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STUDIES ON PHYTOCHEMICAL CONSTITUENTS AND ANTIMICROBIAL ACTIVITY OF SOME ENDANGERED PLANT SPECIES COLLECTED FROM THE WESTERN GHATS

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ABSTRACT Current investigation is aimed to assess seven different endangered plants (*Curcuma zedoaria*, *Cayratia pedata*, *Utleria salicifolia*, *Syzygium travancorium*, *Nothapodytes nimmoniana*, *Rhaphidophora persuto*, *Plectranthus vettiveroides*) in terms of their phytochemical assessment and antimicrobial activity against different pathogens such as *Escherichia coli, Staphylococcus aureus, Streptococcus mutans, Salmonella typhi, Klebsiella pneumonia, Bacillus cereus and Pseudomonas aeruginosa.* Phytochemical analysis showed the variation in presence of bioactive compounds in the leaf, stem and root extracts of different plants. The leaf extract of *Rhaphidophora persuto* showed six positive test among the ten corroborating the extract as an potential origin of herbal medicine. Different parts the plants such as stem, roots and leaves exhibited antimicrobial activity against pathogens which was determined by observing the inhibition zone. Further the minimum inhibitory Concentration (MIC) was evaluated for the leaf extracts of *Utleria salicifolia, Plectranthus vettiveroides and Nothapodytes nimmoniana*. Further investigation with these extracts is beneficial in order to diagnose the therapeutic usage of the selective plant extracts.

KEYWORDS : Endangered plants, Antimicrobial activity, Minimum inhibitory concentration, Phytochemicals

Introduction

With an increase in multi-drug resistant microbes, the appearance of the strains with a reduction in susceptibility to antibiotics are gradually increasing. This results in rapid employment of immunosuppressive agent, intravenous catheters, broad-spectrum antibiotics, (Graybill 1988; Dean and Burchard 1996; Gonzalez et al. 1996). But synthetic drugs are very expensive in developing countries and also leads to several side effects as well. Thus, it is necessary to investigate new blueprint to fight against infection as well as to control microbes (Sieradzki et al. 1999). Global health care has taken an initiative to search for effective, affordable and novel medicines against microbial infection. In developing countries maximum death occurs due to infectious diseasess. Thus, application of those medicine may provide a positive impact (Awouafack et al. 2013; Srivastava et al. 2013). In this context medicinal plants has come up with great success as a source of herbal medicine for the treatment of several diseases. Phytochemicals like flavonoids and pheols are strong antioxidants and have an important role in the health care system. Almost 80% of the world population depends on the conventional medicine to take care of their health (Rout et al. 2013). The World Health Organization (WHO) is encouraging, promoting and facilitating the effective use of herbal medicine in developing countries for health programs. It is already proved that several plant extracts contain bioactive components that show bactericidal and bacteriostatic effects. These extracts can be used as multipurpose drugs. These can act as the resources of traditional medicines as well as natural products for drug development (Rout et al. 2012). Phytochemicals or bioactive compounds occurs naturally in plants and are non-nutritive compounds. These are refined and used in effective drug production (Prejeena et al. 2015). The traditional drugs do not use the whole plant but use specific bioactive compounds as major resources. The bioactive compounds are mainly extracted from various parts of plants such as root, stem, leaf, bark, flower, fruit and seeds. Cayratia Pedata, an important plant shows anti diarrheal activity as observed by Karthik et al. Diarrhea was induced in rat by castor oil and magnesium sulphate. Plant extract was prepared using chloroform and tested for shows anti diarrheal activity. 200 mg/kg and 400 mg/kg dose showed remarkable activity to prevent Diarrhea (Karthik et al. 2011). An aromatic plant namely *Plectranthus vettiveroides* has been reviewed by Nisheeda et al. for its properties, constituents, uses etc. The root of this plant contains an essential oil which act as a source of several Ayurvedic formulations. Different herbal drugs as well as home remedies have been synthesized from this plant. *Plectranthus vettiveroides* shows several essential features such as antimicrobial, antioxidant, antidiabetic, hepatoprotective and anticancer activities. Also it has been used for the treatment of hyperdipsia, diarrhoea, intrinsic haemorrhage, leprosy, nausea, ulcer, genitourinary diseases, quenching thirst, fever, vomiting, eye burning etc. (Nisheeda et al. 2016). On the other hand *Curcuma zeodaria* has been studied for its antimicrobial activity by Lai et al. The essential oil from plant was prepared by stem distillation followed by solvent extraction and analyzed by gas chromatography revealing the presence of curdione and epicurzerenone. The extracted essential oil was assessed for the antimicrobial activity against six different microbes. The result exhibited *Vibrio parahaemolyticus* as the most sensitive bacteria against the oil where as E. Coli was highly resistant. This study also verified the efficiency of the essential oil in inhibition of human leukemia (Lai et al. 2004). Extraction of essential oil from *Syzygium travancoricum* leaf and investigation of its antimicrobial activity against six microbes was carried out in a study. The major component of *Syzygium travancoricum* extracts was found as α-humulene, trans-β-ocemene, α-farnesene, trans-β-caryophyllene. 5 µl essential oil was applied for antimicrobial test and result showed 11, 10, 12, 11,11,12 mm inhibition zone against *Bacillus sphaericus, Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Samonella typhimurium* respectively (Shafi et al. 2002). Antibacterial and antifungal activity of *Curcuma zeodariaa* was evaluated by Wilson et al. Soxhlet based tuber extraction was prepared by chloroform, ethanol, acetone, water and n-hexane. Water aided extract did not show any activity. Antibacterial activity was observed against *Bacillus subtilis*, *Micrococcus luteus, Proteus mirabilis* and Klebsiella pneumoniae whereas antifungal activity was observed against *Candida albicans* and *Aspergillus niger*. Minimal inhibitory concentration of different solvent extracts was obtained in a range of 0.01-0.12 mg/ml (Wilson et al. 2005).

From all these studies it is proved that plants are potential sources of medicinal compounds which play a vital role towards the health of human being. The aim of this study was to evaluate the antimicrobial activity as well as phytochemical assessment of some medicinal plants used in Ayurveda and traditional medicinal system for treatment of manifestations caused by microorganisms. Therefore, stem, leaf and root extracts of seven endangered plants from different families were tested for their potential activity against microbial pathogens.

Materials and Methods

Following 7 endangered plants from different families were collected from Western Ghats.

I. *Curcuma zeodaria* (CZ)
ii. *Cayratia pedata* (CP)
iii. *Utleria salicifolia* (US)
iv. *Syzygium travancorium* (ST)
v. *Nothapodytes nimmoniana* (NN)
vi. *Rhaphidophora persuto* (RP)

vii. Plectranthus vettiveroides (PV)

The plant samples were collected from the following locations, *Utleri salicifolia, Curcuma zeodatia, Nothapodytes nimmoniana* from the Western Ghats of Kerala. Plectranthus vettiveroides from Tamilnadu. *Cayratia pedate* from the Western Ghats bordering Kerala and Tamilnadu and Karnataka states. Rhaphidophora persuta from the Western Ghats of Karnataka and *Syzygium travancoricum* from fresh water Myristica swamps of Kerala and UttarKannada district of Karnataka.

The root, stem and leaf extracts of the plant was prepared by Soxhlet extraction with methanol. The extracts of these plants were tested for their potential activity against microbial pathogens. A total of seven pathogens were taken for antimicrobial test such as *Escherichia coli, Staphylococcus aureus, Streptococcus mutans, Salmonella typhi, Klebsiella pneumonia, Bacillus cereus and Pseudomonas aeruginosa.*

Phytochemical analysis

Phytochemical screenings were done with 10mg/ml plant extracts concentration. For determination of alkaloids 0.2ml test sample was mixed with same volume of HCl. 2-3 drops of Dragendorff reagent was added to the mixture. Appearance of turbid solution and red or orange color precipitate indicates the presence of alkaloid. Carbohydrate was measured by adding few drops of Molisch's reagent (α- napthol dissolved in alcohol) to 0.2 ml test sample in a test tube. 0.2 ml concentrated sulphuric acid was slowly added along the side of test tube. Appearance of purple color ring indicates positive test. For determination of tannins 0.2 ml of plant extract was mixed with 2 ml water and heated for 10 minutes in water bath. The mixture was filtered and ferric chloride was added to the filtrate. If the color of the solution turns into dark green it indicates the presence of tannin. Terpenoid detection was carried out by using chloroform. 0.2 ml plant extract was mixed with 0.2 ml chloroform. Next concentrated sulphuric acid was added slowly to the mixture. Appearance of reddish-brown color at the interface indicates positive test. For glycosides test 0.2 ml extract was mixed with 0.2 ml chloroform. Next 0.2 ml acetic acid was added to the mixture and the resulting solution was cooled on ice. Sulphuric acid was added. Change of color from violet to blue to green indicates glycosides presence. Steroid was also confirmed my mixing the test sample with chloroform followed by sulphuric acid. The appearance of red color in the lower layer of chloroform indicates the presence of steroids. Test for saponins was done by adding 5 ml of distilled water in 0.5 gm of leaf extract in a test tube. The resulting solution was shaken vigorously to observe froth. When stable persistent froth appears, then 3 drops of olive oil was mixed to the froth and mixed vigorously. Formation of an emulsion indicates positive test. To determine flavonoids, 0.2 ml plant extract was mixed with dilute NaOH solution in a test tube. Color of the solution turns to yellow. Dilute HCl was added to the mixture and if the solution turns into colorless it signifies positive test. Phenol was determined by adding 0.4 ml distilled water to 0.2 ml plant extract followed by few drops of 10% aqueous ferric chloride solution. Formation of blue or green color indicated the presence of phenols. Test of cardiac glycosides was carried out by mixing 0.2 ml extract to 10 µl of glacial acetic acid containing one drop of ferric chloride solution. Addition of 40 µl concentrated sulphuric acid to the solution results in brown ring formation at the interface indicating the presence of deoxysugar of cardenoloides. A violet ring may appear beneath the brown ring, while in the acetic acid layer, a greenish ring may also form just gradually throughout the layer.

Preparation of test compound and pathogen cultures

Antimicrobial activity of the test extracts was carried out by Well Diffusion technique. Ciprofloxacin was taken as a standard test compound as it has the potential to inhibit the growth of all the pathogens. All the seven pathogens were grown in peptone broth for 24-48 h at 37°C. The cell suspensions of all the cultures were adjusted to 1-2x 10^5cells/ml.

Root, stem and leaf extracts were prepared from the seven endangered plants. Drug concentration was prepared as 100mg/ml for antimicrobial test. Methanol was taken as control.

Evaluation of antimicrobial activity

At first100 µl of all the seven pathogens was swabbed in seven soya bean casein digested agar plates (90 mm). 5 mm well was created in each agar plate. 100µg/ml test compounds, ciprofloxacin (0.1 mg/ml) and methanol was added to the well and incubated at 37^{0}C for 24-48 hrs. The plates were observed for zone of inhibition around the wells.

Determination of Minimum inhibitory Concentration (MIC):

For determination of minimum inhibitory concentration, 90µl of drug was mixed with different concentration of test compound and inoculated with 10µl inoculum in 96 well plates in triplicate. 100 µl peptone broth without drug was taken as control. Treated Bacterial cultures were incubated at 37^{0}C for 24-48 hr. and O.D was taken at 590 nm. MIC or minimum concentration of drug was measured as the concentration of drug that gives 50% inhibition as compared to control.

Antimicrobial activity of Leaves of Utleria salicifolia, Plectranthus vettiveroides and Nothapodytes nimmoniana

10 mg of test sample was dissolved in 1 mL DMSO (Dimethyl sulfoxide). 24 hr cultured *Bacillus cereus, Escherichia coli, Klebsiella, Pseudomonas, Staphylococcus aureus, Streptococcus mutans* and *Salmonella typhi* were taken as test organism. 200µL of deionized water was added in each of the wells of the microtiter plate (A_1-A_{12}, B_{12}-H_{12}, H_{11}-H_1, and G_1-B_1) to prevent the sample from drying. 100µL of sterilized LB broth was added to all the remaining wells. 30µL of 0.1% of resazurin was added to the wells C_2- F_2 in another plate B_2-D2 and named as colour blank. In wells C_3- $F_3$and in another plate B_3-D_3test organism and 30µL of 0.1% of resazurin was added as culture control.100µL of the samples 1, 2 and 3 were added to respective plates from wells C_4-F_4 and B_4-D_4in another plate and serially diluted by transferring 100µL of the mixture to subsequent wells upto C_{11}-F_{11},B_{11}-$D_{11}$and 100µL of the excess sample was discarded from C_{11}-$F_{11,}$ B_{11}-D_{11} respectively. To test against organism, 100µL of culture was inoculated to serially diluted sample wells. *Bacillus cereus* was inoculated to wells from C_4 –C_{11}. *Escherichia coli* was inoculated to wells fromD_4 –D_{11}.*Klebsiella* was inoculated to wells from E_4 –E_{11}. *Pseudomonas* was inoculated to wells from F_4 –$F_{11}$and in another plate. *Staphylococcus aureus*culturewas inoculated to wells from B_4 –B_{11}. *Streptococcus mutans* was inoculated to wells from C_4 –C_{11}. *Salmonella typhi* was inoculated to wells from D_4 –$D_{11}$and 30µL of 0.1% of resazurin was added to all the test sample wells. The plates were incubated at 37° C for 17hr. Presence of blue color indicates no growth of the organism whereas appearance of pink color indicates growth.

Results and discussion

Phytochemical analysis of plant extracts

Table 1 and table 2 exhibited the phytochemical analysis of the seven endangered species. Almost all the extracts except a few showed presence of saponins and phenols. Only *Rhadophora persuto* stem and *Plectranthus vettiveroides* stem extracts showed positive test for steroids. Tannins are present in leaf extract of *Cayratia pedata, stem and leaf extracts of Syzygium travancoricum,* root and stem extracts of *Utleria salicifolia* and *Nothapodytes nimmoniana,* leaf extract of *Rhaphidophora persuto* and stem extract of *Plectranthus vettiveroides.* Flavonoids were detected by alkaline reagent test and root extract of *Curcuma zeodaria*, root and stem extracts of *Cayratia pedata*, stem extract of *Syzygium travancoricum*, root extract of *Utleria salicifolia*, *Nothapodytes nimmoniana* and stem extract of *Rhaphidophora persuto*, *Plectranthus vettiveroides* showed positive result. Salkowki's test determined the presence of terpenoids by leaf extracts of *Curcuma zeodaria, Cayratia pedata, Syzygium travancoricum, Rhaphidophora persuto* and root extract of *Utleria salicifolia.* For the other test *Curcuma zeodaria* root extract exhibited the presence of Cardiac glycosides, carbohydrates and glycosides but alkaloid was not detected, leaf extract showed positive test for alkaloid whereas stem extracts did not show these three phytochemical properties. The detailed phytochemical characterization of all the extracts were shown in table 1 and 2. Figure 1 shows the images of phytochemical test.

Table 1: Phytochemical analysis of *Curcuma zedoaria, Cayratia pedata, Syzygium travancoricum* and *Utleria salicifolia* extracts

Sl. no.	Phytochemicals	Type of test	*Curcuma zeodaria*	*Cayratia pedata*	*Syzygium travancoricum*	*Utleria salicifolia*							
			Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem
1	Saponins	Foam test	+	+	+	+	+	-	-	+	+	+	+
2	Phenol	$FeCl_3$ test	+	-	+	+	+	+	+	+	+	+	-

3	Steroids	Lieberman Burchardt test	-	-	-	-	-	-	-	-	-	-	-
4	Tannins	Braymer's test	-	-	-	-	-	+	-	+	+	+	+
5	Flavonoids	Alkaline reagent test	+	-	-	+	+	-	-	+	-	+	+
6	Terpenoids	Salkowki's test	-	-	+	-	-	+	-	-	+	+	-
7	Cardiac glycosides	Keller-Killani test	+	-	-	-	-	+	-	+	+	+	+
8	Alkaloids	Dragendoff's test	-	-	+	-	-	+	-	-	+	-	+
9	carbohydrates	Molisch's test	+	-	-	-	+	-	-	+	-	+	+
10	Glycosides	Glycosides test	+	-	-	-	-	-	-	+	+	+	-

Table 2: Phytochemical analysis of *Nothapodytes nimmoniana, Rhadophora persuto* and *Plectranthus vettiveroides* extracts

Sl. no.	Phytochemicals	Type of test	*Nothapodytes nimmoniana*	*Rhaphidophora persuto*	*Plectranthus vettiveroides*			
			Root	Stem	Root	Stem	Leaf	Stem
1	Saponins	Foam test	-	+	+	+	+	+
2	Phenol	$FeCl_3$ test	-	+	+	+	+	+
3	Steroids	Lieberman Burchardt test	-	-	-	+	-	+
4	Tannins	Braymer's test	+	+	-	-	+	+
5	Flavonoids	Alkaline reagent test	+	-	-	+	-	+
6	Terpenoids	Salkowki's test	-	-	-	-	+	-
7	Cardiac glycosides	Keller-Killani test	-	+	-	-	+	-
8	Alkaloids	Dragendoff's test	+	+	+	-	+	+
9	carbohydrates	Molisch's test	+	-	-	+	-	-
10	Glycosides	Glycosides test	-	-	-	-	-	+

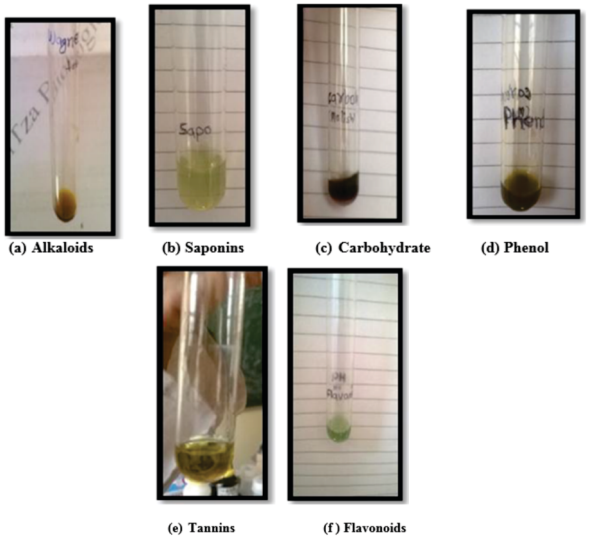

Fig 1: Production of different phytochemicals.

Inhibitory activity of test compound

Fig 2 – fig 7 show the images of the agar plates exploring the inhibitory activity of the test compounds in terms of inhibition zone against seven different pathogens. Table 3 exhibited the diameter of inhibition zone wherever found.

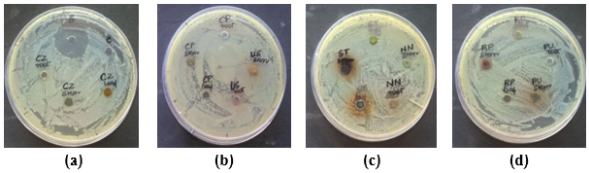

Fig 2: Inhibitory activity of test samples against Escherichia coli

(a) S-standard (Ciprofloxacin); C -Control (methanol); Sample- CZ (root, stem, leaf),
(b) CP (root , stem ,leaf), US(root , stem),
(c) ST (root,stem,leaf), NN(root, stem),
(d) RP (root,stem,leaf) and PU (stem, root).

As observed in Fig 2 only ciprofloxacin inhibited the growth of E. coli. An inhibition zone of 25 mm was found around the well of ciprofloxacin. As a control methanol did not show any inhibitory effect, all the test compounds could not inhibit the growth of E.coli as mentioned in table 3.

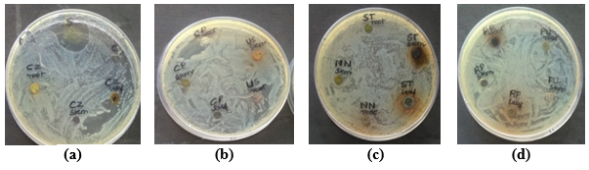

Fig 3: Inhibitory activity of test samples against *Staphylococcus aureus*

(a) S-standard (Ciprofloxacin); C -Control (methanol/water); Sample- CZ (root, stem, leaf),
(b) CP (root , stem ,leaf), US(root , stem),
(c) ST (root,stem,leaf), NN(root, stem),
(d) RP (root,stem,leaf) and PU (stem, root).

Fig 3 showed the activity of test compound against *Staphylococcus aureus*. Leaf extract of *Syzygium travancorium* (ST) showed an inhibition zone of 10 mm whereas the other extracts from other plant species could not inhibit the growth of *Staphylococcus aureus*. Standard showed 20 mm zone of inhibition whereas control could not inhibit the growth.

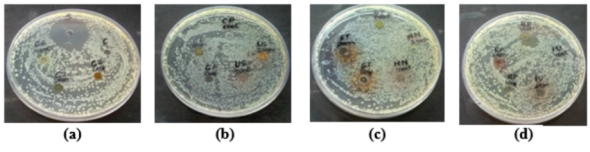

Fig 4: Inhibitory activity of test samples against *Streptococcus mutans*

(a) S-standard (Ciprofloxacin); C -Control (methanol/water); Sample- CZ (root, stem, leaf),
(b) CP (root , stem ,leaf), US(root , stem),
(c) ST (root,stem,leaf), NN(root, stem),
(d) RP (root,stem,leaf) and PU (stem, root).

Root extract of *Rhaphidophora persuto* (RP) and standard showed the inhibition zone of 12 and 24 mm respectively against *Streptococcus mutans* (Fig 4 and Table 3).

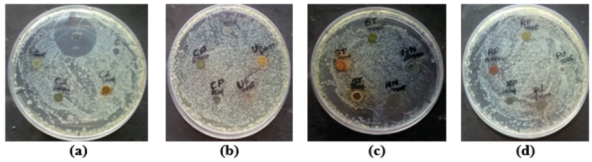

Fig 5: Inhibitory activity of test samples against *Salmonella typhi*

(a) S-standard (Ciprofloxacin); C -Control (methanol/water); Sample- CZ (root, stem, leaf),
(b) CP (root , stem ,leaf), US(root , stem),
(c) ST (root,stem,leaf), NN(root, stem),
(d) RP (root,stem,leaf) and PU (stem, root).

Stem and leaf extract of *Syzygium travancorium (ST)*, root and stem extracts of *Nothapodytes nimmoniana* (NN) showed inhibitory effect against *Salmonella typhi* (Fig 5). An inhibition zone of 7 and 8 mm diameter was observed for stem and leaf extracts of ST respectively whereas 10 and 9 mm diameter inhibition zone was obtained by root and stem extracts of NN respectively. Standard showed 26 mm inhibition zone (Table 3).

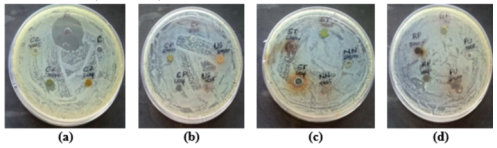

Fig 6: Inhibitory activity of test samples against *Klebsiella pneumoniae*
(a) S-standard (Ciprofloxacin); C -Control (methanol/water); Sample- CZ (root, stem, leaf),
(b) CP (root , stem ,leaf), US(root , stem),
(c) ST (root,stem,leaf), NN(root, stem),
(d) RP (root,stem,leaf) and PU (stem, root).

Stem extract of *Crucuma zeodaria* (CZ) and root extract of *Syzygium travancorium* (ST) showed inhibition zone of 7 mm against *Klebsiella pneumonia* whereas standard resulted in 25 mm zone of inhibition.

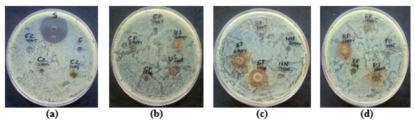

Fig 7: Inhibitory activity of test samples against *Bacillus cereus.*
(a) S-standard (Ciprofloxacin); C -Control (methanol/water); Sample- CZ (root, stem, leaf),
(b) CP (root , stem ,leaf), US(root , stem),
(c) ST (root,stem,leaf), NN(root, stem),
(d) RP (root,stem,leaf) and PU (stem, root).

The stem extract of *Crucuma zedoaria (CZ)* showed positive effect against *Bacillus cereus* by producing 7 mm inhibition zone.

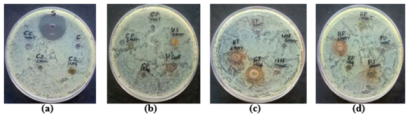

Fig 8: Inhibitory activity of test samples against *Pseudomonas aeruginosa.*
(a) S-standard (Ciprofloxacin); C -Control (methanol/water); Sample- CZ (root, stem, leaf),
(b) CP (root , stem ,leaf), US(root , stem),
(c) ST (root,stem,leaf), NN(root, stem),
(d) RP (root,stem,leaf) and PU (stem, root).
Root extract of *Ulteria salicifolia* (US), stem and leaf extracts of *Syzygium travancorium (*ST) and stem extract of *Plectranthus uettiveroides* (PU) showed inhibition zone of 10, 9, 10 and 6 mm against *Pseudomonas aeruginosa.*

Table 3: Inhibitory activity of test sample against respective Bacteria

Test Compounds	Conc. (µg/well)	Zone of inhibition (mm)							Well reference number
		E. coli	*S. aureus*	*S. mutans*	*S. typhi*	*K. pneumoniae*	*B. cereus*	*P. aeruginosa*	
Control	Methanol	-	-	-	-	-	-	-	C
Ciprofloxacin (Standard)	2.5	25.0±0.0	20.0±0.0	24.0±0.0	26.0±0.0	25.0±0.0	24.0±0.0	26±0.0	S
***Crucuma zeodaria** (CZ)*	100	-	-	-	-	-	-	-	CZ root
		-	-	-	-	7.00±0.0	7.00±0.0	-	CZ stem
		-	-	-	-	-	-	-	CZ leaf
Cayratia pedata (CP)		-	-	-	-	-	-	-	CP root
		-	-	-	-	-	-	-	CP stem
		-	-	-	-	-	-	-	CP leaf
Ulteria salicifolia (US)		-	-	-	-	-	-	10.00±0.0	US root
		-	-	-	-	-	-	-	US stem
Syzygium travancorium (ST)		-	10.0±0.0	-	-	7.0±0.0	-	-	ST root
		-	-	-	7.0±0.0	-	-	9.00±0.0	ST stem
		-	-	-	8.0±0.0	-	-	10.00±0.0	ST leaf
Nothapodytes nimmoniana(NN)		-	-	-	10.0±0.0	-	-	-	NN root
		-	-	-	9.0±0.0	-	-	-	NN stem
Rhaphidophora persuto(RP)				12.0±0.0	-	-	-	-	RP root
		-	-	-	-	-	-	-	RP stem
		-	-	-	-	-	-	-	RP leaf
Plectranthus uettiveroides (PU)		-	-	-	6.0±0.0	-	-	6.00±0.0	PU stem
		-	-	-	-	-	-	-	PU root

Determination of Minimum inhibitory Concentration (MIC):

Table 4: Determination of MIC.

Test Compound	Sample Code	MIC (µg/mL)						Test parameters
		S. aureus	S. mutans	S. typhi	K. pneumoniae	*B. cereus*	*P. aeruginosa*	
Standard	Ciprofloxacin (S)	0.5	0.5	0.25	0.5	0.5	0.25	**Methodology:** Micro broth dilution technique using Culture Medium: Peptone broth respective bacteria. Sample test concentrations 1000, 500, 250, 125, 62.5, 31.25, 15.62µg/ml.
Curcuma zedoaria	CZ **stem**				1000	1000		
Ulteria salicifolia	US **root**						1000	
Syzygium travancorium	ST **root**	1000			1000			
	ST **stem**			1000			1000	
	ST **leaf**			1000			1000	
Nothapodytes nimmoniana	NN **root**			1000				
	NN **stem**			1000				
Rhaphidophora persuto	RP **root**		500					
Plectranthus vettiveroides	PU **stem**			1000			1000	

3.4 Antimicrobial activity of Leaves of *Utleria salicifolia, Plectranthus vettiveroides and Nothapodytes nimmoniana*

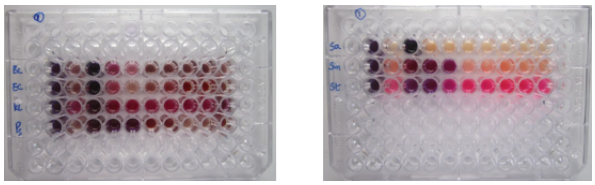

Fig 9: Antimicrobial activity of *Utleria salicifolia(Leaves)* extract

The leaf extract of *Utleria salicifolia* shows minimum inhibition concentration for *Bacillus cereus* (BC)1000µg, *Escherichia coli* (EC) 1000µg, *Klebsiella*(KL)1000µg, *Pseudomonas*(Ps) 250µg, *Staphylococcus aureus*(Sa)1000µg, *Streptococcus mutans* (Sm) 250µg and *Salmonella typhi*(St) 500µg.

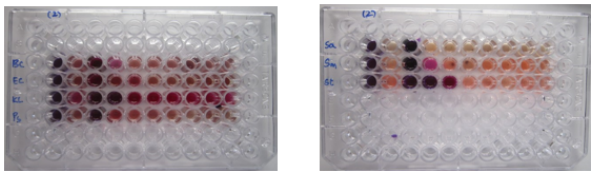

Fig 10: Antimicrobial activity of *Plectranthus vettiveroides (Leaves)* extract

The leaves extract of *Plectranthus vettiveroides showed* the minimum inhibition concentration for *Bacillus cereus*(BC) 1000µg, *E- coli* (EC) 1000µg, *Klebsiella* (KL) 500µg, *Pseudomonas* (Ps)1000µg, *Staphylococcus aureus*(Sa) 1000µg, *Streptococcus mutans*(Sm) 1000µg and *Salmonella typhi*(St) 250µg.

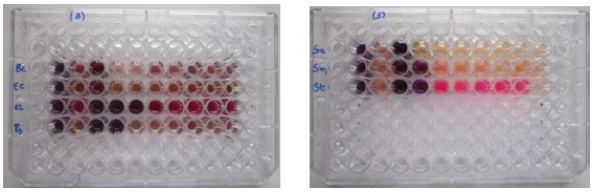

Fig 11: Antimicrobial activity of *Nothapodytes nimmoniana (Leaves)* extract

Leaves extract of *Nothapodytes nimmoniana* showed the minimum inhibition concentration for *Bacillus cereus*(BC) 1000µg, *Escherichia coli*(EC) 1000µg, *Klebsiella* 250µg, *Pseudomonas* (Ps) 500µg, *Staphylococcus aureus*(Sa) 1000µg, *Streptococcus mutans*(Sm) 500µg and *Salmonella typhi* (St) 500µg.

MIC of the plant leaves against the specific concentration of test organism is tabulated in table 5.

Table 5: Minimum Inhibition Concentration (MIC) in µg against organism

Test Organism	Minimum Inhibition Concentration (MIC) in µg		
	Sample-LEAVES		
	Utleria salicifolia	*Plectranthus vettiveroides*	*Nothapodytes nimmoniana*
Bacillus cereus	1000	1000	1000
Escherihia coli	1000	1000	1000
Klebsiella	1000	500	250
Pseudomonas	250	1000	500
Staphylococcus aureus	1000	1000	1000
Streptococcus mutans	250	1000	500
Salmonella typhi	500	250	500

Conclusion

This study evaluated the antibacterial activity of 7 endangered plants of Western Ghats. The zones of inhibition as observed in the plates revealed that *Curcuma zeodaria* stem had inhibitory activity against *Bacillus cereus* and *Klebsiella pneumoniae* whereas *Utleria salicifolia* root showed inhibitory activity against *Pseudomonas aeruginosa*. *Syzygium travancorium* root showed activity against *Staphylococcus aureus* and *Klebsiella pneumoniae*. Whereas stem and leaf extracts showed activity against *Salmonella typhi* and *Pseudomonas aeruginosa*. Root and stem of *Nothapodytes nimmoniana* showed activity against *Salmonella typhi*. Sample *Rhaphidophora persuto* root showed activity against S*treptococcus mutans* and Sample *Plectranthus vettiveroides* stem shows activity against *Salmonella typhi* and *Pseudomonas aeruginosa.* Utleria salicifolia showed lowest minimum inhibitory concentration (MIC) for *Pseudomonos* and *Streptococcus mutans*, whereas, *Plectranthus vettiveroides* and *Nothapodytes nimmoniana* showed lowest MIC for *Salmonella typhi* and *Klebsiella* species respectively. Some of the extracts had a good potential for therapeutic uses against some pathogens. It appears that extracts with high antimicrobial activity against Gram-negative bacteria do not necessarily have high activity against other Gram–negative bacteria compared to Gram-positive bacteria. This may mean that the activity is not related to the differences in cell wall structure. Because there is such a wide range of MICs for different strains of the same bacterial species. Some extracts displayed a potent antibacterial activity, indicating that these plants could be a good source for the antibacterials to combat MDR bacterial infections. In the present study qualitative phytochemical screening showed that plants are rich in secondary metabolites. These phyto-constituents are considered as active compounds that have commercial interest in pharmaceutical industries for the development of natural drugs. Further studies are necessary for these potent plant extracts to evaluate the other parameters of antimicrobial efficacy such as in-vivo efficacy, toxicity, and anti-mycobacterial, antiviral, and antiparasitic activity. Assessment of the pure constituent will be fruitful in order to investigate the actual mechanism of the wound healing activity of the undertaken endangered plants.

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