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Informant consensus factor and antimicrobial activity of ethno medicines used by the tribes of wayanad district kerala

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The ethno botanical investigation of medicinal plants used by the Kurichia, Kuruma, Kattunaika, Adiya and Paniya tribes of Wayanad district, Kerala were recorded. One thousand (1000) ethno medicines derived from 500 plants used by the tribal medical practitioners were documented. Of this, 10 species were frequently used for treating various infectious diseases. An informant consensus factor was calculated for 10 species which are being frequently used. The leaf extracts of these plants were screened for antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus* and *Aspergillus niger.* Among the plants screened *Garcinia gummi gutta* (Clusiaceae) was found to possess highly significant antibacterial activity and significant antifungal activity was observed in *Nothapodytes nimmoniana* (*Icacinaceae*) which possess highest antifungal activity. These two species have shown the highest informant consensus factor values.

Key words: Medicinal plants, informant consensus factor, antibacterial activity, antifungal activity, ethno botany.

INTRODUCTION

Antimicrobials provide the main basis for the therapy of microbial (bacterial and fungal) infections. However, over use of antibiotics has become the major factor for the emergence and dissemination of multidrug resistant strains of several groups of micro organisms. The worldwide emergence of multi drug resistant *Escherichia coli* and many other β- lactamase producers has become a major therapeutic problem (Khan et al., 2004). For this reason, researchers are turning their attentions to herbal products, looking for new leads to develop better drugs against multidrug resistant microbe strains (Kafaru, 1994).

There are over 275,000 species of flowering plants in the world today (Anonymous, 2000). Various plant parts have been used by man for the treatment of diseases, particularly those caused by micro-organisms. Although hundreds of plants have been tested for antimicrobial properties, the vast majority have not been adequately evaluated especially the tribal medicines. There is a need to screen plants used by the tribal people and document them through scientific validation. Wayanad has a great wealth of medicinal plants and tribal people. The tribes have an ancestral tradition about the use of medicinal plants and there are reviews on different types of medicinal plants used by the Wayanad tribes (Nisha and Sivadasan, 2007; Mini and Sivadasan, 2007; Raji and Raveendran, 2011). The modern civilization has put them on the verge of extinction. To rescue this knowledge, it is necessary to explore the scientific information about these medicinal plants in Wayanad district. In this regard,

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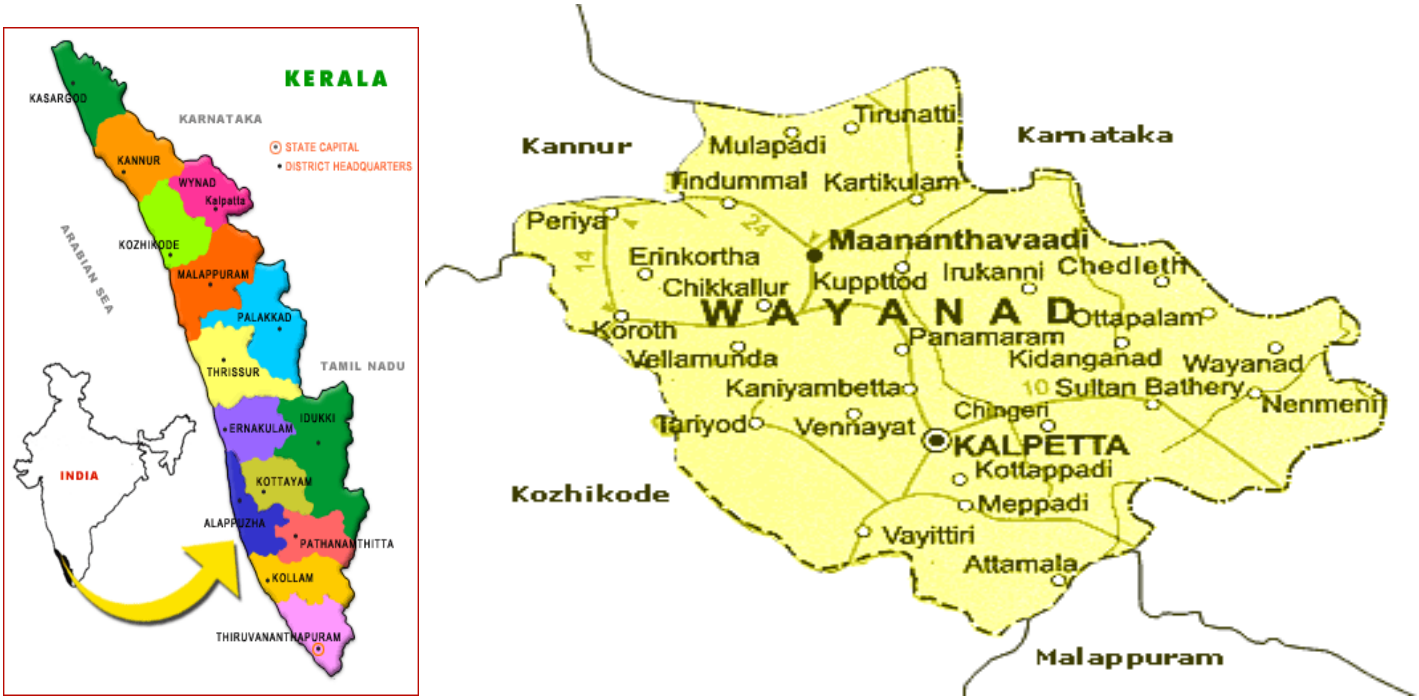

Figure 1. Wayanad district map.

there is ethno directed method of exploitation of these plants. This technique helps in the collection of plants based on the knowledge. According to Cox and Balick (1994) and Cordell (2000), this method plays a fundamental role in biodiversity prospecting. Since time does not allow us to evaluate all existing medicinal plants scientifically selection of the most important taxa is a prerequisite to begin ethno pharmacological, phytochemical and toxicological studies. For this purpose it is necessary to determine the species that are most used to treat a particular illness. A useful tool to find these species is the informant consensus factor (Frei et al., 1998; Heinrich et al., 1998a).

In view of this, the present investigation was undertaken to document the medicinal plants used by the tribes of Wayanad to treat infectious diseases, and also to evaluate the antimicrobial activity considering informant consensus factor.

Study area

The district of Wayanad is situated in the eastern part of Kerala and lies between 11° 27’ and 11° 58’35"N and 75° 47’50" and 76° 26’ 35"E (Figure 1).

Ethnic groups

The district of Wayanad has the largest population of the tribes in Kerala. Wayanad district accounts for one third of the total tribal population in Kerala, which is equivalent to 17.43% of the district population. The total tribal population in Wayanad as per Census 2001 is 136,062. The main tribal groups in Wayanad include Paniya 45%, Kurichiya 17%, Mullu Kuruma 17%, Kattunaikya 10% and other splinter tribal groups 1%.

The name 'paniyan' means 'worker' as they were supposed to have been the workers of non-tribes. They are dark skinned, short stature, long headed with long wavy curly hair and broad nose. The dialect of the paniyan people is paniya language. People of paniya still depend upon the local flora for healing their illness (Raji and Raveendran, 2011). Adiya is one of the slave sects in Kerala. The adiyas speak a dialect of Kannada. Their traditional occupation is agricultural labour. There are mainly distributed in Mananthavady and Vythiri taluks of this district. As their name denote, the kattunayakan were the kings of the jungle regions engaged in the collection and gathering of forest produces. They have all the physical features of a hill tribe. They speak kattunaikyan dialect, which is a mixture of all Dravidian languages, drawing more from Kannada. The kurumas are also known as mullu kuruma. They are mostly found in the Wayanad region. There are three sub-divisions in the kuruma tribal sect: uralIkuruma, 'Mullu Kuruma' and 'Jenu Kuruma' (honey gathering kurumas) tribes. Amongst the kuruma tribes in Wayanad, the Mullu Kurumas consider themselves superior to the other Kuruma sects. The

Kurichias are an agricultural tribal community. They speak Malayalam and use Malayalam script. The Kurichians are rich in their oral tradition.

MATERIALS AND METHODS

Plant collection and ethno botanical interviews

The ethno botanical survey was conducted in the tribal localities of three taluks Mananthavady, Bathery and Vythiri of Wayanad district, Kerala. Field explorations were undertaken during 2006-2010 among the tribal colonies of Mananthavdy, Bathery and Vythiri taluk to identify ethnic groups and the plants used by them as medicines. The confirmation of identification has made in consultation with taxonomic experts of MS Swaminathan Research Foundation, Wayanad and by referring to literature.

A questionnaire was prepared to collect information related to informant consensus factor, method of application, therapeutic use, parts of plants used, name of diseases, symptoms of diseases and causes of diseases, etc.

Informant consensus factor

To estimate use variability of medicinal plants and to determine which plants are particularly interesting in the search for bioactive compounds, the informant consensus factor (F_{ic}) (Heinrich et al., 1998 a) was calculated. This factor estimates the relationship between the number of use reports in each category (n_{ur}) minus the number of taxa used (n_t) and the number of use reports in each category minus 1. F_{ic} is thus calculated using the following formula:

$$F_{ic} = \frac{n_{ur} - n_t}{n_{ur} - 1}$$

The product of this factor ranges from 0-1. A high value close to one indicates the relatively few species used by large proportion of people. While a low value indicates that the informants disagree on the taxa to be used in the treatment within a category of illness (Canales et al., 2005).

Bioassays

The bacterial samples of *Esherichia coli* isolated from stool samples of diseased persons, *Staphylococcus aureus* isolated from infected urine samples and fungal samples of *Aspergillus niger* isolated from decaying vegetables were cultured on MacConkey's agar (Becton Dickinson and company, Microbiology systems, sparks, MD) and nutrient agar media. Fungal cultures were prepared on Sabouraud Dextrose Agar medium (SDA).

The plant extracts obtained using methanol; diethyl ether and cold sterile distilled water were primarily screened for antibacterial activity. The agar well diffusion method (Okeke et al., 2001) was used. In this method, pure isolate of each microbe was sub cultured on the recommended specific media for each microorganism at 37°C for 24 h. From each plate four colonies were touched with a sterile loop and transferred into normal saline (0.85%) under aseptic conditions. Density of each microbial suspension was adjusted equal to that of 10.6 cfu/ml standardized by 0.5 Mc Farland standard and used as an inoculums for performing agar well diffusion assay (Aneja et al., 2010). 100 µl of inoculums of test organisms was swabbed on to the media plates. The agar plates were allowed to dry and wells or cups of 6 mm were made with a sterile borer in the Inoculated plates. The methanol and diethyl ether extracts were prepared at different dilutions (10, 5 and 2.5%) in DMSO solution. The aqueous extract was diluted in water.50 µl volume of the extracts was propelled directly into the well (in triplicate) of each culture plate containing test organisms. The plate was allowed to stand for 10 min for diffusion of extract to take place and incubated at 37°C for 24 h (Aneja et al., 2009a, b; Khokra et al., 2008; Rios et al., 1980). 50 µl volumes of the extracts were propelled directly into the well (in triplicate) of each culture plate containing test organisms. The plate were allowed to stand for 10 min for diffusion of extract to take place and incubated at 37°C for 24 h (Khokra et al., 2008). Sterile DMSO (20%) serves as negative control. Antibiotic discs of chloramphenicol (C)-(100 µg/disc) and cephoperazon (CS)-(100 µg/disc) for bacteria, and miconazole for fungus serves as positive control. MIC is the lowest concentration of compounds/extract/drug that completely inhibit the growth of the microorganisms in 24 h (Thongson et al., 2004). The inhibition zone surrounding the well containing the extracts indicates the anti-microbial activity. The diameter was measured and the experiment was performed in triplicates.

RESULTS AND DISCUSSION

Ethno botanical survey and informant consensus factor

During the survey, a total of 876 answers were obtained concerning the use of 11 medicinal plants (Table 1) which were grouped into 7 categories of medicinal uses (Figure 2 and Table 2). All these categories are more or less involved in the cure of illness of microbial origin. Among them those with the highest number of mentions were psoriasis (22.4%), diarrhoea (21.34%), scabies (19.63%), dysentery (14.20%) and cancerous wound (6%).

The group of illness of possible microbial origin that obtained the highest informant consensus factor value was that of ailments like scabies, psoriasis, dysentery, diarrhoea and cancerous wound with F_{ic} value of 0.98. The species responsible for this high consensus was *Garcinia gummi gutta* (Clusiaceae) with 60 and 75 reports, *Breynia vitis Idaea* (Phyllanthaceae) with 51 and 59 reports, *Gomphostemma heyneanum* (Lamiaceae) with 52 and 50 reports, *Nothapodytes nimmoniana (Icacinaceae)* with 48, 50 and 51 reports, *Embelia tsjeriam-cottam* with 48 reports, *Chilocarpus malabaricus (*Apocyanaceae) with 43 reports, *Chonemorpha fragrans (*Apocyanaceae) with 34 and 36 report , *Hedychium coronarium* with 20 and 25 reported events, *Raphidophora pertusa (Raphidophoraceae)* with 12 and 14 reports and *Alstonia venenata* **(**Apocynaceae) with 12 reported events. The calculation of informant consensus factor allowed a more objective selection of the species for the microbiological study in order to validate traditional knowledge (Canales et al., 2005).

Table 1. The number of use reports of 11 medicinal plants in each category.

Plant species	Disease category and number of reported events by different tribal groups						
	Dysentery	Diarrhea	Psoriasis	Scabies	Cancerous wound	Snake bite	Mumps
Alstonia venenata R.Br				12		50	
Chilocarpus malabaricus Bedd.			43.				
Chonemorpha fragrans			34	36			
Garcinia gummi gutta	60	75					
Gomphostemma heyneanum	52	50					
Raphidophora pertusa	12	14					50
Pittosporum neelgherrense						46	
Nothapodytes nimmoniana			48	50	51		
Breynia vitis Idaea			51	59			
Embelia tsjeriam-cottam		48					
Madhuka longifolia			20	15			
Total	124	187	196	172	51	96	50

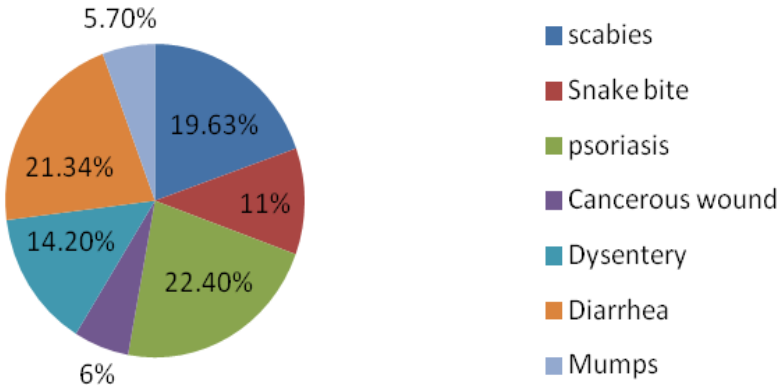

Figure 2. Seven categories of medicinal uses and their percentage.

Table 2. The seven categories of medicinal uses, total reported events and informant consensus factor.

S/N	Disease category	Total reported events	*Fic*
1	Scabies	172	0.98
2	Snake bite	96	1.
3	Psoriasis	196	0.98
4	Cancerous wound	51	1.
5	Dysentery	124	0.98
6	Diarrhea	187	0.98
7	Mumps	50	1.

Antimicrobial activity evaluation

Based on the above results, the anti bacterial and anti-fungal activities were evaluated for the 10 species. The zone of inhibition shown by these medicinal plants were tabulated and shown in Table 3. All plants have shown

Table 3. Antimicrobial activity of plant extracts.

Botanical name	*E. coli*			*Staphylococcus aureus*			*Aspergillus niger*
	Methanol extract (mm)	Diethyl ether extract (mm)	Water extract (mm)	Methanol extract (mm)	Diethyl ether extract (mm)	Water extract (mm)	Methanol extract (mm)
Alstonia venenata R.Br	8	21	-	8	15	-	-
Breynia vitis Idaea	16	-	-	10	24		-
Chilocarpus malabaricus Bedd.	-	13	-	-	16	-	-
Chonemorpha fragrans (Moon)	7	10	-	7	13	-	-
Embelia tsjeriam-cottam	14.3	9	-	7	15	-	-
Garcinia gummi gutta (L.) Robs.*var gummi-gutta*	-	-	30	-	18	-	-
Gomphostemma heyneanum Benth.*var. heyneanum*	15	13	-	9	14	-	-
Madhuka longifolia (Koenig.)J.F.Macbr	-	21	-		22	-	-
Nothapodytes nimmoniana (Graham)Mabb	15	10	-	7	15		27
Raphidophora pertusa (Roxb.)Schott.	8	11	-	6	10		-
Chloramphenicol	29			30			
Cephoperazone	8			9			
Miconazole							26

a. Inhibition zones are the mean including well diameter (6 mm); b. Antibiotic discs of chloramphenicol (C) (100 µg/disc), Cephoperazon (CS) (100 µg/disc) and Amphotericine B, Miconazole (1000 mg/ml)) are antimicrobials used as positive control. Extract quantity is 50 µl/well.

antimicrobial activity both with *E. coli* and *Staphylococcus aureus and* only *N. nimmoniana* showed antifungal activity.

The diethyl ether extracts of almost all plants focused on in the study except *Breynia vitis Idaea* showed antibacterial activity against *E. coli*. The methanolic extract of *Chilocarpus malabaricus* did not show activity against *E. coli* and *S. aureus.* The water extract of *G.gummi gutta* was found to be antibacterial against *E. coli*. The methanolic leaf extract of *N. nimmoniana* showed antifungal property against *A. niger.*

The aqueous leaf paste of *N. nimmoniana (Icacinaceae),* was used by the Kurichia, Kuruma and Kattunaika tribes of Wayanad to make herbal remedies against arthritis, cancer, skin problems like psoriasis and scabies. The results of antimicrobial activity of this species shows a MIC value of 27 mm against *A. niger*, 7 and 15 mm against *S.aureus*, 15and 10 mm against *E. coli* which give support to the plant use by these tribes. *Alstonia venenata* (MIC value 15 mm against *E. coli*) leaf paste were used by the tribes of Wayanad for the treatment of snake poison and skin diseases. The water extract of *G. gummi gutta* showed an excellent result against *E. coli* (MIC 30 mm), which is higher than the inhibition diameter of positive control, chloramphenicol (29 mm.) *G. gummi gutta* wasused by the tribes singly or in combination with other plant parts to cure various ailments. The plant *G. gummi gutta* (Clusiaceae) is a lower risk near threatened plant. The dried fruit piece is an essential ingredient in fish curries in Kerala. Its leaves were used by the Kurichia tribes of Wayanad district to prepare medicine for dysentery, diarrhea, tonsillitis, ulcer and bleeding piles. The diethyl ether extract of this plant showed an inhibition value of 18 mm against *S. aureus* which is less significant as compared to the antimicrobial results of the antibiotic chloramphenicol (29 mm).

Leaf extract of *Breynia vitis Idaea* (Phyllanthaceae) was used for curing body pain and skin problems like psoriasis and scabies. The methanolic extract of this plant showed minimum inhibition of 16 and 10 mm against *E .coli* and *S. aureus*, respectively. Diethyl ether extract of this plant showed a minimum inhibition of 24 mm against *S. aureus* . The aqueous leaf extract of *Madhuka longifolia* is mainly used by the tribes for the treating skin diseases. This extract showed inhibition with both *E. coli* (21 mm) and *S. aureus* (22 mm). The kurichia tribes use the leaves of *Gomphostemma heyneanum*, (Lamiaceae) to cure dysentery, diarrhoea and arthritis.

Table 4. Therapeutic uses of the plants selected for antimicrobial analysis.

Botanical name	Local name	Therapeutic use	Tribes associated
Alstonia venenata R.Br	Theeppala	Scabies, snake bite	Kurichia, Kuruma.
Breynia vitis Idaea	Kurukkankombu	Body pain Psoriasis, scabies,	Kurichia Kuruma Kattunaika
Chilocarpus malabaricus Bedd.	Vallippala	Skin disease	Kurichia.
Chonemorpha fragrans (Moon)	Perumkurumba.	Psoriasis, scabies, blood purification	Kurichia.
Embelia tsjeriam-cottam(Roem.&Schult.)DC	Kattuvizhal	Pneumonia, diarrhoea.	Kurichia Kuruma Kattunaika
Garcinia gummi gutta (L.) Robs.*var gummi-gutta*	Kodampuli	Tonsilites. Ulcer Bleeding piles, dysentery, diarrhoea.	Kurichia Kuruma Kattunaika Adiya Paniya
Gomphostemma heyneanum Benth.var*. heyneanum*	Theepperuku	Dysentery, diarrhoea, arthritis	Kurichia Kuruma Kattunaika
Madhuka longifolia(Koenig.)J.F.Macbr	Elippa	Psoriasis, scabies	Kuruma. Adiyan
Nothapodytes nimmoniana (Graham)Mabb	Ulukkuvetty	Cancerous wound, psoriasis, scabies, arthritis	Kurichia kurichya Kuruma Kattunaika
Pittosporum neelgherrense Wightt.	Analivenga	Snake bite	kurichya
Raphidophora pertusa(Roxb.)Schott.	Anachakkara	Dysentery, diarrhoea, mumps, tonsillitis.	Kattunaika Kurichya

Methanolic extracts of this species showed antibacterial activity against *E. coli* and *S. aureus* (Table 3). Diethyl ether extract showed activity against *E. coli* and *S. aureus* with MIC of 13 and 14 mm, respectively.

Raphidophora pertusa is a climber. Its local name is Anachakkara. Leaf juice is extensively used by the Kattunaika and Kurichia tribes for curing dysentery *and* diarrhea. In the present study, the water extract of the leaves showed no activity against *E. coli* and *S. aureus.* The diethyl ether and methanol extract showed MIC value of 10 and 6 mm against *S. aureus*, respectively. The methanolic and diethyl ether extracts against *E. coli* is less inhibitory and showed MIC values of 11 and 8 mm, respectively. The therapeutic uses of all these plants by the tribes of Wayanad district, Kerala are shown in Table 4. We isolated the tested organisms *E. coli* from stool samples of diseased persons and *S. aureus* from infected urine samples. The tribal populations of Wayanad use the plants mentioned in Table 1 to cure dysentery, diarrhea, psoriasis, wound infections and scabies. *E. coli* are bacteria that normally live in the intestines of humans and animals. Although, most strains of these bacteria are harmless, several are known to produce toxins that can cause diarrhea. The antimicrobial reports showed that the leaves of *G. gummi gutta, G. heyneanum, M. longifolia and Embelia tsjeriam-cottam* used by the tribes in Wayanad were excellent medicines for dysentery. *S. aureus* is the most common cause of staphylococcus infections. It is a spherical bacterium, frequently seen as a part of the skin flora found in the nose and on skin. About 20% of the population is long-term carriers of *S. aureus.* It can cause a wide range of illnesses like minor skin infections. The tribal people of Wayanad use *Alstonia venenata*, *Chonemorpha fragrans, Nothapodytes nimmoniana and Breynia vitis Idaea* for the treatment *of* scabies. We got good results for antimicrobial activity of these leaf extracts against these organisms. So these plant leaves can be used for the preparation of medicines against scabies like skin infections caused by *S. aureus.*

Based on the results of antimicrobial activity and ethno-medicinal values, attention has to be given for scientific bioprospection and conservation of these medicinal plants.

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