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Research Article

TRACE METAL CONCENTRATION AND ANTIMICROBIAL EFFICACY OF SELECTIVE MEDICINAL PLANTS, SOUTHERN INDIA

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ABSTRACT

The present study was evaluated the trace metals load and antimicrobial effect of three important medicinal plants in southern India namely, *Mukia maderaspatana, Kedrostis foetidissima* and *Cayratia pedata*. The phytochemical analysis was tested by using ethanol extract of these plants and the alkaloids, flavonoids, tannins, saponins, terpenoids, steroids and sugars were presented. These plants were challenged against certain pathogens which are obtained from MTCC and NCIM. The antimicrobial effect showed that higher concentration (30 µl) got good effect than lower concentration (15 µl). In trace metal analysis, Cd and Ni was not present (below detectable limit) in all the three plants while Cr, Cu, Fe and Zn was present in the entire sample.

Keywords: *Mukia maderaspatana*, *Kedrostis foetidissima, Cayratia pedata,* Trace metal.

INTRODUCTION

India is a varietals emporium of medicinal plants and is one of the richest countries in the world in regard to genetic resources of medicinal plants. It exhibits a wide range in topography and climate, which has a bearing on its vegetation and floristic composition. Moreover, the agro-climatic conditions are conducive for introducing and domesticating new exotic plant varieties (Martins, 2001). In India, different parts of several medicinal plants or their extracts are used for the treatment of various diseases. Several antibiotics used for the treatment of human infections, which have limited antimicrobial spectrum. They could develop drug resistance in pathogens and lead to serious ill effect. Hence plant derived antimicrobial properties have received considerable attention in recent years. Several plants have been indicated in folk and other traditional system of medicine as aseptic agents. More than a hundred species of therapeutically important higher plants are listed and described in ancient Indian treatise to have the antimicrobial activity, spectrum of antimicrobial property with no ill effects (Agrawal, 1986). The medicinal properties of the plants could be credited to the presence of one or more of the active constituents of the plant (Egwaikhide, 2007).

Many infectious diseases have been known treated with herbal remedies throughout the history of mankind. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases (Rojas, 2003). The increasing failure of chemotherapeutics and antibiotic resistance exhibited by athogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Colombo and Bosisio, 1996; Iwu et al. 1999). It is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections (Balandrin et al.. 1985). Even today, scientists and the general public recognize their value as a source of new and complimentary medicines owing to their versatile applications (Liu, 2012). The World Health Organization (WHO) noted that the majority of the world's population depends on

traditional medicine for primary healthcare. Medicinal and aromatic plants are widely used as medicine and constitute a major source of natural organic compounds.

Considering all these in mind, the present study is concentrated on the medicinal plants such as *Mukia maderaspatana*, *Kedrostis foetidissima*, and *Cayratia pedata* and their derived products. And also the phytochemical and trace metal levels were analysed which is help ful for their future applications.

MATERIALS AND METHODS

Plant Material

The plant materials were collected from Kolli hills, Namakkal district of Tamil Nadu in India during the period of August to October 2013. The shade dried plant powders (100 g) were successively extracted with ethanol by soxhlet apparatus and is used as test sample.

Phytoconstituents screenings by qualitative method

The solvent extracts were subjected to routine qualitative secondary metabolites analysis to identify the nature of phytochemical constituents present in sample (Koperuncholan et al. 2011). Steroids: Three ml of test solution and minimum quantity of chloroform was added with 3-4 drops of acetic anhydride and one drop of concentrated H_2SO_4. Purple color thus formed changes into blue or green color indicating the presence of steroids. Triterpenoids: A 3 ml of test solution was added with a piece of tin and 2 drops of thionyl chloride. Formation of violet or purple colour indicates the presence of triterpenoids. Reducing Sugars: A 3 ml of test solution was added with a 2 ml of Fehling's reagent and 2 ml of water. Formation of reddish orange colour indicates the presence of reducing sugar. Sugars: A 3 ml of the test solution was added with very small quantity of anthrone reagent and a few drops of concentrated H_2SO_4 and heated. Formation of green or purple color indicates the presence of sugars. Alkaloids: A 3 ml of test solution was taken with 2N HCl. Aqueous layer formed was decanted and then added with one or a few drops of Mayer's reagent. Formation of white precipitate or turbidity indicates the presence of alkaloids. Phenols: A 3 ml of test solution in alcohol was added with one drop of neutral ferric chloride (5%) solution. Formation of intense blue color indicates the presence of phenols. Flavonoids: A 3 ml of test solution in alcohol was added with a bit of magnesium and one (or) two drops of concentrated HCl and heated. Formation of red or orange color indicates the presence of flavonoids. Saponins: A 3 ml of test solution was added with water and shacked. Formation of foamy lather indicates the presence of Saponins. Tannins: A 3 ml of test solution was added with water and lead acetate. Formation of white precipitate indicates the presence of tannins. Anthroquinones: A 3 ml of test solution was added with magnesium acetate. Formation of pink color indicates the presence of anthroquinones. Amino Acids: A 3 ml of test solution was added with 1% ninhydrin in alcohol. Formation of blue or violet color indicates the presence of amino acids. Catechins: A 3 ml of test solution in alcohol was added with Ehrlich reagent and a few drops of concentrated HCl. Formation of pink color indicate the presence of catechins.

Testing of antimicrobial activity

The test strains were: *Bacillus subtilis* NCIM 2920 (B1), *Escherichia coli* NCIM 2931 (B2), *Proteus mirabilis* NCIM 2241 (B3), *Pseudomonas aeruginosa* NCIM 5029 (B4), *Staphylococcus aureus* NCIM 5021 (B5), *Staphylococcus epidermidis* NCIM 2493 (B6), *Candida glabrata* MTCC 3984 (F1), *Epidermophyton floccosum* MTCC 613 (F2), *Microsporum canis* MTCC 3270 (F3), *Trichophyton rubrum* MTCC 3272 (F4). The cultures were obtained from MTCC, Chandigarh and NCIM, Pune, India. Microbial strains were tested for antimicrobial sensitivity using the disc diffusion method (Bauer, 1966). The antibacterial and antifungal activity of test samples was analyzed against certain microorganisms on muller hinton agar (MHA) and potato dextrose agar (PDA), respectively. A sterile cotton swab was used to inoculate the bacterial suspension on surface of agar plate. The 15 and 30 µL of sample coated disc were placed in agar plates, separately. For negative control study, the sterile triple distilled water was used. The plates were incubated at 37±1°C for 24–48 h (for bacteria) and 25 ±1°C for 48-72 h (for fungus). After incubation, the zone of inhibition was measured with ruler. The assays were performed in triplicate and the average values are presented. Methicillin – 10mcg (for bacteria) and Itraconazole – 10mcg (for fungus) was used as positive control. All the media, standard discs and sterile disc were purchased from Hi-Media (Mumbai, India).

Trace metals estimation in plants

The three different plant samples such as *Mukia maderaspatana* (KP1), *Kedrostis foetidissima* (KP2), and *Cayratia pedata* (KP3) were collected from the Kolli hills, Namakkal district, Tamil Nadu. The three plant leaves were carefully removed and washed with sterile distilled water, separately. The cleaned leaves were dried

in shadow area and were grinned with mortar and pestle. The powered plant samples were stored in sterile plastic container. The 1 g of powdered plant samples was treated with aqua-regia mixture in Teflon bomb and was incubated at °C for 2-3 days. After incubation, the reaction mixture was filtered with Whatman No.1 filter paper. Then the extraction was test for trace metals (Fe, Cu, Zn, Pd, Cd, Cr and Ni) analysis. The extraction of the studied metals in the solutions was determined by the 797 VA Computrace voltametry, Metrohm. To avoid the contamination, the devices were rinsed with acidified water (10% HNO_3) and weighted to dissolve metals before analysis. And all the equipments and containers were soaked in 10% NHO_3 for 24 h then rinsed thoroughly in de-ionized water before use. Also find the below detectable limit of the instruments

RESULT AND DISCUSSION

Qualitative secondary metabolites screening Qualitative analysis is very essential for identifying the compounds present in the medicinal plants. Hence, in our present study secondary metabolites screening was performed on selected medicinal plants leaves are *Mukia maderaspatana*, *Kedrostis foetidissima* and *Cayratia pedata*. The results of plants leaf ethanolic extracts were revealed that Steroids, Triterpenes and Flavonoids were commonly present in all the plant while Alkaloids, Phenols, and Tannins were presence in *Kedrostis foetidissima* and *Cayratia pedata*, except *Mukia maderaspatana*. Sugar molecules were present in *Mukia maderaspatana,* and *Cayratia pedata* except *Kedrostis foetidissima*. Whereas Catechins, Anthraquinones, Amino acids were absent in these extracts.

Flavonoids have been shown to have antibacterial, anti-inflammatory, anti-allergic, anti-viral activity (Alan and Miller, 1996). Saponins have been reported to show hypocholesterolemia and tumour inhibiting activity on experimental animals (Johns, 1996). Saponins may also enhance nutrient absorption and in animal digestion. Alkaloids often have pharmacological effects and are used as medication and recreational drugs (Roger and Wink, 1998). Saponins may also enhance nutrient absorption and in animal digestion. Alkaloids often have pharmacological effects and are used as medication and recreational drugs (Roger and Wink, 1998). Tannins and phenols, which together constitute the polyphenolic group are known to have antioxidant, anticancer and antimicrobial activities (Rice et al. 1996).

Ethanolic leaf extract of *M. maderasapatana* is found to contain maximum of 75.38 µg of ascorbic acid/mg of extract. The other potentially beneficial antioxidant vitamin i.e. vitamin E has been determined to the extent of 0.03 µg/mg of leaf acetone extract. In addition to its antioxidant activities, vitamin E might be involved in anti inflammatory processes, inhibition of platelet aggregation, enhanced immune function and help prevent or delay coronary heart disease, cancer, age related macular degeneration and neurodegenerative diseases (Ahamed et al. 2010). *Kedrostis foetidissima,* which is very effective in the treatment of asthma, chest pain and urinary tract infection (Giday, 2001), diarrhoea, HIV (Otieno et al. 2008), small pox, skin diseases (Tabuti et al. 2003), snake bite (Dymock, 1891) and livestock problems (Ole Miaron, 2003). With this background, the present study was carried out to evaluate the antimicrobial potential of different solvent extracts of leaf, stem and tuber of *Kedrostis foetidissima*. The *Cayratia pedata* leaves were used in the treatment of ulcers and diarrhea. The decoction of the leaves was used to check uterine and other fluxes (Patil and Honrao 2000). The plant has also found to possesses anti-inflammatory (Veeradass Rajendran et al. 2011) and antinociceptive activities (Veeradass Rajendran et al. 2011a)

Antibacterial and Antifungal screening

The antimicrobial activity of *Mukia maderaspatana*, *Kedrostis foetidissima* and *Cayratia pedata* were examined with various microorganisms using the disc diffusion test. The results of the antimicrobial activities are summarized in Table 3. The two tested concentrations such as 15 and 30 µl /disc produce zone of inhibition on MHA and PDA plates for bacteria and fungi, respectively. In the present study, higher (30 µl/disc) concentration of sample got greater sensitivity than (15 µl/disc) lower concentration in most of the microorganisms. In bacteria, the test sample was most effective against *Staphylococcus a*ureus NCIM 5021 (B5) while smaller effect was noticed from *Pseudomonas aer*uginosa NCIM 5029 (B4). In fungi, which was effective against *Trichophyton rubrum* MTCC 3272 (F4) whereas smaller effect was observed in *Epidermophyton floccosum* MTCC 613 (F2). All the microbial strains depict higher sensitivity to the higher concentration (30 µL) for the test sample when compared to the positive control except B3, B4 and B6. There is no antimicrobial activity in solution devoid of sample used as a vehicle control (sterile triple distilled water), reflecting that antimicrobial activity was directly related to the sample.

Trace metals detection in plants species

Some of the trace metals are essential for plant growth whereas many of them affect the plant physiology (Shaw, 1990). Especially, the role of trace metal pollutants causing injury to plants either by direct toxic effect or modifying the host physiology rendering it more susceptible to infection (Gupta and Mishra 1994) which leads to affects the photosynthesis process, growth and their efficiency (Larcher, 1995). Metal contents of plant samples, Cd, Cr, Cu, Fe, Ni, Pb and Zn concentrations are between BDL – BDL, 0.03 – 0.05, 0.07 – 0.25, 0.66 – 0.8, BDL – BDL, BDL – 0.02 and 0.32 – 0.6 mg kg^{-1}, respectively. Contaminated sites often support some plant species, which are able to accumulate or tolerate high concentrations of metals such as Pb and Zn (Kumar et al. 1995). A small number of species are capable of growing on soils containing high levels of metals, and also accumulate these pollutants in high concentrations in the parts above ground. These plants are known as hyper accumulators (Brooks et al. 1977). The uptake of these heavy metals by plants is an avenue of their entry into the human food chain with harmful effects on health (Ihekoronye and Ngoddy, 1985).

CONCLUSION

The potential for developing antimicrobials from higher plants appears rewarding, as it will lead to the development of a phytomedicine to act against microbes. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. The property of active phytoconstituents responsible for the antimicrobial activity cannot be altered. A small number of species are capable of growing on soils containing high levels of metals which may also cause serious threats to plants.

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Table 1: Qualitative phytochemical constituent of selected medicinal plants

Phytochemical Constituents	*Mukia maderaspatana*	*Kedrostis foetidissima*	*Cayratia pedata*
Steroids	+	+	+
Triterpenes	+	+	+
Reducing sugars	-	-	-
Sugars	+	-	+
Alkaloids	-	+	+
Phenolics	-	+	+
Catechins	-	-	-
Flavonoids	+	+	+
Saponins	+	+	-
Tannins	-	+	+
Anthraquinones	-	-	-
Amino acids	-	-	-

+ = Present
- = Absent

Table 2: Concentration of trace metals in selected medicinal plants

Sampling Site Name	Sampling Site No.	Sample Name / Family	Sample No.	S. Code	Cd	Cr	Cu	Fe	Ni	Pb	Zn
Kolli hills, Tamil Nadu	S1	*Mukia maderaspatana*	P1	KP1	BDL	0.03	0.25	0.78	BDL	BDL	0.6
Kolli hills, Tamil Nadu	S2	*Kedrostis foetidissima*	P2	KP2	BDL	0.05	0.07	0.8	BDL	BDL	0.4
Kolli hills, Tamil Nadu	S3	*Cayratia pedata*	P3	KP3	BDL	0.05	0.12	0.66	BDL	0.02	0.32

BDL – Below detectable limit

Table 3: Antimicrobial activity of the ethanolic extracts of leaves in selected medicinal plants

S.No	Test Microorganisms	Zone of inhibition (mm)							
		Mm		Kf		Cp		PC	Remarks
	Bacteria	15 µL	30 µL	15 µL	30 µL	15 µL	30 µL	10 µg	
1.	*Bacillus subtilis* (B1)	14	17	13	15	12	16	14	> PC
2.	*Escherichia coli* (B2)	12	15	11	14	13	17	8	> PC
3.	*Proteus mirabilis*(B3)	11	14	12	15	12	14	28	< PC
4.	*Pseudomonas aeruginosa* (B4)	12	16	11	14	13	15	38	< PC
5.	*Staphylococcus aureus* (B5)	14	17	13	15	12	16	0	> PC
6.	*Staphylococcus epidermidis* (B6)	11	14	12	15	11	13	16	< PC
	Fungi								
7.	*Candida glabrata* (F1)	12	14	11	13	12	13	10	> PC
8.	*Epidermophyton floccosum* (F2)	13	17	11	14	12	16	9	> PC
9.	*Microsporum canis* (F3)	11	15	13	16	12	14	9	> PC
10.	*Trichophyton rubrum*(F4)	12	15	11	14	15	17	7	> PC

Mm- *Mukia maderaspatana*, Kf- *Kedrostis foetidissima*, Cp- *Cayratia pedata*, *PC* -Positive Control
(Using antibiotic disc; Bacteria – Methicillin (10mcg/disc) ; Fungi – Itraconazole (10mcg/disc),
Samples - 15 µL / disc & 30 µL / disc; > PC – greater than positive control; < PC – less than positive control

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