International Journal of Herbal Medicine
Available online at www.florajournal.com

E-ISSN: 2321-2187
P-ISSN: 2394-0514
www.florajournal.com
IJHM 2023; 11(4): 38-46
Received: 28-04-2023
Accepted: 05-07-2023

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Indiginous knowledge, phytochemical analysis, antimicrobial activity and *in vitro* conservation of some medicinal plants of Bhimkund and its adjoining regions in Mayurbhanj district of Odisha, India

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DOI: https://doi.org/10.22271/flora.2023.v11.i4a.878

Abstract
Mayurbhanj district in the state of Odisha is much rich with indigenous knowledge about the medicinal plants used by various ethnic groups. Bhimkund and its adjoining region in this district are endowed with rich biodiversity. People of these areas are utilizing various plants and plant products for the ailments of various diseases. The rich traditional knowledge about the use of medicinal plants for the treatment of various diseases is on the verge of extinction in these areas which should be properly documented for their use in the compilation of Traditional Knowledge Digital Library. These areas are endowed with large number of medicinal plants along with plants of other socio-economic importance. During the present investigation, the phytochemical analysis of some common medicinal plants have been conducted which gives evidence on the medicinal properties of these plants. Antimicrobial activity of some selected medicinal plants of this region such as *Cissampelos pareira* L., *Nyctanthes arbor-tristis* L., *Terminalia chebula* Retz., *Curcuma angustifolia* Roxb., *Rauvolfia serpentina* Benth. ex Kurz shows that the plants have inhibitory effects on the growth of the bacterium *Lactobacillus*. It gives an idea that medicines prepared from various parts of these plants will be much effective for the treatment of various diseases caused by the infection of various microorganisms. The *in vitro* conservation through the techniques of tissue culture of the medicinal plants *Curcuma angustifolia* Roxb. Collected for these region seems to be an effective method for the *ex situ* conservation.

Keywords: Indigenous knowledge, phytochemical analysis, antimicrobial activity, *in vitro* conservation, medicinal plants, traditional knowledge

Introduction
India is one of the major countries in term of biodiversity and it occupies tenth position in plant diversity area wise in the whole world. Odisha state in India is endowed with very rich vegetable wealth due to its peculiar geographical location, topography and diverse climatic conditions. Mayurbhanj district in the state of Odisha is rich with luxuriant forests and has different types of medicinal plant resources. About 46 tribal groups inhabit in the district which is 58.7% of the total population of district. It is seen that some tribal groups reside in less accessible areas of the district and lead a primitive life. Each tribe has a different ancient culture and tradition of utilization and conservation of plant resources. Only the medicine man popularly called Vaidya, or Kaviraj, old men and women know much more about the traditional uses of their surrounding vegetation. Bhimkund is present on the river Baitarani which is 15 kms away from the Thakurmunda Block of Mayurbhanj district. Along with the river basin and its adjoining regions such as Ranibhul, Saleibeda, Khaparkhai, Sanamahuldiha are rich with medicinal plants. People of these tribal dominated areas depend on traditional system of healing of their diseases than modern allopathic system of medicines.
From time immemorial people learn the application of different plants and plant parts for the treatment of various diseases. Various medicinal properties have been attributed to natural herbs. Medicinal plants constitute the main source of new pharmaceuticals and healthcare products [1]. Herbal medicines have become more popular in the treatment of many diseases, as these are safe, easily available and with less side effects. Because of excess use there is loss of genetic diversity of some medicinal plants. As such some of these species are on the verge of extinction. Indeed, the market and public demand has been so great that nowadays there is a great risk to many medicinal plants for their extinction or loss of genetic diversity. This fact is evident from the ethnomedicinal studies conducted by different investigators in the state of

Odisha and other parts of India [2, 16].

In rural and tribal areas people depend on traditional medicines based on Indigenous knowledge as their major primary health care system. Medicinal plants have been used since time immemorial for the treatment of human as well as animal diseases [17]. Ethno medicine is one of the systems of medicine that is widely practiced for cure of ailments [8]. According to World Health Organisation as many as 80% of the world's population depends on traditional medicines and in India, 65% of the population in the rural areas especially those residing in the remote forests mainly rely on the traditional health practices as it is cost effective [12]. Ethnomedicines have gained new dimensions in the present days through phytochemical researches in India and abroad, as information on medicinal plants and folk drugs recorded during field-works are now being subjected to investigations in the search for new biodynamic compounds of therapeutic value [5]. The use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs from these plants as well as from traditionally used folk medicine. The extraction and characterization of several active phytocompounds from the green factories have given birth to some highly effective [18].

Phytochemicals can be defined as the chemical compounds that are produced by plants. Natural product is a source of bioactive compounds and has potential for developing some novel therapeutic agent. Over the last decade there has been a growing interest in drugs of plant origin and such drugs have become an important method for control of diseases. Secondary plant metabolites (phytochemicals), with known pharmaceutical activities, have been extensively investigated as a source of medicinal agents [19]. Higher plants as the sources of medicinal compounds play a dominant role in the maintenance of human health care since ancient times. Over 50% of all modern clinical drugs are of natural product origin [20]. The alkaloids, flavonoids, glycosides, phenols, tannins, saponins etc. are different phytochemicals which are isolated from plants. Generally phytochemicals are responsible for producing different colours, flavours and smell of plants. They form part of a plant's natural defense mechanism against diseases. Their therapeutic values to human health have been reported in the different systems of medicines. Natural products such as pure phytoconstituents and plant extracts offer enormous opportunities for new drug development due to the unmatched availability of chemical diversity [21]. Medicinal plants contain components of therapeutic values and hence they are used as remedies for human diseases. Phytochemical analysis was performed using standard procedures [22-23].

Antimicrobial activity refers to the ability of an agent that kills microorganisms or suppresses their multiplication or growth. There is an urgent need to discover new antimicrobial compounds with different chemical structures and novel mechanisms of action for new and re-emerging infectious diseases. There has been considerable interest in the use of plant extracts as multi-drug complex and an alternative method to control pathogenic microorganisms. There are many reports that different plant parts such as bark, leaves, peel, seed and stem potentially have some antimicrobial property. Most of the medicinal and aromatic plants are rich sources in antibacterial compounds which can be an alternative method to combat bacterial diseases [24]. The discovery of antibiotics is more than 70 years ago and since then different initiatives have been done for the development of new drugs for use in human, animal health and agriculture. These discoveries were tempered and questioned in all cases with the emergence of new resistant microbes. For which, we are now facing the threat of superbugs, i.e. pathogenic bacteria resistant to most or all available antibiotics. It was warned by the World Health Organization that those multiple antibiotic-resistant pathogens would very likely bring the world back to the pre-antibiotic era. This alarming situation imposes the need for a search and development of new drugs and discovery of new medicinal agents with novel modes of activity. This clearly highlights the need for new antibacterial agents with fundamentally different modes of action than that of traditional antibiotics. The enormous demand has triggered worldwide efforts in developing novel antibacterial alternatives, particularly the screening of several medicinal plants for their potential antimicrobial activity [25]. An ultimate goal is required to eradicate microbes to offer appropriate and efficient antimicrobial drugs to the patients. Plants are valuable sources of natural products for maintaining human health care. Plants have attained the status of natural sources of new and potent antimicrobial agents. About 20% of the plants, which were found in the world had been submitted to biological tests and sustainable number of new antibiotics introduced in to the market was obtained from nature [26].

In vitro techniques have been increasingly applied for mass propagation and conservation of germplasm as it is the best method over other conservation methods of propagation and offers some distinct advantages over alternative strategies. Micropropagation of plants through tissue culture is an alternate method to prevent the loss of important medicinal plants from the natural habitat [27]. *In vitro* multiplication rate was low and the genetic fidelity of regenerated plantlets was not assessed either at cell or molecular level [28]. The farming and conservation of the plant could be based on its *in vitro* regeneration. Plant tissue culture is a well-known biotechnological tool for the mass propagation of rare, endangered and threatened medicinal plants which are facing the danger of extinction. *In vitro* propagation is also a powerful tool for the production of medicinal secondary metabolites as well as for the purpose of conservation [29]. Development of in vitro plant regeneration protocols is not only essential for propagation but also it is a pre-requisite for genetic transformation studies. *In vitro* propagation derived plants exhibit huge genetic variation that could be exploited for developing superior clones or varieties particularly in vegetative propagated plant species.

Methodology

Study area: Mayurbhanj is a hilly district and present in the North part of Odisha state. It is the largest district of Odisha and extending over an area of 10,418 sq. km. It lies between 21º 17' and 22º 34' N latitude and between 85º 40' to 87º 10' E longitude. This district shares its border with Singhbum district on the North, West with West Singbhum District, South East with Balasore, South West with Keonjhar and on the East shares boundary with Medinipur District of West Bengal. The total area of forest cover in the district is about 4392.13 sq. km. It is endowed with some unique features such as Simlipal Biosphere Reserve, Barehipani, Joranda and Debkund waterfalls and some mining activity areas like Badampahad and Garumahisani mines. Tropical moist deciduous forests and the sal forests (dry decidious) are the dominant vegetation of the district. In the present study, a part of the Similipal biosphere reserve including different areas of Bhimkund and its adjoining regions such as Ranibhul, Saleibeda, Khaprakhai and Sanamahuldiha of the Thakurmunda Block have been surveyed for the presence of different medicinal plants (Figure-1).

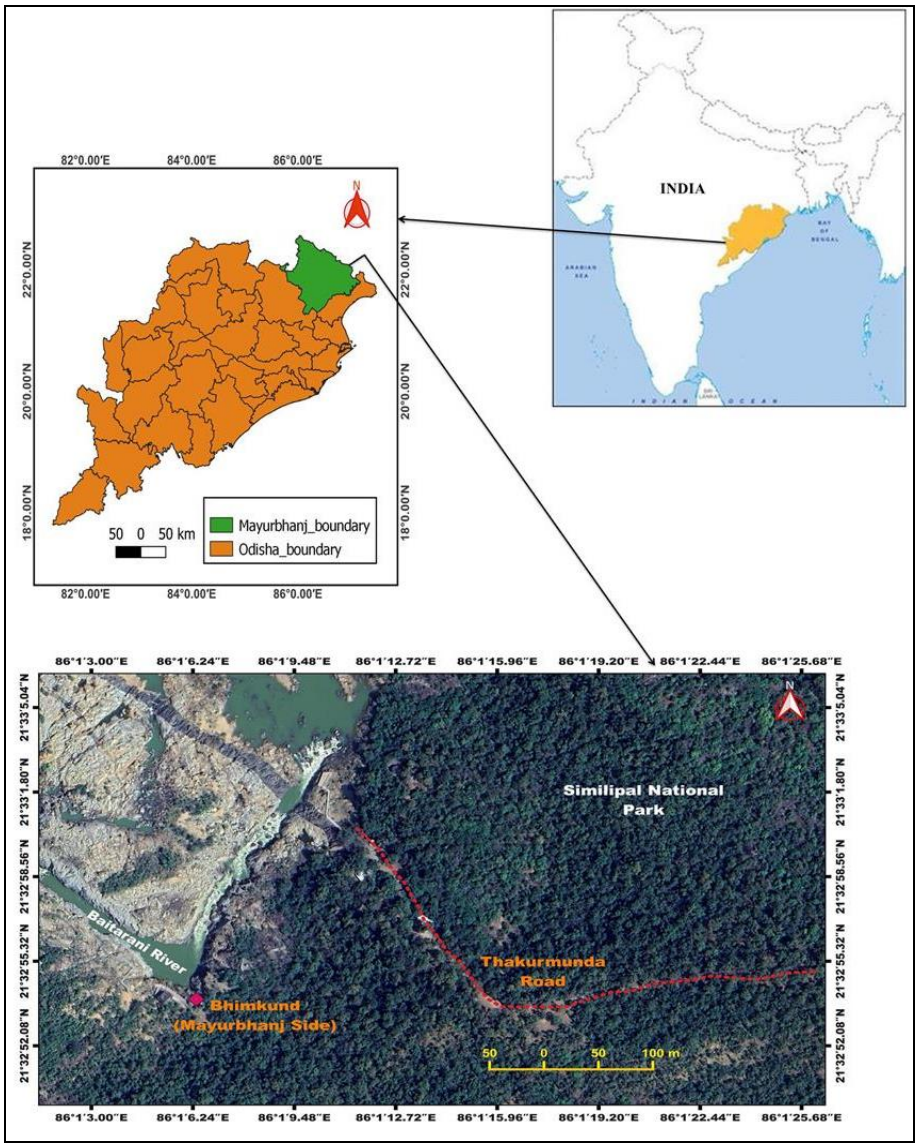

Fig 1: Study Area

Plant Collection and Identification

Plant species were mostly collected in flowering and fruiting conditions and with reproductive characters which are required for the exact identification. The specimens were collected and identified with the help of regional flora book [30-31].

Local Names

Vernacular names as used by the local people in Odia and tribal languages were recorded during the field visit and plant sample collection.

Ethnomedicinal noting

Ethnomedicinal uses of various plants by the local tribal people have been recorded during the field study. Every care has been taken to avoid ambiguity as regards to the use of plant parts, quantity, number of doses per day, method of preparation and mode of application etc.

Phytochemical Analysis

The phytochemical analysis has been done by standard procedures with slight modification [22-23].

Extraction of plant materials

Near about 30 gm of air dried plant material were powdered and were taken in a conical flask containing 200ml of ethanol or n hexane or distilled water and was plugged with cotton wool. Then it was kept on orbital shaker for 48 hrs. With speed of 150 rpm at room temperature. After that extracts were filtered with Whatman No.1 filter paper and the supernatant was collected. Then it was stored at 4 °C in air tight containers. The tests for different phytochemicals were carried out for all the three different type of extracts.

Test for Alkaloids

2 ml of plant extract was taken in a test tube and 2 ml of 1% HCL and 6 drops of both Mayer's reagent and Dragendroff's reagent were added to it. An organic precipitate was formed which indicated the presence of alkaloid in the sample.

Test for Flavonoids

About 10 drops of aqueous extract of plant material was taken in a test tube and 5ml of dilute ammonium solution was added to it. Then little amount of concentrated H_2SO_4 was added slowly. A yellow colour was observed which confirmed the presence of flavonoids and it disappeared on standing.

Test for Glycosides

5ml of plant extract was taken in a test tube and was treated with 2ml of glacial acetic acid with one drop of ferric chloride solution. Then1ml of concentrated H_2SO_4 was added over it

gently. A brown ring of the interface was formed which indicated the presence of a deoxy sugar of glycosides. A violet ring might appear below the brown ring whereas in the acetic acid layer, a greenish ring might form just gradually through thin layer.

Test for Phenols
2ml of plant extract was taken in a test tube and 3ml of ethanol was added to it. Then a pinch of $FeCl_3$ was added to it. A greenish yellow color was formed which indicated the presence of phenols.

Test for Tannins
5ml of plant extract was taken in a test tube and few drops of 1% of lead acetate were added to it. A yellow precipitate was formed which indicated the presence of tannins.

Test for Saponins
The plant extract with 20 ml of dist. H_2O was agitated in a graduated cylinder for about 15mins. The formation of 1cm layer of foam indicated the presence of Saponins.

Description of different reagents used for the phytochemical analysis
- Dragendorff's reagent = A+B
- A = Bismuth nitrate 1.7 gm + Tartaric acid 6 gms with 80 ml dist. H_2O
- B = KI 6 gm + 40 ml water
- Mayer's reagent = 1.36 gm mercury chloride + 5 mg potassium Iodide with 100 ml water
- 1% HCL
- 1% Fecl3 = 0.25 gm Fecl3 + 25 ml H_2O
- 1% Lead acetate = 0.25 gm lead acetate + 25 ml H_2O

Antimicrobial Activity
The glass wares used for investigating the antimicrobial activity include conical flasks, beaker, measuring cylinder, petri-dish, test tubes. The instruments used in the present investigation are Balance (Contect Instruments electronic balance), Laminar chamber, Autoclave, Orbital shaker, Incubator. Other thing used in this experiment includes Beef extract (0.2 g), Peptone (0.5 g), Agar powder (3.0 g) and Distilled water.

Collection of Plant Material and Preparation of Ethanol Extract
Mature and healthy plant parts of *Cissampelos pareira* L., *Nyctanthes arbor-tristis* L., *Terminalia chebula* Retz., *Curcuma angustifolia* Roxb. and *Rauvolfia serpentina* (L.) Benth. ex Kurz which used for this study were collected from the Bhimkund and its adjoining regions of Mayurbhanj, Odisha. The plant parts were washed thoroughly with tap water and dried in shade condition. The dried plant parts were placed into a blender to be ground into powder. Five grams of each sample powder were weighed and dissolved in 50 ml of solvents in a conical flask. After 24 hour solvent extract was collected in sterile test tubes wrapped with aluminium foil.

Culture of Bacterial Strains from curd
For testing antimicrobial activity of the herbal extracts, disc diffusion method was followed. In the present study *Lactobacillus* strain isolated and cultured from curd has been used as a model organism. First of all, nutrient broth culture was prepared by adding 0.2gm of beef extract, 0.5gm of peptone per 100 ml of medium prepared with distilled water. The culture medium was sterilised by autoclaving and cooled to normal temperature and then 1 ml of fresh curd was added to the medium in a laminar hood and then it was kept in an orbital shaker for overnight for growth of the bacteria.
In the second step the disc diffusion assay was carried out on the nutrient agar plates which were prepared by adding 0.2gm of beef extract, 0.5gm of peptone and 3.0gm of agar per 100 ml of medium prepared with distilled water. The culture medium along with 5 pair of petri-dishes, forceps, and some cotton wool swab (match sticks wrapped with cotton at the tip which looked like ear bud) was sterilized by autoclaving. After sterilization all the things were transferred to laminar air flow and the nutrient agar plates were prepared by pouring the warm sterilized medium on to the petri-dishes and allowing them to solidify.
When broth culture (discussed in the first step) achieved the exponential growth stage, a sterile cotton wool swab was dipped into the bacterial suspension and the excess liquid was removed by turning the swab against the side of the container. The inoculums were spread evenly over the entire surface of the nutrient agar plate. The agar plates were allowed to dry. Small filter paper discs of 1 mm radius were prepared, and they were dipped in the ethanol plant extract, Dettol (positive control) and ethanol (solvent or negative control). Then the discs were allowed to dry, and then they were applied on the agar plates. On each agar plate five discs were applied. Out of the five discs, one of each served as positive or negative control marked with (+) and (-) signs respectively and the remaining three served for the plant extract (3 replicates).
Then the plates were incubated at 30 ºC in the incubator for 24 hour. After 24 hour of growth, formation of zone of inhibition was monitored and the size of the zone was measured with the help of calliper and ruler.

***In vitro* conservation**
Source of explants
For *in vitro* conservation of plants species here *Curcuma angustifolia* Roxb. Were taken as explant for the experiment. These samples were collected form the Bhimkund and its adjoining regions of Mayurbhanj district, Odisha. The fresh rhizomes and stems were collected from field site and brought to the laboratory of Post Graduate Department of Environmental Science for *in vitro* conservation.

Surface sterilization
The rhizomes were collected from hilly areas and kept in a closed bag. In the laboratory rhizomes of *Curcuma angustifolia* Roxb. were washed for 10 to 15 minutes under running tap water followed by washing 2 to 3 times in distilled water. After washing, the rhizomes were taken to the laminar air flow for sterilization. Excised shoot tip (1 to 2 cm long) was surface sterilized with 70% alcohol (ethanol) for 30 seconds and then it was rinsed in sterile distilled water by the help of a sterile forceps. Then it was soaked with 0.1% (W/V) Mercuric chloride solution for about 30 seconds. Finally it was washed 7 to 8 times with sterilized distilled water.

***In vitro* callusing, shooting and rooting**
This section deals with the protocol for *in vitro* shoot and root regeneration starting form explant of the species. The rhizome and immature stem tips were cultured on nutrient media. Formation of proto-corm like bodies (PLBs), induction and proliferation of calli, regeneration of shoots and roots were different steps of this protocol.
The base nutrient medium formulated by Murashige & Skoog

(1962), generally known as MS medium, was used for this experiment. The effects of MS medium without growth regulators and with growth regulators such as IAA & NAA (auxins) and BAP (cytokinin) were observed and evaluated. MS medium was supplemented with one of the auxins (either IAA or NAA) and BAP. The pH of the medium was adjusted to 5.6 by adding either 1.0 N NaOH or 1.0 N HCl. The media were made to semi-solid by adding 8.0g of agar per one litre of medium. The media were autoclaved at 1200 ºC and 15 lb pressure for 20 minutes. The prepared media were transferred in to the conical flasks of size 250ml volume. The surface sterilized rhizomes and young thin stem tips were cut in to a very thin section of 1mm size by the help of a surgical scalpel blade. After cutting in to a thin section, the sections with young green stem tip were placed in the conical flask containing MS media with or without growth regulators. Then the flasks were kept on the culture racks. Suitable conditions for growth of the explant like temperature range 25±10 C, 14-h of photoperiod at 35-50 µ Em-2s-1 intensity with the help of white florescent tubes (Philips, India) and 60-70% relative humidity was maintained constantly throughout the culture experiment. After the formation PLBs, these were separated out by the help of a sterilized scalpel in an aseptic condition and transferred on to fresh medium. The MS medium with or without growth regulators were used. The small PLBs were converted to clump like structure. These clumps like structure are called as callus. After 3 weeks, very small shoots with minute leaves were seen to develop from the callus. These were then transferred to the fresh medium with the help of sterilized forceps in an aseptic condition inside a Laminar Air Flow chamber. Then the conical flasks were capped with aluminium foil and stored in the culture room on the culturing racks. The shoots with 2-3 expanded leaves were excised gently from the shoot clumps and were transferred to the fresh medium supplemented with different concentrations of auxins and cytokinin. After two weeks these were developed into small plantlets and then transferred to field condition.

Results and Discussion

Ethnomedicinal uses

Ethanomedicinal uses of the medicinal plants which are available in and around the study area have been recorded during the field study. It has been observed that various plants and their parts such as root, stem, leave etc. have been used by different ethnic groups for the treatment of various diseases (Table-1). These data will be much useful for the development of Traditional Knowledge Digital Library (TKDL) and attract different herbal medicine companies to make medicines for the treatment of common ailments.

Phytochemical analysis

Phytochemical analysis of some potential medicinal plants (Fig.2) has also been done by following standard methods [31-32]. The qualitative estimation of plant materials such as alkaloids, flavonoids, glycosides, phenols, tannins and saponins were carried out for all the three different type of extracts (ethanol or n hexane or distilled water) which gives evidence on the medicinal properties of these plants (Table-2). The quantitative estimation of these phytochemicals will provide more relevant information on the effectiveness of the medicines prepared from these plants. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites. They possess biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities. Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds. Natural antioxidant mainly come from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc.Tannins bind to proline rich protein and interfere with protein synthesis. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall. These are effective antioxidant and show strong anticancer activities. The plant extracts were also revealed to contain saponins which are known to produce inhibitory effect on inflammation. Saponins have the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity. Several workers have reported the analgesic antispasmodic and antibacterial properties of alkaloids. Glycosides are known to lower the blood pressure according to many reports [31].

Antimicrobial activity

Results of the antimicrobial activity of some selected medicinal plants collected from Bhimkund and its adjoining regions of Mayurbhanj district *viz*. *Cissampelos pareira* L., *Nyctanthes arbor-tristis* L., *Terminalia chebula* Retz., *Curcuma angustifolia* Roxb., *Rauvolfia serpentina* Benth. ex Kurz shows that these plants have inhibitory effects on the growth of the bacterium *Lacto bacillus* (Table-3, Fig-3 and 4). It gives an idea that medicines prepared from various parts of these plants will be much effective for the treatment of various diseases caused by the infection of various microorganisms. Further study on the antimicrobial activity of these plants will provide more relevant information on the medicinal uses of these plants.

In vitro conservation

The result of the *in vitro* conservation carried out through the techniques of tissue culture of the medicinal plant *Curcuma angustifolia* Roxb. shows better results for the conservation of these plants (Fig-5). It also indicates that such method can be used for the conservation of rare and endangered medicinal plants from this region. The techniques of tissue culture can also be used for the mass propagation of some selected medicinal plants which have much demand in herbal drug industry.

Table 1: Ethnobotanical uses of common medicinal plants of Bhimkund and adjoining regions in Mayurbhanj district, Odisha

S. No.	Botanical Name	Family	Local name	Uses
1	*Cissampelos pareira* L.	Menispermaceae	Akandabindu	It is used in the treatment of chronic non healing ulcers and sinuses. It is also used in the treatment of chronic skin diseases and poisonous bites.
2	*Nyctanthes arbor-*	Oleaceae	Gangaseuli	It is used medicinally to provoke menstruation. An extract is given to children for the

	tristis L.			expulsion of roundworms. The leaf is mostly used for the treatment of malaria fever.
3	*Terminalia chebula* Retz.	Combretaceae	Harida	It helps in prevention of hair loss, constipation, removes acne, prevents cough and cold, diabetes and fights against skin allergies.
4	*Curcuma angustifolia* Roxb.	Zingiberaceae	Palua	It is used to soothe coughs and to treat bronchitis. Essential oils extracted from this plant are used in antifungal medications.
5	*Rauvolfia serpentina* (L.) Benth. ex Kurz	Apocynaceae	Patala garuda	The root is widely used in the preparation of Ayurvedic medicines for the treatment of snake bites.

Table 2: Phytochemical analysis of common medicinal plants of Bhimkund and its adjoining regions in Mayurbhanj district, Odisha

S. No.	Botanical Name Family	Local Name	Plant Parts	Fraction	Alkaloids	Flavonoids	Glycosides	Phenols	Tannins	Saponins
1	*Cissampelos pareira* L. (Menispermaceae)	Akandabindu	Root	Ethanol	+	+	+	-	+	+
				N- Hexane	+	-	-	+	+	+
				Dist. Water	+	-	-	+	+	-
2	*Nyctanthes arbor-tristis* L. (Oleaceae)	Gangaseuli	Leaf	Ethanol	+	+	+	+	+	-
				N- Hexane	-	-	+	+	+	-
				Dist. Water	-	-	-	-	+	-
3	*Terminalia chebula* Retz. (Combretaceae)	Harida	Fruit	Ethanol	+	+	+	-	+	+
				N- Hexane	-	-	-	+	-	-
				Dist. Water	+	-	-	-	+	+
4	*Curcuma angustifolia* Roxb. (Zingiberaceae)	Palua	Stem	Ethanol	+	+	+	+	+	-
				sN- Hexane	+	-	-	+	+	-
				Dist. Water	-	+	-	-	+	+
5	*Rauvolfia serpentina* (L.) Benth. ex Kurz (Apocynaceae)	Patala garuda	Root	Ethanol	+	+	+	+	+	+
				N- Hexane	+	-	-	+	-	-
				Dist. Water	+	+	-	+	+	-

N.B: +: presence of a particular phytochemical -: absence of a particular phytochemical

Table-3: Antimicrobial activities of some common medicinal plants of Bhimkund and its adjoining regions in Mayurbhanj district, Odisha

Sr. No.	Botanical Name Family	Local Name	Plant Parts	Zone of inhibition (C.M.)			Mean (C.M.)	-ve/ Solventcontrol (C.M.)	Zone of inhibition-ve control corrected (C.M.)	+ve control (C.M.)
1	*Cissampelos pareira* L. (Menispermacea)	Akandabindu	Root	0.7	0.5	0.6	0.6	0.4	0.2	1
2	*Nyctanthes arbor-tristis* L. (Oleaceae)	Gangaseuli	Leaf	0.8	0.6	0.7	0.7	0.5	0.2	1
3	*Terminalia chebula* Retz. (Combretaceae)	Harida	Fruit	1.6	1.6	1.4	1.53	0.6	0.93	2
4	*Curcuma angustifolia* Roxb. (Zingiberaceae)	Palua	Stem	0.8	0.7	0.7	0.73	0.6	0.13	1.8
5	*Rauvolfia serpentina* (L.) Benth. ex kurz (Apocynaceae)	Patala garuda	Root	0.6	0.6	0.8	0.66	0.5	0.16	0.8

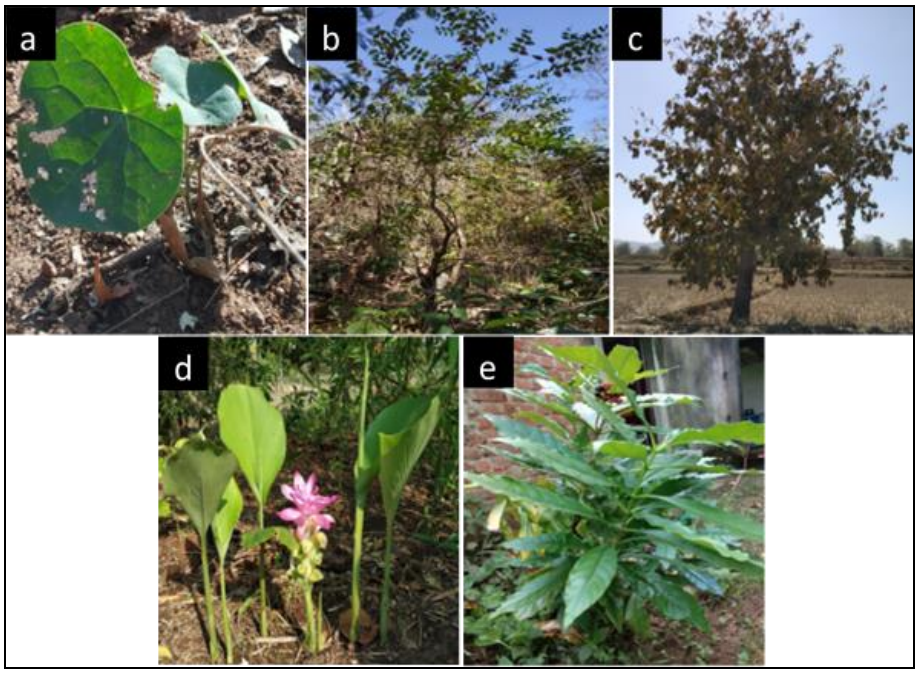

Fig 2: Medicinal plants in Bhimkund and its adjoining regions of Mayurbhanj district, Odisha: (a) *Cissampelos pareira* L. (b) *Nyctanthes arbor-tristis* L. (c) *Terminalia chebula* Retz. (d) *Curcuma angustifolia* Roxb. (e) *Rauvolfia serpentine* (L.) Benth. ex Kurz

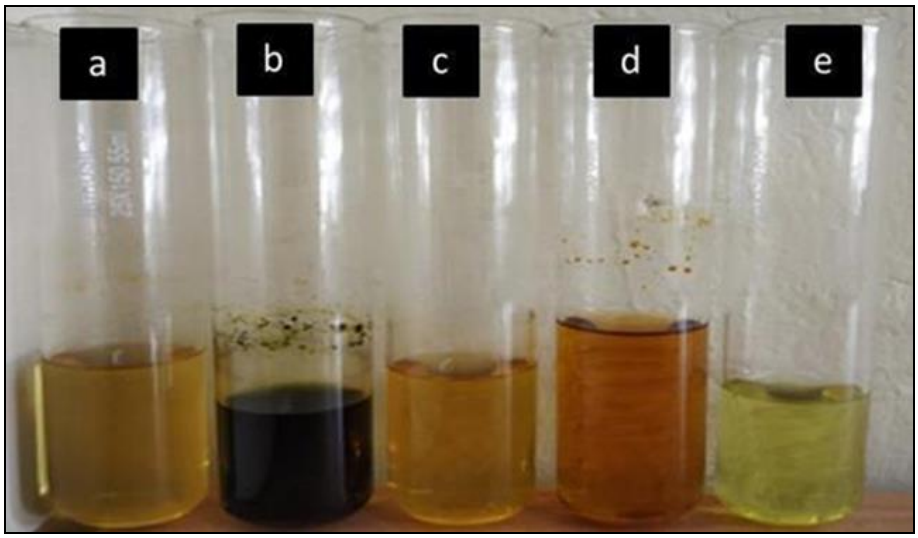

Fig-3: Preparation of Ethanol Plant Extract for Antimicrobial activity (a) *Cissampelos pareira* L. (b) *Nyctanthes arbor-tristis* L. (c) *Terminalia chebula* Retz. (d) *Curcuma angustifolia* Roxb. (e) *Rauvolfia serpentine* (L.) Benth. Ex Kurz

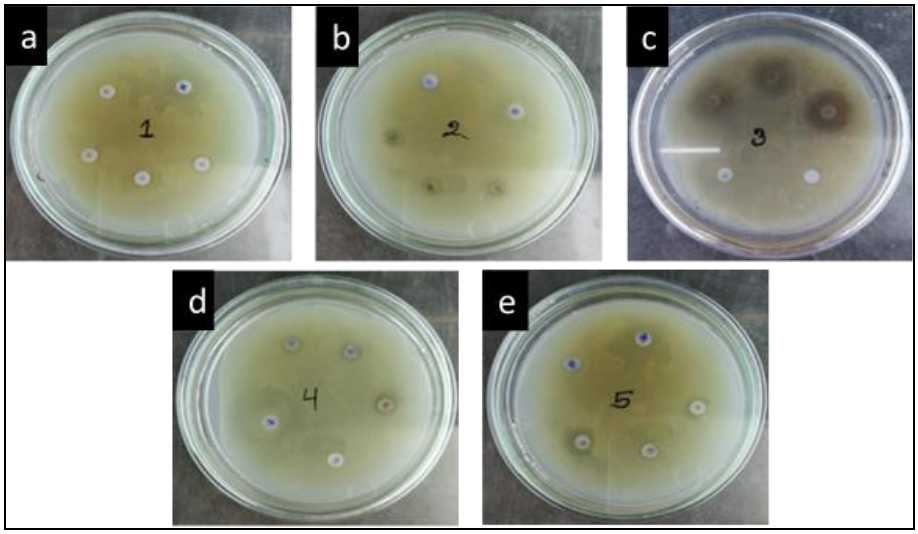

Fig-4: Zone of inhibition of 3 replicates (Plant extract), +ve control (Dettol), -ve/solvent control (Ethanol): (a) *Cissampelos pareira* L. (b) *Nyctanthes arbor-tristis* L. (c) *Terminalia chebula* Retz. (d) *Curcuma angustifolia* Roxb. (e) *Rauvolfia serpentina* (L.) Benth. ex Kurz

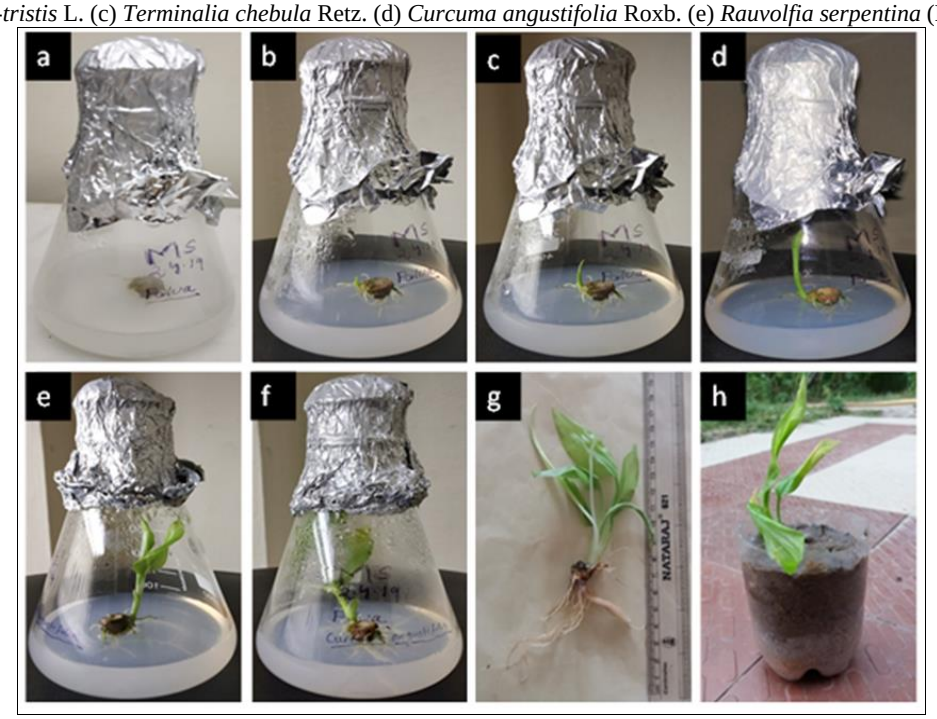

Fig-5: *In-vitro* regeneration of *Curcuma angustifolia* Roxb.: (a) Day 4(Formation of callus) (b) Day 10 (Initiation of root) (c) Day 15 (Initiation of shoot) (d) Day 25 (Development of plantlet) (e) Day 35 (Development of plant) (f) Day 45 (Development of plant) (g) Day 60 (Taken out for

Hardening) (h) Day 70 (Hardening continues)

Conclusion

During the present investigation an in depth study has been carried out on the ethnomedicinal study and antimicrobial activity of some common medicinal plants of Bhimkund and its adjoining regions of Mayurbhanj District in Odisha. From this investigation it is found that the indigenous and traditional knowledge of the people in this area on medicinal plants is very rich. People of different ethnic groups of these areas have been utilizing various plants and plant products for the treatment of various diseases.

During the analysis of phytochemicals it has been observed that there is presence of various chemicals such as alkaloids, flavonoids, glycosides, phenols, tannins and saponins in various parts of plants which have much medicinal value. Quantitative estimation of these Phytochemicals will be much useful for the preparation of new drugs in the herbal drug industry. Quantitative estimation of the phytochemicals will also be useful to assess the action of the drugs obtained from the particular parts of the medicinal plants against a specific disease. The ethanol extraction of *Rauvolfia serpentine* (L.) Benth. ex Kurz root showed the presence of all experimental phytochemical active compounds such as alkaloids, flavonoids, glycosides, phenols, tannins and saponins.

Results of the antimicrobial activity of some selected medicinal plants collected from the study area shows that the plants have inhibitory effects on the growth of the bacterium *Lactobacillus*. It gives an idea that medicines can be prepared from various parts of these plants which will be much effective for the treatment of various diseases causes by the infection of various microorganisms. Further study on the antimicrobial activity of these plants will provide more relevant information on the medicinal uses of these plants.

The result of the *in vitro* conservation carried out through the techniques of tissue culture of in medicinal plant *Curcuma angustifolia* Roxb. Shows promising results for the conservation of these plants. It also indicates that such method can be used for the conservation of rare, endangered medicinal plants and plants of other socioeconomic importance of this region. The techniques of tissue culture can also be used for the mass propagation of some selected medicinal plants to fulfill the demand in the herbal drug industry.

Acknowledgement

Authors of this paper express their gratitude for the support provided by the Centre of Excellence for Bio-resource Management and Energy Conservation Material Development established under the Odisha Higher Education Programme for Excellence and Equity (OHEPEE) for providing necessary research support.

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