

Volume 3, Issue 2, 2476-2492.

Research Article

ISSN 2277 – 7105

ANTIMICROBIAL ACTIVITY OF TRIBAL MEDICINES COLLECTED FROM WAYANAD DISTRICT, KERALA

Devi Prasad A.G.^{1*}, Raghavendra M.P². and Shyma T.B¹

¹Department of Studies in Environmental Science, University of Mysore, Manasagangotri,

Mysore 570 006, Karnataka, India

²Postgraduate Department of Microbiology, Maharani's Science College for Women, Jhansi Lakshmi Bai Road, Mysore 570 005, Karnataka, India.

Article Received on 11 December 2013 Revised on 09 January2014, Accepted on 12 February 2014

*Correspondence for Author Dr. Devi Prasad A.G., Department of Studies in Environmental Science, University of Mysore, Manasagangotri, Mysore, Karnataka, India

ABSTRACT

The study reports the antibacterial and antifungal activity of aqueous, diethyl ether and methanol extracts of leaves of 20 medicinal plants belonging to 16 different families against *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger* and *Microsporum racemosum*. These plants were regularly used for the treatment of various ailments by the kurichia, kuruma, kattunaika, adiya and paniya tribes located in three taluks Mananthavady, Bathery and Vythiri of Wayanad district, Kerala. Shade dried leaves of all the plants were subjected to extraction using diethyl ether, methanol, and water and the extracts were used in the study. Documentation and analysis of tribal medicines, phytochemical analysis and antimicrobial activity of all the extracts by well diffusion method were done for all twenty plants. Among the

plant extracts, aqueous extract of *Garcinia gummi gutta* (L.) Robson and diethyl ether extract of *Alstonia vernata* R.Br. showed significant inhibitory activity against *E. coli*. The diethyl ether extract of *Garcinia gummi gutta*, *Breynia vitis-idaea*, *Madhuka longifolia* and *Strobilanthes alata* revealed inhibitory activity against *Staphylococcus aureus*. Methanolic extract of *Nothapodytes nimmoniana* Graham recorded significant antifungal activity against *Aspergillus niger* and *Microsporum racemosum* even compared to micanozole. The study is the first to document the ethno medicines used by five tribal communities and an attempt has been made to scientifically validate the antimicrobial activity of these tribal medicines.

Keywords: Antifungal, Antibacterial, Traditional healers, Wayanad, Ethnomedicines.

INTRODUCTION

Antibiotics are used for the therapy of microbial infections and its overuse is resulted in the emergence of multidrug resistant strains of microorganisms. The world wide emergence of multidrug resistant E. coli and many other β lactamase producers has become a major therapeutic problem.^[1, 2] Due to the rapid spread of resistant microbes, a need to find new antimicrobial agents is essential. Traditional medicine has been defined by Bhushan^[3] as diverse health practices, approaches, knowledge and beliefs incorporating plants applied singularly or in combination to maintain well-being as well as to treat diagnose or prevent illness. The district Wayanad in Kerala has the largest population of the tribes in the state which is equivalent to 17.43% to the district population. And majority of the tribes in the district have been using the medicinal plants mostly for infectious diseases and related problems.^[4, 5] Udayan^[6] have reported 48 traditional plants used by the kurichia tribes inhabiting Thirunelly forest of Wayanad district. Silia^[7] reported 136 medicinal plants used by the mullukuruma tribes of Wayanad district. The ethno medicinal knowledge from a collection of literature forced us to conduct an survey on ethno medicine practiced among the kurichia, kuruma, kattunaika, adiya and paniya tribes of Wayanad and documented 1000 medicine formulations from 500 plant species.^[8, 9, 10] In continuation of the survey we observed 20 plants were frequently used by tribals for preparation of antimicrobial remedies. It is reported that natural antimicrobials can be derived from barks, stems, leaves, flowers and fruits of plants, various animal tissues or from microorganisms.^[11] These are known to play an important role in both drug discovery and chemical biology. In fact many of the current drugs either mimic naturally occurring molecules or have structures that are fully or in part derived from the natural motifs.^[12] All these information prompted us to engage in documentation, antimicrobial evaluation of extracts of all the 20 medicinal plants frequently used by the tribal people and their phytochemical analysis.

MATERIALS AND METHODS

Study area

The district of Wayanad is situated on the eastern portion of Kerala. It lies between north latitude 11° 27' and 11 ° 58'35" and the east longitudes 75 ° 47'50" and 76 ° 26' 35". The district is bounded on the north by Kodagu district of Karnataka state, on the east by Mysore district of Karnataka state and Nilagiri district of Tamilnadu state, on the south by Ernad taluk of Malappuram district and Kozhikode taluk of Kozhikode district, on the west by Vadakara and Quilandy taluks of Kozhikode district and Thalassery taluk of Kannur district.

The total area of the district is 2131 sq.km. i.e., 5.48 % of the total geographical area of the state. Figure 1 shows the map of study area.





Collection and identification of plant material

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 among the tribal colonies of Mananthvady, 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. Methodology suggested by Jain^[13] was followed to collect the data from the tribals. Information related to method of application, therapeutic use, parts of plants used, name of diseases, symptoms of diseases, and causes of diseases was collected through questionnaire, interviews and discussions with tribal people and practitioners in their local language. The voucher specimen of all the plants are deposited in Department of Studies in Environmental Science, University of Mysore, Manasagangotri, Mysore 570 006, Karnataka, India.

Extraction

The fresh plant leaf samples selected for antimicrobial analysis were washed with tap water followed by sterilized distilled water and shade dried at room temperature and powdered using sterile mixer grinder and stored in airtight bottles. Dried plant material was subjected to diethyl ether, methanol and aqueous extraction using Soxhlet extractor. All the extracts were evaporated to dryness using rotary flash evaporator and used for further studies.

Phytochemical screening

The qualitative analysis of phytochemical constituents such as tannin, phlobatannin, saponin, flavonoids, terpenoids and cardiac glycosides of all the plants was done following procedures of Oguyemi.^[14]

Test microorganisms

The bacterial samples of *E. coli* isolated from stool samples of diseased persons, *Staphylococcus aureus* isolated from infected urine samples and fungal sample *Aspergillus niger* isolated from decaying vegetables were cultured on nutrient agar and MacConkey's agar (Becton Dickinson and company, Microbiology systems, sparks, MD) media respectively. *Microsporum racemosum* isolated from infected nail was cultured on Sabouraud Dextrose Agar medium (SDA). All the cultures were confirmed through preliminary microscopic and biochemical studies.

Antimicrobial activity assay

Methanol, diethyl ether and sterile distilled water (aqueous) extracts were subjected to both antibacterial and antifungal activity using agar well diffusion method.^[15, 16] Pure cultures of test bacteria and fungi were sub cultured on respective media as explained earlier and incubated. From each plate colonies were transferred separately in to 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 standards) and used as an inoculums for performing agar well diffusion assay. The agar plates were allowed to solidify and cups of 6 mm diameter were made using sterile cork borer. 100μ l of inoculum previously adjusted with the known density was swabbed on to the media plates. Evaporated and dried methanol and diethyl ether extracts were prepared at different dilutions (2.5%, 5% and 10%) in Dimethyl Sulphoxide (DMSO) solution. The aqueous extract was diluted in water to obtain the said concentrations. 50 µl volumes of the extracts were propelled directly into the well (in triplicate) of each petriplate containing test organisms. The plates were incubated at 37°C for

24hours and 7 days for bacteria and fungi respectively, whereas *A. niger* was incubated at room temperature. DMSO (10%) served as negative control. Antibiotic discs of Chloramphenicol (100μ g/disc) and Cephoperazon (100μ g/disc) for bacteria and Miconazole (10mg/ml DMSO) for fungus served as positive control. The inhibition zone if any around the well containing the extracts indicates the antimicrobial activity. The diameter was measured in millimeter.

Statistical Analysis

All experiments were conducted in triplicates. Data analysis was done according to the standard statistical method SPSS package for windows.

RESULTS AND DISCUSSION

The ethno botanical survey revealed the frequent use of these 20 species of medicinal plants for the treatment of infectious diseases. They were shown in table 1 with their medicinal uses and tribes associated with them. The tribes in different villages have been using the same plant for curing a particular disease. Medicines prepared by leaves of these plants were used as treatment against skin infections, diarrhea, dysentery and pneumonia by the tribes. Plants used by the tribes belongs to 16 different families among which four plants belong to Apocynaceae and two belongs to Amaranthaceae, where as other 14 plants belongs to 14 different families of plant kingdom (Table 1).

Phytochemical analysis revealed that phytochemical constituents varied among the plants tested (Table 2) whereas all the plants reported positive for cardiac glycosides. Terpenoides test was also found positive for all the plants except *Embelia tsjeriam-cottam*. Here it is important to note that the therapeutic effects of most of the plants are not the direct effect of a single group or compound, but rather that the compounds possibly act in combination to bring out an effect generally represented as synergism.^[17] Further studies on synergistic effect if any, of individual plant constituents and combination of plants is in progress.

The present study reports the antimicrobial activity of three different solvent extracts of plants listed in table 1 against enteropathogenic *Escherichia coli*, *Staphylococcus aureus*, *Aspergillus niger* and *Microsporum racemosum*. Inhibitory activity of all the extracts against test organisms is reported in table 3. Diethyl ether and water extract of all the plants tested did not record antifungal activity against both the test fungi. The water extracts of *Garcinia gummi gutta* and *Pittosporum neelgherrense* are inhibitory against *E. coli*. Water extracts of

Madhuka longifolia, Breynia vitis-idaea, Fagraea ceylanica and Rhaphidophora pertusa were inhibitory to Staphylococcus aureus.

Methanolic leaf extract of *Nothapodytes nimmoniana*, *Gomphostemma heyneanum*, *Breynia vitis-idaea*, *Pittosporum neelgherrense* and *Chilocarpus malbaricus* recorded significant inhibitory activity against *Microsporum racemosum* where as inhibitory activity against *A*. *niger* was observed only in *Nothapodytes nimmoniana*. Leaf paste of this plant was used by the kurichia tribes of Wayanad to make herbal remedies against cancer, skin problems and arthritis. The results of the present antimicrobial studies and the phytochemical screening tests indicated the presence of tannin, saponin, flavonoids, terpenoids and cardiac glycosides supports the ethno medicinal treatment practiced by the tribes.

Tannin, saponin, flavonoids, terpenoids, and cardiac glycosides were also observed in *Alstonia venenata*, leaf paste of this plant is used for the treatment of snake poison and skin diseases by the tribes. Present study reports inhibitory activity of this plant against both *E. coli* and *Staph. aureus. Breynia vitis-idaea* was used for curing body pain and skin diseases. Aqueous extract of *Breynia vitis-idaea* recorded highly significant inhibitory activity against *Staphylococcus aureus*. The leaf juice of *Madhuka longifolia* is abundantly used by the tribes for the treatment of diarrhea and skin diseases. The present study also reveals the inhibitory activity of leaf extract against *Staph. aureus* associated with skin infection, but *E. coli* used in the present study was found resistant.

Gomphostemma heyneanum is used to prepare medicine for rheumatism, dysentery and diarrohea by the kurichia, kuruma and kattunaika tribes. The methanol and diethyl ether extract of this plant recorded significant inhibitory activity against both the bacteria, where as it was found not significant against *M. racemosum* compared to positive control. Phytochemical analysis revealed the presence of tannin, saponin, terpenoides and cardiac glycosides.

Garcinia gummi gutta is a lower risk near threatened plant used by the tribes singly or in combination with other plant parts to cure various ailments. 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. Phytochemical screening of crude extract of *Garcinia gummi gutta* leaves showed the presence of tannin, phlobatannin, saponin, flavanoids, terpenoids, and cardiac glycosides.

Several authors have linked the presence of these bioactive compounds to the antimicrobial properties of the crude extracts.^[18, 19, 20] The presence of glycosides moieties like saponins, cardiac glycosides and flavanoids which are known to inhibit tumor growth and serve also to protect against gastrointestinal infections are of pharmacognostic importance and give evidence to the use of the plant in ethno medicine. Diethyl ether and methanolic extract of the leaves of this plant did not record inhibitory activity against *E. coli* whereas aqueous extract recorded highly significant inhibitory activity against *E. coli* compared to chloramphenicol. It is interesting to note that only diethyl ether extract was inhibitory to *Staphylococcus aureus*, this indicates presence of more than one active principle which can act on Gram positive and Gram negative bacteria separately. These observations support the traditional knowledge of tribes in curing infectious diseases.

Rhaphidophora pertusa is a climber and it is known with a local name Anachakkara. Leaf juice is extensively used by the kattunaika and kurichia tribes for curing dysentery and diarrhea. In the present study the aqueous extract of the leaves showed inhibitory activity against *Staphylococcus aureus*. The methanol extract of leaves showed no activity against both fungi.

The Kurichia tribes use the leaves of *Pittosporum neelgherrense* for the treatment of snake poison and skin infections. The present study reveals antimicrobial activity of this plant against both bacteria. The methanolic extract did not record inhibitory activity against *Aspergillus niger*, but was found effective against *Microsporum racemosum*.

Several reports are already available on antimicrobial activity of plants selected in the present study. Antimicrobial activity of *Achyranthes bidentata*,^[21] *Gomphostemma*,^[22] *Madhuka longifolia*,^[23] *Nothapodites nimmoniana*,^[24, 25, 26] *Rouvolfia tetraphylla*,^[27] *Alstonia* spp.,^[28] *Saraca*,^[29] *Raphidophora pertusa*,^[30] *Garcinia gummi gutta*^[31] and *Hedychium coronariu* ^[32] reported by earlier workers was also observed in the present study. But inhibitory activity varied significantly compared to earlier workers.

TABLE 1: Ethno botanical information of some traditionally used medicinal plant species from Wayanad district, Kerala selected for antibacterial and antifungal activity.

Sl No	Botanical name	FamilyIndigenous namesPartTherapeutic useFormulations		FamilyIndigenous namesPartTherapeutic useFormulations		Part Therapeutic F use		IndigenousPartTherapeuticFenamesuse		Indigenous namesPartTherapeutic useFormulations		Indigenous namesPartTherapeutic useFormulationsN a		Mode of administrations	Tribes associated
1	Alstonia venenata Br.	Apocynaceae	Theeppala Pambinekollim ara.	Leaf	Skin diseases Snake bite	Grind the leaves with turmeric and apply on the affected part	Smearing externally	Kurichia, Kuruma.							
2	Hedychium coronarium Koening	Zingiberaceae	Kalyanasaugan thikam	Leaf	Skin diseases	The leaf paste is applied on the affected part	Smearing externally	Kuruma. Adiyan Kurichia							
3	Chonemorpha fragrans (Moon)	Apocynaceae	Perumkurumba	Leaf	Skin diseases Blood purification	The juice intake purifies blood. The leaf paste is applied on the affected part	Oral intake	Kurichia.							
4	Chilocarpus malabaricus Bedd.	Аросупасеае	Vallippala	Leaf	Skin disease	The resin of the leaf is applied on the affected part	Smearing externally	Kurichia.							
5	Garcinia gummi gutta (L.) Roxb. var. gummi–gutta	Clusiaceae	Kodampuli Meenpuli	Leaf	Tonsillitis. Ulcer Bleeding piles Dysentery Diarrhoea.	Drinking the leaf juice cures tonsillitis, ulcer and bleeding piles, dysentery and diarrhoea	Oral intake	Kurichia Kuruma Kattunaika Adiya Paniya							
6	Gomphostemma heyneanum Benth.var. heyneanum),	Lamiaceae	Theepperuku	Leaf	Dysentery Diarrhoea. Arthritis	Drinking the leaf juice cures dysentery and diarrhoea.	Oral intake	Kurichia Kuruma Kattunaika							

TABLE 1: Ethno botanical information of some traditionally used medicinal plant species from Wayanad district, Kerala selected for antibacterial												
	and antifungal activity (Continued)											
7	Breynia vitis Idaea	Euphorbia ceae	Kurukkanko mbu	Leaf	Body pain Skin problems	The leaf paste is applied on the affected part cures skin problems.	Smearing externally	Kurichia Kuruma Kattunaika				
8	<i>Madhuka</i> <i>longifolia</i> (Koenig.)J.F.Macb r	Sapotaceae	Elippa	Leaf	Dysentery Diarrhoea. Fever Rheumatism	Drinking the leaf juice cures dysentery and diarrhoea.	Oral intake	Kurichia Kuruma Kattunaika.				
9	Naringi crenulata (Roxb.) Nicols,	Rutaceae	Narinarakam	Leaf	Dysentery Diarrhoea. Bone crack	Drinking the leaf juice cures dysentery and diarrhoea.	Oral intake	Kurichia Kuruma Kattunaika Adiyan Paniya				
10	Nothapodytes nimmoniana (Graham)Mabb	Icacinaceae	Ulukkuvetty	Leaf	Cancer Skin problems Arthritis	The leaf juice is an ingredient in the medicine for cancer, skin problem and arthritis	Smearing externally	Kurichya Kuruma Kattunaika				
11	Pittosporum neelgherrense Wightt.	Pittosporaceae	Analivenga	Leaf	Snake bite Skin disease	The leaf paste is applied on the affected part cures skin problems and snake bite	Smearing externally	Kurichya				
12	<i>Psilotrichum elliotii</i> Baker&Clarke	Amaranthaceae	Pancharachedy	Leaf	Antidiabetic	The leaf juice drinking cures diabetics	Oral intake	Kurichya				

TAB	TABLE 1: Ethno botanical information of some traditionally used medicinal plant species from Wayanad district, Kerala selected for antibacterial										
	and antifungal ac	tivity (Continue	ed)	1	I	1	ſ				
13	Raphidophora pertusa (Roxb.)Schott.	Raphidophora ceae	Anachakkara	Leaf	Dysentery Diarrhoea. Mumps Tonsillitis.	The young leaf juice along with salt drinking cures dysentery and diarrhea. Smearing of leaf paste cures tonsillitis and mumps.	Oral intake	Kattunaika Kurichya			
14	Fagraea ceylanica Thunb	Logaceae	Modakam	Leaf	Dysentery Diarrhoea.	The leaf juice drinking cures dysentery and diarrhoea	Oral intake	Kurichya			
15	Achyranthes bidentatae Blum	Amaranthacea e	Vankadalady	Leaf	Dysentery Diarrhoea.	The leaf juice drinking cures dysentery and diarrhoea	Oral intake	Kuruma Paniya			
16	<i>Embelia tsjeriam- cottam</i> (Roem.&Schult.) DC.	Myrsinaceae	Anjanam Kattuvizhal	Leaf	Pneumonia, Diarrhoea.	The leaf juice drinking cures pneumonia and diarrhea.	Oral intake	Kurichia Kuruma Kattunaika			
17	Strobilanthes alata Nees	Acanthaceae	Kurinji	Leaf	Skin disease	The leaf paste is applied on the affected part cures skin problems and snake bite	Smearing externally	Kurichia.			
18	<i>Gnetum edule</i> (Willd.)Blume ula Brongn	Gnetaceae	Karanakkody	Leaf	Arthritis	The leaf paste is applied on the affected part cures Arthritis	Smearing externally	Kurichia.			

TABLE 1: Ethno botanical information of some traditionally used medicinal plant species from Wayanad district, Kerala selected for antibacterial and antifungal activity (Continued)

								1	
19	Rouvolfia	Apocynaceae	Pambinkaya	Leaf	Blood pressure,	The leaf paste is	Smearing	Kurichia.	
	tetraphylla L.				for good sleep	applied on the affected	externally		
					and	part cures skin	-		
					Skin disease	problems			
20	Saraca asoka	Caesalpiniace	Asokam	Leaf	Skin disease	The leaf paste along	Smearing	Kurichia	
	(Roxb.) deWilde	ae				with calcium carbonate	externally		
						is applied on the	externally		
						affected part cures skin	-		
						problems.			

 TABLE 2: Qualitative analysis of the phytochemicals of twenty medicinal plants

Sl. No.	Plant	Tannin	Phlobatannin	Saponin	Flavanoid	Terpenoid	Cardiac
							glycosides
1	Achyranthes bidentatae	+	-	+	+	+	+
2	Alstonia venenata	+	-	+	+	+	+
3	Fagraea ceylanica	-	-	-	-	+	+
4	Chilocarpus malabaricus	-	-	+	+	+	+
5	Chonemorpha fragrans	+	-	+	+	+	+
6	Rouvolfia tetraphylla	+	-	-	+	+	+
7	Embelia tsjeriam-cottam	+	-	+	+	-	+
8	Garcinia gummi gutta	+	+	+	+	+	+
9	Gomphostemma heyneanum	+	-	+	-	+	+
10	Gnetum edule	+	-	+	+	+	+
11	Hedychium coronarium	+	-	+	+	+	+
12	Breynia vitis Idaea	-	-	+	+	+	+
13	Madhuka longifolia		-	+	+	+	+

www.wjpr.net

Devi Prasad et al.

World Journal of Pharmaceutical Research

14	Naringi crenulata		-	+	-	+	+
15	Nothapodytes nimmoniana	+	-	+	+	+	+
16	Psilotrichum elliotii	+	-	+	-	+	+
17	Pittosporum neelgherrense	+	-	+	+	+	+
18	Raphidophora pertusa	-	-	+	+	+	+
19	Strobilanthes alata	+	-	+	-	+	+
20	Saraca asoka	+	-	+	+	+	+

+: Present

-: Absent

TABLE 3: The Antibacterial and antifungal activity of aqueous, methanolic and diethyl ether extracts of medicinal plants

	Zone of inhibition measured in mm								
	Bacteria		Fungi						
Botanical name	E. coli			Staphylococcus aureus			Aspergillus niger	Microsporum racemosum	
	Methanol extract	Diethyl ether extract	Water	Methanol extract	Diethyl ether extract	Water extract	Methanol extract	Methanol extract	
Alstonia venenata Br.	08±0.08	21±0.29	-	08±0.08	15±0.41	-	-	-	
Hedychium coronarium Koening	-	09±0.22	-	9±.008	2±0.163	-	-	-	
Chonemorpha fragrans (Moon)	7±0.37	10±.25	-	07±0.37	13±0.33	-	-	-	
Chilocarpus malabaricus Bedd.	-	13±0.08	-	-	16±0.24	-	-	18±0.163	
Garcinia gummi gutta (L.) Roxb. var. gummi-gutta	-	-	30±0.05	-	-	-	-	-	
Gomphostemma heyneanum Benth.var. heyneanum),	15±0.22	13±0.16	-	9±0.22	14±0.16	-	-	16±0.16	
Breynia vitis-idaea	16±0.43	-	-	10±0.16	24±0.05	25±0.05	-	21±0.05	
Madhuka longifolia (Koenig.) J.F.Macbr	-	-	-	-	20±0.05	12±0.05	-	-	
Naringi crenulata (Roxb.) Nicols,	08 ± 0.08	-	-	08±0.08	-	-	-	-	

www.wjpr.net

Devi Prasad et al.

World Journal of Pharmaceutical Research

Nothapodytes nimmoniana (Graham)Mabb	12±.51	10±0.05	-	07±0.37	15±0.05		32±0.05	30±0.05
Pittosporum neelgherrense Wightt.	16±.43	10±0.05	15±0.05	07 ± 0.05	19±0.05	-	-	17±0.05
Psilotrichum elliotii Baker&Clarke	07±0.65	13±0.73	-	07 ± 0.6	10±0.4	-	-	-
Raphidophora pertusa (Roxb.)Schott.	08±0.16	11±0.60	-	06±0.5	10±0.24	23±0.33	-	-
Fagraea ceylanica Thunb	15±0.41	10 ± 0.81	-	08 ± 0.6	14±0.73	13±0.4	-	-
Saraca asoka (Roxb.) deWilde	-	15±.16	-	-	14±.24	-	-	-
Achyranthes bidentatae Blum	-	09±0.16	-	-	17±0.08	-	-	-
Embelia tsjeriam-cottam (Roem.&Schult.)	14.2 ± 0.05	09 ± 0.82	-	$07 \pm .08$	15±0.08	-	-	-
DC.								
Strobilanthes alata Nees	-	-	-	-	20±0.16	-	-	-
Gnetum edule (Willd.)Blume ula Brongn	-	10±0.54	-	-	14±0.16	-	-	-
Rouvolfia tetraphylla L.	-	10±0.36	-	-	16±0.51	-	-	-
Chloramphenicol	29 ±.54			30±.51				
Cephoperazone	08±0.70			09±0.22				
Miconazole							26±0.69	29±0.36

Values are mean of three replicates \pm S.E.

The analysis of questionnaire collected from tribal people revealed that the educational status of both male and female interviewers was very low. However the percentage of the male with tertiary education was higher when compared with that of female interviewers. Most of the interviewers (75%) inherited the practice from their grandparents and parents. But few males learned it through informal training and dreams. Similar findings on traditional practitioners' socio demographic characteristics such as educational level, age and the source of traditional knowledge have been reported in the other cultures.^[33]

They have general knowledge about human diseases. And for prescribing medicines blood in urine and pricking pain spreading from back to abdomen are some of the symptoms used for identifying urinary infections and kidney stone. Increased turbidity in the urine is the symptom of diabetics. Skin discoloration, softening and itching were the symptoms of skin diseases. A sore on tongue and buccal cavity shows digestive disorders.

CONCLUSION

Historically ethno medical studies have been the best way of discovering new plant derived medicines. In the present investigation plant extracts used by tribal community recorded significant antibacterial and antifungal activity. From this evidence it can be rationally suggested that further work needs to be done to identify the chemical nature of active principles as well as their modes of action on bacterial cells and their roles in disease curing. The results of the present study also provide evidence that medicinal plants continue to play an important role in the healthcare of tribal community. The data collected show that majority of the remedies are taken orally and smearing for the external application. Most of the reported preparations are drawn from a single plant and mixtures are used rarely. In other parts of the country, the use of mixtures of plant species in treating a particular ailment is fairly common.

ACKNOWLEDGMENT

The authors are grateful to the tribal people of Wayanad district, Kerala for their help and cooperation during the present work. Also thanks the taxonomists of M.S. Swaminathan Research Foundation, Wayanad and Dr. Vijayan, Head of the Department, Microbiology Department, Pazhassi Raja College Pulpally, Wayanad for their help and support.

REFERENCES

- Khan AU, Musharraf A. Plasmid mediated multiple antibiotic resistances in *Proteus* mirabilis isolated from patients with urinary tract infection. Med Sci Monit, 2004; 10: 598-602.
- 2. WHO. The evolving threat of antimicrobial resistance: Options for action. GPS publishing, France: 2012.
- Bhushan P. Traditional medicine: A novel approach for available, accessible and affordable health care. Paper for regional consultative on medicine. WHO regional office for South East Asia: 2005.
- 4. Nisha VM, Sivadasan M. Ethnodermatologically significant plants used by traditional healers of Wayanad District, Kerala. Ethnobotany, 2007; 19: 55-61.
- Raji, R, Raveendran K. Medicinal plants used by the forest tribe of Mananthavady taluk, Wayanad district, Kerala South India. Life Sci Leaflets 2011; 13:421-426.
- 6. Udayan PS, Sateesh G. Thushar KV, Indira B. Ethnomedicine of Chellipale community of Namakkal district, Tamil Nadu. Ind J Trad Knowl, 2005; 4: 437-442.
- 7. Silja VP, Samitha VK, Mohanan KV. Ethno medicinal plant knowledge of the Mullukuruma tribe of Wayanad District, Kerala. Indian J Trad Knowl, 2008; 4: 604-612.
- Benny ST, Prasad AGD. An ethno botanical survey of medicinal plants used in Bathery taluk of Wayanad, Kerala, India. Asian J Microbiol Biotechnol Environ Sci, 2011; 13: 685-694.
- 9. Prasad AGD, Shyma TB. *Nothapodites nimmoniana* (Graham) Mabb. leaf extract a remedy for *Aspergillus niger* infections. Int J Environ Eng Manage, 2011; 2: 213-220.
- Shyma TB, Prasad AGD. Traditional use of medicinal plants and its status among the tribes in Mananthavady of Wayanad district, Kerala. World Res J Med Aro Plants, 2012; 1: 22-26.
- Gordon MC, David JN. Natural product drug discovery in the next millennium. Pharm Biol, 2001; 39: 08-17.
- Cheesbrough M. Medical laboratory manual for tropical countries, Butterworth, Oxford: 2000, pp. 260.
- 13. Jain SK. The role of botanist in folklore research. Folklore 1964; 5: 145-150.
- 14. Oguyemi AO. In Sofowora, A. (ed) Proceedings of a conference of African medicinal plants. Ife-Ife: Univ life: 1979, pp. 20-22.
- 15. Aneja KR, Joshi R, Sharma C. The antimicrobial potential of ten often used mouthwashes against dental caries pathogens. Jundishapur J Microbiol, 2010; 3: 15-27.

- Okeke MI, Iroegbu CU, Eze EN, Okoli AS, Esimone CO. Evaluation of extracts of the root of *Landolphia owerrience* for antibacterial activity. J Ethnopharmacol, 2001; 78: 119-127.
- Ncube B, Finnie JF, Staden VJ. *In vitro* antimicrobial synergism within plant extracts combinations from three South African medicinal bulbs. J Ethnopharmacol. 2012; 139: 81–89.
- 18. Adesokan AA, Akanji MA, Yakubu MT. Antibacterial potentials of aqueous extract of *Enantia chlorantha* stem bark. Afr J Biotechnol, 2007; 6: 2502-2505.
- Oyeleke SB, Dauda BEN, Boye OA. Antibacterial activity of *Ficus capensis*. Afr J Biotechnol, 2008; 17: 1414-1417.
- 20. Sahm DF, Washington JA. Antibacterial susceptibility tests: Dilution methods. In manual of clinical microbiology Lennette, EH (ed.) fifth edition. American Society of Microbiology, Washington, DC: 1990. pp. 1105-1118.
- Uma DP, Murugan S, Suja S, Selvi S, Chinnaswamy P, Vijayanand E. Antibacterial, *in vitro* lipid per oxidation and phytochemical observation on *Achyranthes Bidentata* Blume. Pakistan J Nutr, 2007; 6:447-451.
- 22. Deka H, Gogoi D, Gogoi HK, Handique PJ. *In vitro* evaluation of antimicrobial property of two species of genus *Gomphostemma*. J Cell Tissue Res, 2006; 6: 787-91.
- 23. Khond MJD, Bhosale AT, Mandal TK, Padhi MM, Dabur R. Screening of some selected medicinal plants extracts for *in vitro* antimicrobial activity. Middle-East J Sci Res, 2009; 4: 271-278.
- Namdeo AG, Ajay S, Fulzele DP, Mahadik KR. Influence of geographical and climatic conditions on camptothecin content of *Nothapodytes nimmoniana*. Rec Nat Prod, 2010; 4(1): 64-71.
- 25. Padmanabha BV, Chandrashekar M, Ramesha BT, Hombe Gowda HC, Gunaga RP, Suhas S, Vasudeva R, Ganeshaiah KN, Uma Shanker R. Patterns of accumulation of camptothecin, an anti-cancer alkaloid in *Nothapodytes nimmoniana* Graham in the Western Ghats, India: Implications for identifying high-yielding sources of the alkaloid. Curr Sci, 2006; 90: 95–100.
- 26. Roja G. Comparative studies on the camptothecin content from *Nothapodytes nimmoniana* and *Ophiorrhiza* species. Nat Prod Res, 2006; 20: 85-88.
- 27. Suresh K, Saravana BS, Harisaranraj R. Studies on in *vitro* antimicrobial activity of ethanol extract of *Rauvolfia tetraphylla*. Ethnobot. Leaflets, 2008; 12: 586-90.

- 28. Khyade MS, Vaikos NP. Vaikos phytochemical and antibacterial properties of leave of *Alstonia scholaris* R. Br. Afr J Biotechnol, 2009; 8: 6434-6436.
- 29. Nayak S, Sahoo AM, Chakraborti CK. Phytochemical screening and antibacterial activity study of *Saraca indica* leaves extract. Int Res J Pharm, 2011; 2: 176-179.
- 30. Kalairasan A, Ahmed JS. Evaluation of *Rhaphidophoral pertusa*, Schott (Araceae) for antibacterial activities. J Pharm Res, 2011; 4: 1-2.
- Varalakshmi KN, Sangeetha CG, Shabeena AN, Sunitha SN, Vapika J. Antimicrobial and cytotoxic effects of *Garcinia indica* fruit rind extract. American-Eurasian J Agri Environ Sci, 2010; 7: 652-656.
- 32. Aziz MA, Rowshanul HM, Rezaul KM. Antibacterial and cytotoxic activities of *Hedychium coronarium* J. Koenig. Res J Agri Biol Sci, 2009; 5: 969-972.
- 33. Adebo GM, Alfred SDY. Gender dimension of herbal medicine's knowledge and practice in Ekiti and Ondo States, Nigeria. J Med Plants Res, 2011; 5: 1284–1290.