Shrub medicinal plants and their uses

Botanical name	Family	Local name	Parts used	Medicinal Uses
Adhatoda vesica	Acanthaceae	Banshoo	Flowers, leaves, roots	Flowers juice is useful in pulmonary infections. Leaves are used in fever. Roots are infusion useful in bronchitis, fever, tuberculosis and bronchiole disorders. Leprosy, blood disorders, heart troubles, vomiting, and loss of memory, Lecco derma, jaundice, tumors, mouth troubles, sore-eye and gonorrhea.
Berberis asiatica	Berberidace	Kingore	Roots, leaves	Roots extract used in ophthalmic. The leaves juice used in jaundice. *Berberine,* marked antibacterial affect.
Cannabis sativa	Cannabinaceae	Bhangulu	Leaves, flowers	Leaves are applied on insect bite. Extract of leaves and flowers used in diarrhea.
Hedera nepalensis	Araliaceae	Lagali	Leaves, fruits	Leaf and fruit paste applied on ulcers. Leaf juice used in dyspepsia.
Prinsepia utilus	Rosaceae	Cherry prinsepia	Seeds	Juice used in rheumatism and muscular pain, colds cough and stomach ache.
Ricinus communis	Euphorbiaceae	Arand	Seeds	Castor oil used as purgative and laxative.

Rosa brunonii	Rosaceae	Kunja	Leaves, flower	Leaf and flower juice used in wounds and ophthalmic. Dried flower-powder given in diarrhea.
Rubus ellipticus	Rosaceae	Hinssar	Root	The juice of the root used in fever, gastric troubles, diarrhea and dysentery.
Rubus niveus	Rosaceae	Anchu	Root, Fruit	Root juice used as an antidote of snakebite. Fruit used in dysmenorrheal.
Tinospora cordifolia	Menispermaceae	Giloy	Stem	Used in diabetes.
Vitex negundo	Lamiaceae	Shiwali	Root and leaves	Roots and leaves used in eczema, ringworm, skin diseases, liver disorders, spleen enlargement, rheumatic pain, gout, abscess, backache. Control population of mosquitoes.
Xanthoxylum armatum	Rutaceae	Timroo	Stem, leaf, seeds	Stem and fruits used in chronic rheumatism, typhoid, skin diseases, blood purifier digestive ailments, toothache, cold, cough and fever.
Ziziphus mauritiana	Rhamnaceae	Ber	Root, fruits	Root juice used in anxiety, asthma, diarrhea, fatigue, fever, high blood pressure, inflammation, stress. Fruit juice enhance liver health.

Table 3: Trees medicinal plants and their uses

Botanical name	Family	Local name	Parts used	Medicinal Uses
Callistemon viminails	Myrtaceae	Cheel	Leaves, stem	Leaf juice is used as antibacterial and for relieving problems of the urinary tract. Women as douche used to clean the genitourinary tract from excessive menstruation or mucosal discharge as leucorrhea, used for urinary incontinence, gastroenteritis, diarrhea and skin infections.
Cassia fistula	Fabaceae	Simaroo	Seeds	Cold, skin disorder, promote cardiac functioning, wands, dyspepsia and diabetes.
Cedrus deodar	Pinaceae	Deodar		Wood possesses to be a good remedy in remittent and intermittent fever, diarrhea and dysentery, ulcers. *Cedrus* wood posse's anti-fungal properties.
Emblica officinails	Euphorbiaceae	Alma	Fruits	Fruits used as *Triphala* which is a laxative and diuretic used in diarrhea, dysentery and eye diseases. It is ingredient of chavanprash. Rich source of vit. C.

Eucalyptus globules	Myrtaceae	Safada	Fruits	Oil help in cold, cough lozenges, inhalants, fungal infections and skin wounds.
Ficus religiosa	Moraceae	Pipal	Leaves	Leaves and stem have purgative properties.
Juglans regia	Julandaceae	Akroot	Leaves	It is astringent, antifungal, and antiseptic. Stimulate the production of thyroid hormones. Used externally walnut can help in the treatment of various skin diseases such as acne, eczema, dermatitis, itching etc.
Melia azedarach	Meliaceae	Dainkan	Leaves, fruits	It is antifungal, antiviral and antibacterial. Leaf extract used in various skin diseases.
Morus alba	Moraceae	Sahtoot	Roots, fruits	The roots are astringent, anthelminthic and purgative. The luscious fruits are edible, used in digestive disorders.
Myrica esculenta	Myricaceae	Kaifal	Bark	Bark extract used in fever, cold cough, ulcers, diarrhea, inflammation and paralysis and in the infection of throat. Oil dropped used to stop earache.
Pinus roxburghii	Pinaceae	Chir	Root	Root paste used in foul ulcers, giddiness, asthma, gonorrhea, and epilepsy.

Prunus cerasoides	Rosaceae	Aru	Fruits, bark	The fruit juice of the bark applied externally to treat backaches. It stimulates respiration, improves digestion and gives a sense of wellbeing.
Pyrus pashia	Rosaceae	Mahal	Fruits	Juice of the ripped fruit used to treat conjunctivitis by putting it in the eye of the diseased animal. Six tea spoons twice a day used to treat diarrhea and reduce the risk of the cancer.
Quercus leucotrichophora	Fagaceae	Banj	Seeds, bark	Used in treatment of gonorrhea and asthma. Bark used in the treatment of hemorrhages, chronic diarrhea and dysentery.
Rhododendron arboreum	Ericaceae	Burans	Flowers	Juice used for respiratory disorder, diarrhea and dysentery. Paste applied on forehead to alleviate headaches curing chronic rheumatism and syphilis.
Robinia pseudoacacia	Fabaceae	Black locust	Leaves, flowers, bark, root	Flowers are antispasmodic, aromatic, diuretic, emollient and laxative. They cooked and eaten for the treatment of eye ailments. The inner bark and the root bark are emetic, purgative and tonic. The root chewed to

				induce vomiting or held in the mouth toothaches.
Toona ciliata	Meliaceae	Toon	Bark	The bark is useful in chronic infantile, dysentery, cough, bronchitis, intermittent fever, leprosy and ulcers.
Taxus bacata	Taxaceae	Thuner	Root, leaves	Root and leaves used for cancer treatment.

Nature has been an important source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Valley of flower in Uttarakhand state is one of the famous and important place where various number of medicinal plant species are found. Economically weaker section of the community collects medicinal plants from the forests for commercial use as livelihood option in hills of Uttarakhand. Since the beginning of human civilization, medicinal plants have been used by mankind for its therapeutic value. The local persons mainly depend on medicinal plants for their health care. They treat themselves with traditional medical system. Actually traditional medicine is the sum of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different culture, whether explicable or not, used in the maintains of health as well in the prevention, diagnosis, improvement or treatment of physical and mental illness. Traditional way local people use medicinal plants. Today about 65% of the Indian population depend on the traditional system of medicine (WHO 2002).

Appropriate information of all observed medicinal plants was taken from 500 people. Except some people, majority has provided same type of information regarding their medicinal value. But community only belief on the traditional practitioner for their health care. The knowledge of utilization of medicinal plants is very high among Himalayan people. The basic ingredients in the traditional medicine are the medicinal plants, which are depleting at a faster rate due to the increase in consumption and indiscriminate drawl of resources from the wild (Kumari *et al.*, 2012). Traditional practitioner prescribes the parts of plants as medicine on the basis of their disease symptoms after investigation. It is very

important to check the quality and quantity of medicine before giving it. Otherwise, sometimes it can be harmful. Nascimento *et al*., 2000 has also suggested that the quantity and quality of plants should be investigated to better understand their properties, safety and efficacy. Some medicinal plants found here are poisonous, and the local people are not aware of these poisonous plants and their life can be danger after using such plants through traditional way. Knowledge of using native plants and its associated medicinal practices have now become a part of the local tradition, culture, art, belief and folklore. Some medicinal plants and their traditional knowledge on the verge of extinction today in the hilly areas due to lack of maintenance, management and documentation.

Continuous erosion in the traditional knowledge of many valuable plants for medicine in the past and the renewal interest currently, the need existed to review the valuable knowledge with the expectation of developing the medicinal plants sector (Kala *et al*., 2004). Due to the pungent taste of medicinal plants, sometimes people are careless in taking medicines. The property and quality of medicinal plants depends on its biochemical's. The medicinal properties of plants could be based on the antioxidant, antimicrobial, antipyretic effects of the Phytochemicals in them (Cowman 1999; Adesokan *et al*., 2008). Poor growth rate was noticed in all observed plants during survey. It seems to be that it can be directly related to climate change. If this situation continues, some plants may become extinct. Population densities, and narrow geographic ranges (Nautiyal *et al.*, 2002), therefore they are more prone to extinction (Jablonski 2004). Proper maintenance can prove to be boon for plant growth and their utilization in hills. Due to which it will have a good effect on people's health as well as their income. Each medicinal plant has different properties and each medicine is obtained from different parts of the plants. Major contributions of medicinal plants in present investigation relate to document action of inventories, which include information on the list of species, parts used and distribution range. Medicinal plants with the most stem and leaves were recorded in present investigation. The "herbal drug" determines the parts of a plant (leaves, flowers, seeds, roots, barks, stems, etc.) used for preparing medicines (Anonymous 2003). There is a need to protect of medicinal plants in the hilly areas of Uttarakhand. In addition to the requirement for conservation of medicinal plants, it has also become essential to protect and patent the traditional knowledge (Raghupathy 2001). Depending on the availability of medicinal plants in the hilly

areas of Uttarakhand, commercially medicinal plant centres can be set up at many places.

12.4. CONCLUSION

Garhwal Himalaya has diverse form of medicinal plants, which is of high medicinal values. Many botanists from our country and entire the world have done their research work on these medicinal plants and research work is going on at present. However, looking at the current status of the medicinal plants in Uttarakhand, it seems that there is a strong need for their protection and management. Even the local people here are not aware of their conservation and utilization. In such a situation, there is a need to tell the local people scientific methods. The medicinal plants are also a means of employment for the local people. However, overexploitation of medicinal plants also requires a strategy among the local people. There is also a need to set up medicinal plant centers in the hilly area of Uttarakhand. So that the economy of the people here can be strengthened and medicinal plants can be supplied all over the country.

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