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Ethnobotanical survey of antimicrobial flora of Manipur: A biodiversity hotspot region of North East India

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ABSTRACT

The present paper reports the Ethnobotanical survey carried out in 5 villages (Khangshim Village, Minou Village, Nungourok Village, Molnoi Village and Khoibu Khullen) of Manipur (North East India), stressing on medicinal plants used for ailments related to microbial infections. Interviews of the traditional healers, elders and informants were conducted for collecting and documenting medicinally important antimicrobial plants. The authenticities of the usages of the acclaimed medicinal plants were repeatedly verified by contacting various local healers and elders. Plants used for their antimicrobial properties were collected with due permission from the tribal leaders through the guidance of elders and informants. Herbariums of the important plant samples were also prepared for future verifications. The collected plants were identified by using the books Flora of Manipur, and Herbal Medicines of Manipur. In total 5 healers, 4 youth leaders, 6 village elders, 4 church pastor, 6 tribal leaders, 2 NGO workers and 3 informants were interviewed. Most of the traditional healers (4/5) were women, only one (1/5) was male. The healers were of the age group of 55-70 years. A total of 26 plants which were used for ailments related to microbial diseases were documented and collected. Our study concluded that the Uipo (Khoibu) speaking tribe residing in Khangshim Village and Khoibu Khullen was found to have more knowledge and dependency on the use of plants as therapeutic agents.

Key Words: Ethnobotany, Manipur, antimicrobial, anticancer, medicinal plants, tribal.

INTRODUCTION

Antibiotic resistance in bacteria has become one of the most dreadful alarming threats in public health management. In low income countries and people living in interior remote areas, affordability of allopathic drugs and reduced accessibility to modern healthcare managements can restrict the use of modern antibiotics, resulting in growing concerns for increased morbidity and mortality

from various infectious diseases. Reports about the six WHO regions namely African region, region of the Americas, South-East Asia region, European region, Eastern Mediterranean region and Western Pacific region, have shown that in five out of six (5/6) WHO regions, there is more than 50% increase in resistance to third-generation cephalosporins and fluoroquinolones among *Escherichia coli*, 6/6 and 2/6 regions reported greater than 50% increase in resistance to third-generation cephalosporins and carbapenems respectively, in *Klebsiella pneumoniae*, while 5/6 regions reported more than 50% increase in resistance to methicillin in *Staphylococcus aureus*. All six WHO regions were reported to have more than 25% increase in resistance to penicillin in *Streptococcus pneumoniae*, 3/6 regions reported more than 25% increase in resistance to fluoroquinolones and third-generation cephalosporins in non-typhoidal *Salmonella* and *Neisseria gonorrhea* respectively, and 2/6 regions reported more than 25% increase in resistance to fluoroquinolones in *Shigella spp* (WHO, 2014). Studies also reported that infections caused by antimicrobial-resistant pathogens result in higher mortality than those caused by susceptible strains Falagas *et al*., 2014, DiazGranados *et al*., 2005). Developments such as these have led to much intense search for broad spectrum antibiotics that can tackle the problems of antibiotic resistance in bacteria. Thus, many researchers are now turning into traditional and folklore plants for investigating of alternative antimicrobial compounds. One of the best approaches for investigation of alternative drugs to counter this global health problem would be to screen for bioactive compounds present in medicinal plants reported in traditional practices, as it would be more cumbersome to analyze plants whose medicinal importance are not documented in any form. Medicinal plants are important for pharmacological research and drug development not only for using directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as leads for pharmacologically active compounds, and also as pharmacological probes (Newman and Cragg, 2007). Manipur (Fig 1), which is one of the states of North-East India is a treasure house of vast variety of plant resources being a part of the Indo-Burma Biodiversity Hotspot. This State is located between 23.83^0 N and 25.68^0 N latitudes, and 93.03^0 E and 94.78^0 E longitudes. It covers a geographical area of 22,327 sq. km, of which 17,233 Sq. Km., which is 77.20 % of the total geographical area of the State being covered with forest (Chatterjee *et al*., 2006), and is home to 4000 angiospermic plants. The region is affluent in medicinal plants and many other rare and endangered flora and fauna. High endemism in higher plants, vertebrates and avian diversity in this region has qualified it to be a biodiversity 'hotspot' region (Myers *et al*., 2005). It is believed that the Indo-Myanmar region of India was used as a crucial corridor for human migrations including, perhaps, the first migrations from Africa towards East Asia and Australia more than 40,000 years ago (Richard *et al*., 2004). Manipur has 33 recognized Scheduled Tribes (ST), and many unrecognized tribes (India state of forest report, 2015). The use of medicinal plants plays a very vital role in the health care of tribal peoples. These tribes use various medicinal plants to cure varieties of ailments, but they keep no records of them, and the knowledge is mostly passed down orally from generations to generations (Talukdar, 2009). As many have reported about the use of vast numbers of wild plants for foods and therapeutic purposes, and due to the availability of diverse forms of plants in the Indo-Myanmar hotspot region of Manipur, India, the present ethnobotanical survey was conducted to document the medicinally important floras used by the tribals residing in this region in relation to their applications as antimicrobial agents (Singh *et al*., 2015, Mikawlrawng *et al*., 2014, Devi *et al*., 2014, Athokpam *et al*., 2014, Singh, 2011: Sharma *et al*., 2015, Das and Tongbram, 2014: Singh *et al*., 2014, Ningombam *et al*., 2014, Lokho, 2012: Singson *et al*., 2015, Devi *et al*., 2014, Yuhlung and Bhattacharyya 2014: Pfoze *et al*., 2011, Mao & Hynniewta 2011).

MATERIAL AND METHODS

Ethnobotanical survey was carried out to various places of Chandel District, Manipur inhabited by tribal communities. Prior consents and clearances were taken from the headman of the village/ church leaders/youth leaders/and tribal leaders for the visit, and also the purpose of the visit was discussed with them. Upon receiving clearance, five villages (Khangshim Village, Minou Village, Nungourok Village, Molnoi Village and Khoibu Khullen) were selected for ethnobotanical survey. Interviews were conducted with the healers, elders and informants in Manipuri/tribal languages in informal setting along with a semi-structured questionnaire (Fig 2). Each interview sessions lasted for about 1–2 hrs. The interviews were also video recorded for future translations. In total 5 healers, 4 youth leaders, 6 village elders, 4 church pastor, 6 tribal leaders, 2 NGO workers and 3 informants were interviewed (Fig 3).

At the end of each interview sessions, the healers and informants lead the interviewing team to various locations for collection of medicinally important plants (Fig 4). With the permission of the healers and village elders, approximately 500 g of each plant samples were collected for future assays. Global Positioning System (GPS) reading of the plants were recorded for future plant collection purposes.

The plants collected were dried under the shade, and also three herbarium specimens were made for each of the plant samples for future authentication and identification with the local floras (Fig 5).

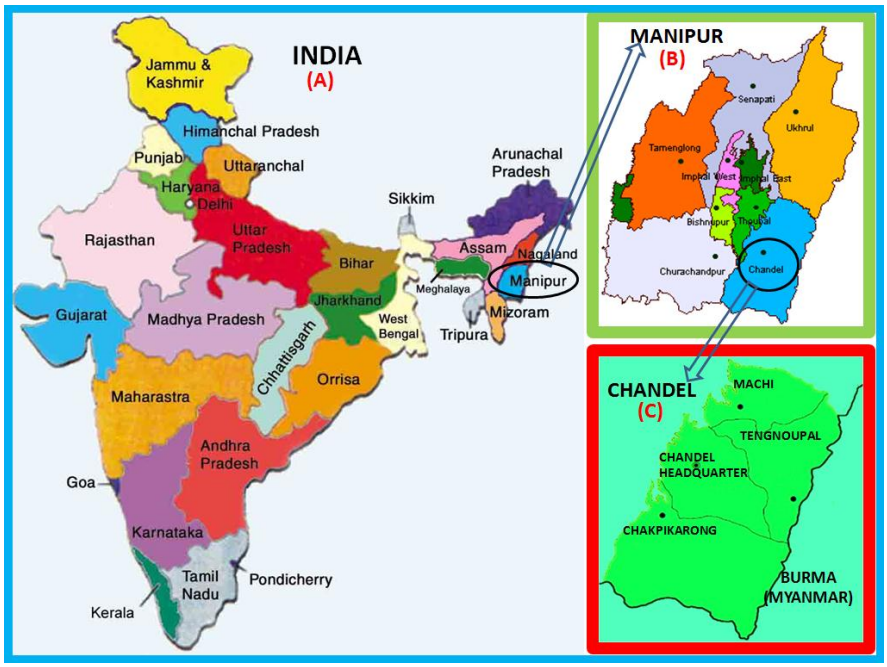

Fig 1. (A) Map of India, Manipur is encircled. (B) Enlarged Map of Manipur, Chandel district is encircled. (C) Map of Chandel district.

Questionnaire for Ethnobotanical Field Study (Innovation Project RC-308, 2015-2016)

Information provided by informants will be used for research purposes only

DETAILS OF THE INFORMANT **Date:-**_____

Name of the Informant……Tribe's/ Community's Name……

Gender (Male/ Female).Age……Occupation……

Education……Location/Residence……

DATA ABOUT THE MEDICINAL PLANT

Local or common name of the plant……

Habit (Tree/ Herb/ Shrub/ Climber/ Runner/ Rhizome/ Tuber/ Bulb/ Corm)

Plant part(s) used……

Cultivated/ Wild……

If cultivated, cultivated for……

If wild, availability in natural resources (easy/ difficulty/ very difficult)

Conservation needs (yes/no)……

Conservation efforts made by Government and local residents……

Method of collection and storage……

Name of disease(s) treated……

Method(s) of crude drug preparation……

Mode(s) of administration……

Dosage……

Other uses (if any)……

Any complications ……

Remarks:

Plant identified as (Botanical name)…. ……Family……

Signature of Researche

Fig. 2. Copy of semi-structured questionnaire used in ethnobotanical survey.

Fig. 3. Photographs show interviews with healers (A,B) and elders (C). A tribal healer (D) giving herbal preparation to one of his patients.

Fig 4. Research team collecting approximately 500 g of medicinal plants with the help of elders and informants, encircled here in red.

RESULTS AND DISCUSSION

At the end of the ethnobotanical survey 26 plants which have various applications in treating microbial diseases were collected and documented (Table 1). Plants belonging to 22 angiosperm families were collected, in which those belonging to Rutaceae, Fabaceae and Acanthaceae have more in number with respect to their use as antimicrobial sources (Figure 7), whereas plants belonging to Poaceae, Phyllanthaceae, Xanthoruhoeceae, Lamiaceae, Crassulaceae, Cannabaceae, Caryophyllaceae, Zingiberaceae, Solanaceae, Saururaceae, Moraceae, Arecaceae, Punicaceae, Elaeocarpaceae, Bignoniaceae, Euphorbiaceae, Asteraceae, Anacardiaceae and Polygoniaceae represented less in number for antimicrobial use among the tribals. In most of the cases, the leaves are used for their antimicrobial properties, followed by fruits, inflorescences, roots, barks and whole plants (Table 2). Among the 5 villages studied (Khangshim Village, Minou Village, Nungourok Village, Molnoi Village and Khoibu Khullen), people residing in Khangshim Village and Khoibu Khullen were found to have more knowledge on the use of plants as therapeutic agents. In all the villages that were surveyed, the elders at the age group of 55-70 years old were found to have more expanded information on the use medicinal plants. Interactions with the youth leaders, village elders and NGO workers alluded that there is increasing erosion of traditional knowledge among the tribals. The younger generations are less enthusiastic in knowing the traditional use of plants for treating diseases, which could be due to very meager incomes associated with this practice, and therefore a less promising source of livelihood. It was also brought up by elders that this could also be due to lesser interactions between younger generations and the elders. If this trend is continued without any intervention, it is highly probable that in the coming decades, most of the knowledge of folklore medicines will be slowly blurred and finally lost from the tribals. In addition to this problem, the elders and traditional healers reported that due to urbanization and anthropogenic interventions the numbers of medicinal plants available are greatly diminishing as compared to last approximately 15 years. The ever increasing demand of medicinal plants in the domestic market has also lead to discriminated collection and overexploitation of herbal medicines from their wild state.

Table 1: List of medicinal plants used for ailments related to antimicrobial infections, along with the GPS reading of the plants. H= Hindi, K= Khoibu, M=Manipuri, Ma=Maring.

S. No.	Botanical Name	Local Name/ Vernacular Name	Family	Part (s) Use	Uses	GPS Location
1.	*Adhatoda vasica* (Linn.) Nees.	Phalup Kangou (Kh.)	Acanthaceae	Leaves and Inflorescence	Fresh leaves are crushed and used for microbial infections of skin. The extract of boiled leaves is used for curing body and joint pains, cough and cold.	N24^{0}29’46.25” E94^{0}00’46.68” (726m above)
2.	*Aloe vera* (Linn.) Burm.f.	Ghrita Kumarai (H)	Xanthoruhoeceae	Leaves	Used in treating antibacterial, stomach ulcer and burns.	N24^{0}29’47.6” E94^{0}00’52.0” (746m above)
3.	*Artocarpus lakoocha* Roxb.	Kataathei (Kh.)	Moraceae	Fruits	Used for treating constipation, fever and skin diseases.	N24^{0}29’41.78” E94^{0}0’46.24” (749m above)
4.	*Bauhinia purpurea* Linn.	Chingthrao (M)	Fabaceae	Leaves and Bark	The concoction of both leaves and barks are used in Dysentery, Skin diseases, Small Pox, Sores, cancerous growth in stomach and diarrhea.	N24^{0}29’48.24” E94^{0}00’40.91” (740m above)
5.	*Bryophyllum pinnatum* (Lam.) Kurz.	Manahidak (M)	Crassulaceae	Leaves	Fresh leaf juice is taken orally against dysentery; leaf paste is applied externally in cuts and wounds and on forehead to reduce headache.	N24^{0}29’48.34” E94^{0}00’44.91” (740m above)

6.	*Calamus floribundus* Griff.	Rui (Kh.)	Arecaceae	Young shoot	Cooked with milk to cure Tuberculosis.	N24^{0}30'29.63" E94^{0}2'20.04" (992m above)
7.	*Cannabis sativa.* Linn.	Kanja (Kh.)	Cannabaceae	Leaves and inflorescence	Eaten raw to cure dysentery and diarrhea, and to treat diabetes.	N24^{0}29'48.25" E94^{0}00'45.85" (726m above)
8.	*Citrus maxima* (Burm.) Merr.	Nobab (Kh.)	Rutaceae	Fruits and Leaves	The leaves are used in Epilepsy and Cholera, cancer and liver problems. Oil from fresh leaves is used to treat skin diseases and fungal diseases.	N24^{0}30'34.45" E94^{0}0'10.24" (734m above)
9.	*Curcuma caesia* Roxb.	Ai Khamang (Kh.)	Zingiberaceae	Rhizome	The juice extract of raw rhizome is used to treat dysentery, cough, tumors, diarrhea, asthma, epilepsy, toothache, skin problems, tonsillitis and piles.	N24^{0}29'47.32" E94^{0}0'48.45" (745m above)
10.	*Cymbopogon citrates* (DC.) Stapf.	Haona (K)	Poaceae	Leaves	Decoction of leaves use as antifungal agent and antidysenteric.	N24^{0}29'46.15" E94^{0}00'50.11" (746m above)
11.	*Drymaria cordata* (Linn.) Willd.	Tandan-pambi (M)	Caryophyllaceae	Whole plant	Used as analgesic, antipyretic, anti-inflammatory and as antibacterial.	N24^{0}29'48.34" E94^{0}00'44.92" (740m above)
12.	*Elaeocarpus serratus* Linn.	Chorphon (M)	Elaeocarpaceae	Fruit and Leaves	The fruits are used as antidiabetic, and in dysentery and diarrhea The leaves are used in rheumatism and as an antidote for poison.	N24^{0}30'22.63" E94^{0}2'21.73" (973m above)
13.	*Elsholtzia blanda* (Benth.) Benth.	Anrei-in (Kh.)	Lamiaceae	Whole plant except roots	The extract of leaves boiled in water is used for throat pain.	N24^{0}29'46.15" E94^{0}00'50.11" (746m above)
14.	*Euphorbia hirta* Linn.	Pharunlou (Kh.)	Euphorbiaceae	Whole plant	Diarrhea, dysentery, colic Pain, and bleeding piles.	N24^{0}30'34.65" E94^{0}0'20.21" (734m above)
15.	*Houttuynia cordata* Thunb.	Keirim (Kh.)	Saururaceae	Whole plant	The whole plant has antibacterial, antiviral and diuretic property. The decoction is used internally in the treatment of many ailments including cancer, coughs and dysentery.	N24^{0}29'47.32" E94^{0}0'48.45" (745m above)
16.	*Murraya koenigii* (Linn.) Spreng.	Karipatta (H)	Rutaceae	Leaves and Roots	Green leaves are eaten raw for cure of dysentery, diarrhea and for checking vomiting. Leaves and roots are also used traditionally as anthelmintic, analgesic, curing piles, inflammation, itching and as antidiabetic.	N24^{0}29'47.32" E94^{0}00'48.45" (745m above)
17.	*Oroxylum*	Shamba	Bignoniaceae	Fruit and	The bark is used to treat	N24^{0}30'29.6

	indicum (Linn.) Vent.	(M)		Bark	intestinal worms, vomiting, dysentery, diarrhea and cancer. The bark of the root is used as antiulcer.	3" E94^{0}2'20.04" (992m above)
18.	*Parkia speciosa* Hassk.	Yongchak (Kh.)	Fabaceae	Fruits	Used as antidiabetic and antibacterial food. Seeds are also known to have anticancer property.	N24^{0}29'48.19" E94^{0}00'43.97" (740m above)
19.	*Persicaria odorata* (Lour.) Soják	PhakPhai (Kh.)	Polygonaceae	Leaves	The extracts of leaves are used in loose motion and indigestion.	N24^{0}30'12.11" E94^{0}0'34.13" (734m above)
20.	*Phlogacanthus thyrsiformis* (Roxb. ex Hadrw.) Mabb	Phalup Kasan (Kh.)	Acanthaceae	Leaves	Fresh leaves are crushed and used for microbial infections of skin. The extract of boiled leaves is used for curing body and joint pains.	N24^{0}30'34.45" E94^{0}0'10.24" (734m above)
21.	*Phyllanthus emblica* Linn.	Pugluhei (Kh.)	Phyllanthaceae	Fruit	The decoction of the fruit is used in cough and cold.	N24^{0}29'48.8", E94^{0}1'7.42" (767m above)
22.	*Punica granatum* Linn.	Pohei (Ma.)	Punicaceae	Leaves and Bark	Used for treating dysentery and diarrhea.	N24^{0}30'29.63" E94^{0}2'20.04" (992m above)
23.	*Rhus chinensis* Mill.	Khongmahei (Kh.)	Anacardiaceae	Fruits and Young leaves	The young leaves are eaten directly or boiled in water to stop dysentery. Leaves also has antiviral, antibacterial, anticancer, hepatoprotective, and antioxidant activities.	N24^{0}30'25.34" E94^{0}1'67.23" (754m above)
24.	*Solanum indicum* Linn.	Saamtrogkha (Kh.)	Solanaceae	Fruits	The fruits are boiled with water and used for treating cough and sore throat.	N24^{0}29'47.85" E94^{0}0'45.26" (745m above)
25.	*Tamarindus indica* Linn.	Mangae (Kh.)	Fabaceae	Leaves and Fruits, and seeds	Used in treating microbial fungal diseases.	N24^{0}30'34.45" E94^{0}0'10.24" (734m above)
26.	*Xanthium strumarium* Linn.	Samprakpi (M)	Asteraceae	Leaves and Root	The leaves are used in fever and cough. The root decoction is used in cancer, urinary stone and purple pains.	N24^{0}26'34.32" E94^{0}1'42.16" (777m above)

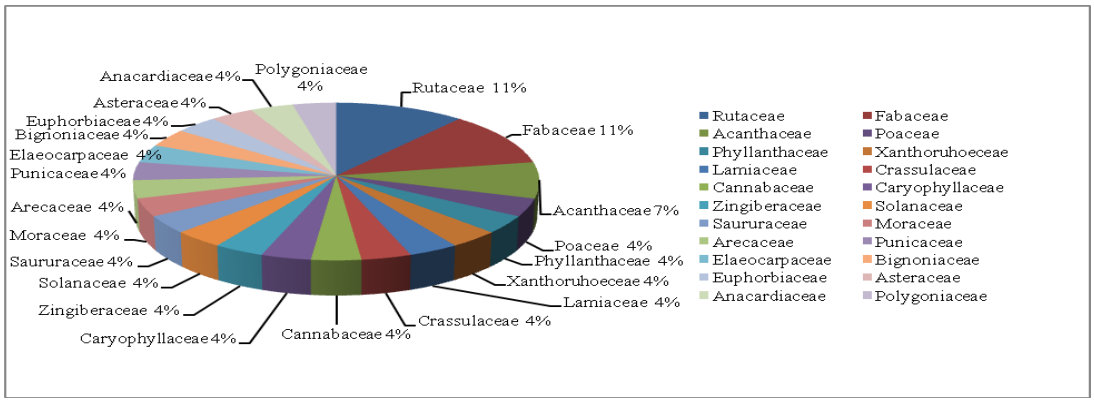

Figure 6: Distribution of antimicrobial plants in different families of plants. The family Rutaceae, Fabaceae and Acanthaceae showed more representation of plants that has antimicrobial properties.

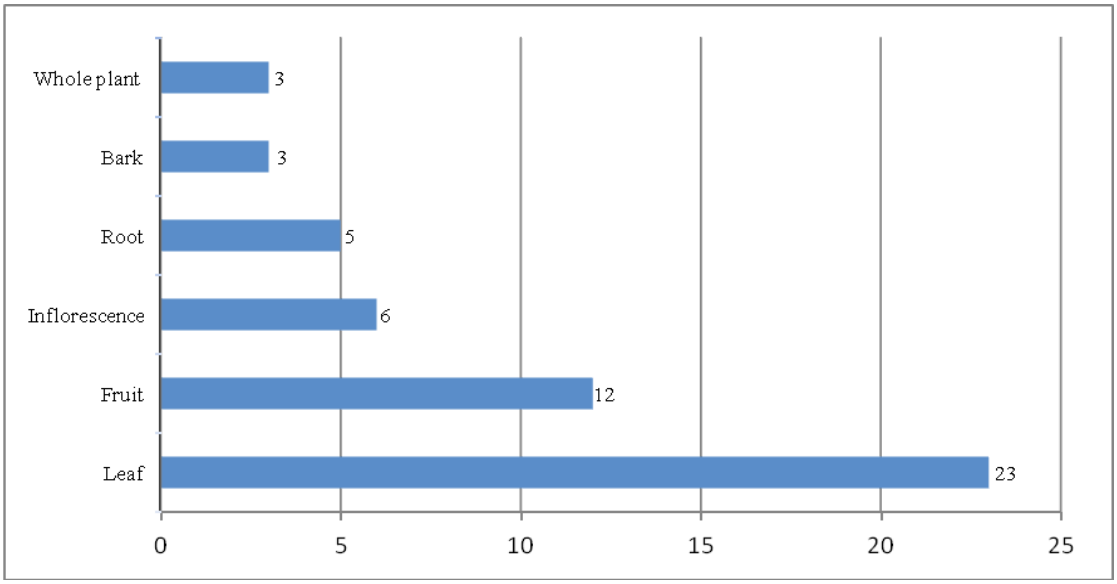

Table 2: Data showing plant parts used for antimicrobial properties. In most plants leaves were used for treating microbial infections.

CONCLUSION

Ethnobotany is one of the in important sources of drug discovery. The present ethnobotanical surveys provide a sense of pride among the tribals to treasure and safeguard their traditional medicinal knowledge for future applications. Interactions between researchers and locals also brought about understanding among the tribals the need to value and document their traditional knowledge before this practice of orally handing down their traditional knowledge is lost. Documentation and preservation of the traditional knowledge are the great challenges of the hour, so that the vast knowledge of the ethnic communities residing in this biodiversity hotspot region of the country can still be preserved for future research in pharmaceuticals and drug discovery, along with a proper provision of benefit sharing with the ethnic communities who have played a tremendous role in contributing towards drug discovery. Traditional knowledge has the potential of being transformed into commercial opportunity, providing useful leads for development of products and processes. Hence, a share of benefits must be accrued to the creators and holders of this knowledge. Field surveys such as this are important double-edged sword, as this type of interactions helps in mutual sharing of knowledge, wherein the knowledge shared by the locals are complemented by sharing of various awareness tips for conservation and sustainable development from the researchers who interact with the tribals. With about 70% of Indian population relying on traditional medicine for various primary health care practices, the present scenario necessitates the need to establish evidence-based do's and don'ts while using them so as to avoid adverse effects associated with unregulated use of medicinal plants. With a long history of providing therapeutic compounds since ages, it is believed that with constant efforts on ethnobotanical surveys and analysis of the bioactive compounds of the plants used by various tribes, researchers would in the near future find a

means for encountering current problems of antimicrobial resistance.

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