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PHYTOCHEMICAL SCREENING AND COMPARATIVE STUDY OF ANTIMICROBIAL ACTIVITY OF SELECTED CONSTITUENTS OF SOME MEDICINAL PLANTS.

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ABSTRACT

Medicinal plants are a rich source of bioactive phytochemicals. The phytochemical screenings of chloroform, aqueous and alcoholic plant extracts of rhizome of *Curcuma longa* and leaves of *Azadirachta indica, Ocimum sanctum, Momordica charantia* were performed using standard phytochemical techniques for the determination of the presence of alkaloids, steroids, terpenoids, tannins, proteins, carbohydrates, saponins etc.. It showed that chloroform, methanol, ethanol and aqueous extracts gave positive tests for alkaloids, proteins and phytosterols. Methanol and ethanol extracts were found to contain phenolic compounds. Aqueous extract gave positive test for saponins. Methanol, ethanol and aqueous extract gave positive test for carbohydrates. The in vitro antimicrobial activity of selected plant extracts was investigated against soil bacteria. The ability of rhizome of *Curcuma longa*, and leaves of *Azadirachta indica, Ocimum sanctum, Momordica charantia* extracts to inhibit the growth of test pathogen is an indication of their broad spectrum antimicrobial potential which may be employed in the management of microbial infections. The seasonal variations of total ash and acid insoluble ash have been investigated in selected constituents of plants, which are medicinally important. Comparative account of total ash and acid insoluble ash content showed higher value of total ash of leaves of Momordica charantia (15.86%) and lower value of total ash of rhizome of Curcuma longa (8.36%) while the acid insoluble ash showed higher value of leaves of Momordica charantia (5.96%) and lower value of leaves of Ocimum sanctum (1.22%).

KEYWORDS: Phytochemical, Antimicrobial activity, Ash value, Curcuma longa, etc.

INTRODUCTION

Plants are the primary source of medicines, food and shelter for humans. Plant derived natural products can be used for the treatment of diseases, thus can act as a base for development of natural blueprint of new drugs.[1]

In recent decades, many researchers are interested in medicinal plants for evaluation of antioxidant phytochemicals such as phenols, flavonoids and tannins which have received more attention for their potential role in prevention of human diseases.[2]

Curcuma longa (C. longa), a perennial herb, is a member of the *Zingiberaceae* family. These plants are said to be medicinally important by having anti-inflammatory, anticancer, antioxidant, antibacterial and antifungal properties. They are being used for the treatment of jaundice, diabetes and kidney related problems in traditional medicinal practice in India.

The *Momordica charantia* belongs to the *Cucurbitaceae* family and it has common names such as bitter melon, karela and bitter gourd. More than thousand herbal products of *Momordica charantia* are used for treatment of diabetic patients and also helpful in lowering of glucose level in the blood.

Azadirachta indica, also known as Neem, and Indian Lilac is a tree in the mahogany family *Meliaceae*. In India, the Neem tree is affectionately called Nature's Drugstore since all parts of the tree are used in various forms for herbal healing. Neem leaf, bark extracts and neem oil are commonly used for therapeutic.

Ocimum sanctum, holy basil, or tulsi, is an aromatic plant in the family Lamiaceae which is native to the Indian subcontinent and widespread as a cultivated plant throughout the Southeast Asian tropics. Tulsi has many traditional health uses, it is used as an antibiotic, an immune system booster, an anti-inflammatory, antiasthmatic, antikaphic drugs and a stress reducer.

These plants are said to be medicinally important by having anti-inflammatory, anticancer, antioxidant, antibacterial and antifungal properties. They are being used for the treatment of jaundice, diabetes and kidney related problems in traditional medicinal practice in India. However, no much scientific validation has been made for these species for their medicinal

uses. To address this lacuna, the present study was carried out for qualitative and antimicrobial activities of leaf and root parts of these plants using various alcoholic and aqueous extracts.

Therefore the aim of present study was analysis of phytochemicals and to evaluate the antimicrobial activity of extracts of A.indica, O.sanctum, M.charantia and C.longa.

MATERIAL AND METHODS

Chemicals

In the present study, all the chemicals used were of analytical grade.

Collection and identification of plant material

The leaves of Azadirachta indica, Ocimum sanctum, Momordica charantia and rhizome of Curcuma longa were collected, cleaned and dried at room temperature under shade for about two weeks. After drying the leaves were then blended using a household electric blender. This fine powder was transferred into airtight containers with proper labelling for further use.

Preparation of plant extracts

5gm of dried finely powdered plant material was taken in a beaker and 200ml of distilled water, ethanol, chloroform, and methanol was added separately and kept it for 12 to 14hr. Then these extracts were filtered through whatmann filterpaper and the filtrate was used for the phytochemical analysis. These extracts was kept in refrigerator when not use.

Test microorganisms

The strains of soil bacteria used as test organisms were obtained from Department of zoology, VBU. Cultures of bacteria were grown on nutrient agar at 37^0c for 24hr.

Preliminary qualitative phytochemical analysis

Preliminary phytochemical screening of plant was done following the standard procedures adapted by the various workers.[3,4,5] The extracts were subjected to phytochemicals tests for determination of plant secondary metabolites such as tannins, saponins, alkaloids, and glycosides in accordance with.[4]

Determination of Ash values

Air dried leaves was used for the quantitative determination of water soluble ash values and acid insoluble ash values via standard methods. The total ash value for a crude drug is not

always reliable, since there is a possibility of presence of non-physiological substances such as earthy matters. So, the parameters such as acid insoluble, water soluble were performed.

Antibacterial activity

The antibacterial activity of the crude extracts was determined by agar disc diffusion method (Bauer et al, 1960). The bacterial isolates were first grown in a nutrient agar for 18hr at 37^0c in an incubator. The inoculated extracts were then examined for inhibition zones (in mm) by zone reader, which indicates antimicrobial activity. The discs (6mm in diameter) were impregnated with samples extracts and placed on inoculated agar. After 24hr it was then observed for the zone of inhibition occurs around paper discs (se in figures 1).

RESULTS AND DISCUSSION

The present study revealed that the various alcoholic, chloroform and aqueous extracts of leaf parts of *Azadirachta indica*, *Ocimum sanctum*, *Momordica charantia* and root parts of *Curcuma longa* contained alkaloids, flavonoids, saponins, tannins (see in table 1). It showed that chloroform, methanol, ethanol and aqueous extracts gave positive tests for alkaloids, proteins and tannins. Methanol and ethanol extracts were found to contain phenolic compounds. Aqueous extract gave positive test for saponins. Methanol, ethanol and aqueous extract gave positive test for carbohydrates.

TABLE 1(a): PHYTOCHEMICAL ANALYSIS OF AQUEOUS EXTRACT DIFFERENT PLANT

Phytochemicals	Aq. Extract of A.indica	Aq. Extract of O.sanctum	Aq. Extract of C.longa	Aq. Extract of M.charantia
Carbohydrates				
Molisch test	+ve	+ve	+ve	+ve
Fehling's test	+ve	+ve	-ve	+ve
Alkaloids				
Mayer's test	+ve	+ve	-ve	+ve
Flavonoids				
Alkaline reagent test	+ve	+ve	+ve	+ve
Proteins				
Biuret test	-ve	+ve	-ve	-ve
Xanthoproteic test	-ve	+ve	-ve	-ve
Saponins				
Froth test	+ve	+ve	+ve	+ve
Terpenoids				
Salkowaski test	+ve	+ve	-ve	+ve
Tannins				
Ferric chloride test	+ve	+ve	-ve	+ve

Quinones				
Sulphuric acid test	-ve	+ve	-ve	+ve
Emodins	-ve	+ve	-ve	-ve
Cardiac glycosides				
Keller-Killani test	+ve	+ve	+ve	-ve
Fixed oils & fats	+ve	-ve	+ve	+ve

Table 1(b): PHYTOCHEMICAL ANALYSIS OF METHANOLIC EXTRACT OF DIFFERENT PLANTS

Phytochemicals	**Methanol Extract of *A.indica***	**Methanol Extract of *O.sanctum***	**Methanol Extract of *C.longa***	**Methanol Extract of *M.charantia***
Carbohydrates				
Molisch test	+ve	+ve	+ve	+ve
Fehling's test	+ve	+ve	+ve	+ve
Alkaloids				
Mayer's test	+ve	+ve	+ve	+ve
Flavonoids				
Alkaline reagent test	+ve	+ve	+ve	+ve
Proteins:				
Biuret test	-ve	+ve	-ve	-ve
Xanthoproteic test	-ve	+ve	-ve	-ve
Saponins				
Froth test	+ve	+ve	+ve	+ve
Terpenoids				
Salkowaski test	+ve	+ve	-ve	-ve
Tannins				
Ferric chloride test	+ve	+ve	+ve	+ve
Quinones				
Sulphuric acid test	+ve	+ve	-ve	+ve
Emodins	-ve	-ve	-ve	-ve
Cardiac glycosides				
Keller-Killani test	+ve	+ve	+ve	-ve
Fixed oils & fats	+ve	-ve	+ve	+ve

TABLE 1(c): PHYTOCHEMICAL ANALYSIS OF ETHANOLIC EXTRACT OF DIFFERENT PLANTS

Phytochemicals	**Ethanol. Extract of *A.indica***	**Ethanol. Extract of *O.sanctum***	**Etanol. Extract of *C.longa***	**Etanol. Extract of *M.charantia***
Carbohydrates				
Molisch test	+ve	+ve	+ve	+ve
Fehling's test	+ve	+ve	+ve	+ve
Alkaloids				
Mayer's test	+ve	+ve	+ve	+ve
Flavonoids				
Alkaline reagent test	+ve	+ve	+ve	+ve

Proteins				
Biuret test	-ve	+ve	-ve	-ve
Xanthoproteic test	-ve	+ve	-ve	-ve
Saponins				
Froth test	+ve	+ve	+ve	+ve
Terpenoids				
Salkowaski test	-ve	+ve	+ve	+ve
Tannins				
Ferric chloride test	+ve	+ve	+ve	-ve
Quinones				
Sulphuric acid test	+ve	+ve	-ve	+ve
Emodins	-ve	-ve	-ve	-ve
Cardiac glycosides				
Keller-Killani test	+ve	+ve	+ve	+ve
Fixed oils & fats	-ve	-ve	+ve	+ve

TABLE 1(d): PHYTOCHEMICALANALYSIS OF CHLOROFOMIC EXTRACT OF DIFFERENT PLANTS

Phytochemicals	**Chloroform Extract of *A.indica***	**Chloroform Extract of *O.sanctum***	**Chloroform Extract of *C.longa***	**Chloroform Extract of *M.charantia***
Carbohydrates				
Molisch test	+ve	+ve	+ve	+ve
Fehling's test	+ve	+ve	+ve	+ve
Alkaloids				
Mayer's test	-ve	+ve	-ve	+ve
Flavonoids				
Alkaline reagent test	-ve	+ve	+ve	+ve
Proteins				
Biuret test	-ve	+ve	-ve	-ve
Xanthoproteic test	-ve	+ve	-ve	-ve
Saponins				
Froth test	+ve	+ve	+ve	+ve
Terpenoids				
Salkowaski test	+ve	+ve	-ve	+ve
Tannins				
Ferric chloride test	+ve	+ve	+ve	-ve
Quinones				
Sulphuric acid test	-ve	+ve	-ve	+ve
Emodins	-ve	+ve	-ve	-ve
Cardiac glycosides				
Keller-Killani test	+ve	+ve	+ve	+ve
Fixed oils & fats	-ve	-ve	+ve	+ve

The extraction yield calculated for chloroform, methanol, ethanol and aqueous extracts of selected constituents showed that methanol extract registered higher percentage of yield. It

may be due to high polarity of methanolic solvent which can draw high variety of plant constituents than the other solvents did.

Generally, majority of the secondary metabolites studied in selected parts of medicinal plants have present with higher amount in methanolic extract than that of the other alcoholic, chloroform and aqueous solvents. It is explained that the polarity level and species nature are playing major role in extracting the secondary metabolites.

The results of physio-chemical parameters such as total ash, acid insoluble ash, are shown in (Table 2). In each case total ash value was higher than the acid insoluble ash value. Comparative account of total ash and acid insoluble ash content showed higher value of total ash of leaves of *Momordica charantia* (15.86%) and lower value of total ash of rhizome of *Curcuma longa* (8.36%) while the acid insoluble ash showed higher value of leaves of *Momordica charantia* (5.96%) and lower value of leaves of *Ocimum sanctum* (1.22%).

Table 2: Ash Analysis Values

S.No.	Plant name	Colour of Ash	Total ash(%)	Acid insoluble ash (%)
1	*A.indica*	White	8.9219	2.2692
2	*O.sanctum*	Light brown	13.5180	1.2148
3	*C.longa*	White	8.3642	1.3548
4	*M.charantia*	Light grey	15.8593	5.9522

The seasonally varied total ash range of *A.indica* from (8.6779% to 8.9219%), *O.sanctum* (13.2847% to 13.5180%), *C.longa* (8.3642% to 8.1309%), *M.charantia* (15.6261 to 15.8593) show that percentage of total ash is higher in summer then monsoon and then winter. Similar results are observed in acid insoluble ash (%) (see in table 3).

Table 3: Seasonal Variation Of Ash Values

Season	Total ash (%)				Acid insoluble(%)			
	A.indica	O.sanctum	C.longa	M.charantia	A.indica	O.sanctum	C.longa	M.charantia
Summer	8.9219	13.5180	8.3642	15.8593	2.2692	1.2148	1.3548	5.9522
Monsoon	8.2663	13.0957	7.9219	14.9952	1.8572	0.8366	0.9386	5.1428
Winter	8.6779	13.2847	8.1309	15.6261	2.1742	1.024	1.1852	5.5410

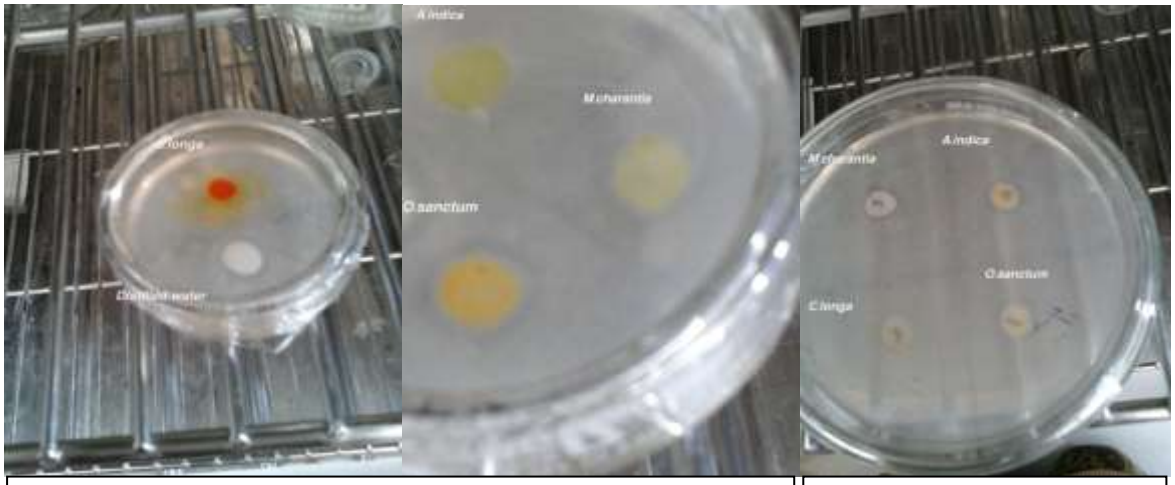
In vitro analysis of extracts

Antimicrobial susceptibility tests of methanol and aqueous extracts of *C.longa*, *A.indica*, *O.sanctum*, *M.charantia* against soil bacteria showed that methanolic extracts of all plants is more active towards it than aqueous extract showing more zone of inhibition(see in table 3).

Table 3: Minimum Inhibitory Concentration of Different Fractions Of Plants

S.No.	Name of plant	Zone of inhibition by aqueous extract	Zone of inhibition by methanol extract
1	*A.indica*	3mm	4mm
2	*O.sanctum*	4mm	6mm
3	*C.longa*	4mm	14mm
4	*M.charantia*	2mm	3mm

The range of Zone of inhibition of aqueous and methanolic plant extracts are from 3.0mm to 4.0mm for *A.indica*, 4.0mm to 6.0mm for *O.sanctum*, 4.0mm to 14.00mm for *C.longa*, 2.0mm to 3.0mm for *M.charantia*. These observations revealed that aqueous extract is least active towards soil bacteria showing less zone of inhibition than methanolic extract (see in figure 1 and graph).



(a) methanolic plant extract | **(b) aqueous extract**

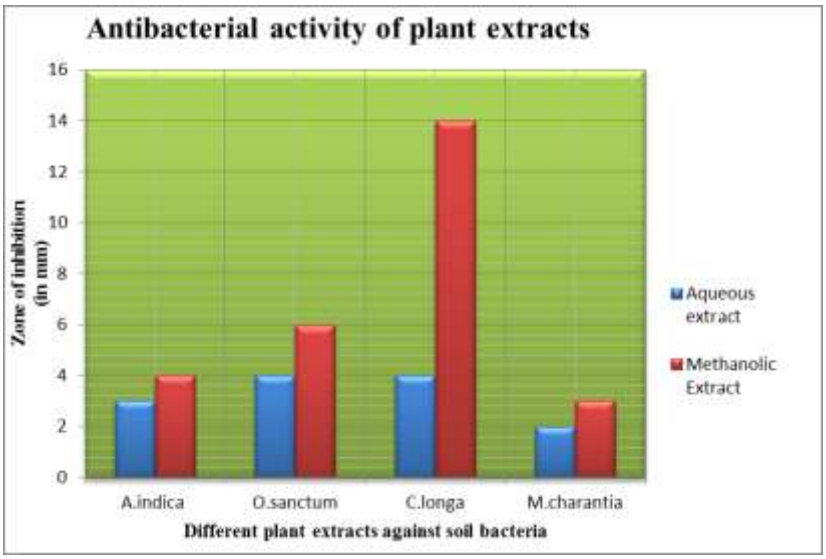


FIG: 2. GRAPHICAL REPRESENTATION OF MIC OF DIFFERENT FRACTIONS OF PLANT EXTRACTS ON SOIL BACTERIA

In aqueous extract *M.charantia* is less active whereas *O.sanctum* and *C.longa* is more active against soil bacteria. And, in methanolic extract also *M.charantia* is less active whereas *C.longa* is more active than all.

There are also some previous studies are available which shows that methanolic extract of plants is more active against most of the bacteria than aqueous extract.[1,2,3]

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REFERENCES

1. Daniel M. Method in plant chemistry and economic Botany, Kalyani publishers, New Delhi, India, 1991.
2. Harborane J.B. Phytochemical methods- A Guide to modern Techniques of Plant Analysis, Chapman and hall, London, 1998; 182-190.
3. Apisariyakal A. Vamittana Kom N. Buddhasukh D. J. Ethanopharmacol, 1995; 49: 163-169.
4. Negi PS, Jayaprakash G.K, Jagan MRL. Sararian KK. Antibacterial activity of turmeric oil: a by product from curcumin manufacture. J.Agri Food Chem, 1995; 47: 163-169.
5. Majumdan AM, Naik DG, Dandge CN, Puntambekar H.M. Antiflammatory activity of curcumamodain albino rats. Indian J. Pharmacol, 2000; 32: 375-377.
6. A.Gupta et al. Biotechnology Reports, 2015; 66: 51-55.
7. Kokate CK, Purohit AP, Gokhale SB, Practical Pharmacogonasy, second ed., Vallabh Prakashan, Delhi, 2004.
8. Akinpelu DA, Kolawole DO. Phytochemical and antimicrobial activity of leaf extract of Piliostigma thonningii (Schum.). Sci. Focus J, 2004; 7: 64-70.
9. Anderson D. Persistence of antibiotic resistant bacteria. Curr. Opin. Microbiol, 2003; 6: 452-456.
10. Ankita S, Parminder K, Ruby G. Phytochemical screening and antimicrobial assay of various seeds extract of Cucurbitaceae family. Int. J. appl. Biol. Pharm. Technol, 2012; 3(3): 401-409.
11. AOAC. Official methods of analysis, Association of Official Analytical Chemists. 15th Edition., Washington DC, USA; Baker C, Thormsberg C, 1990; 807-928. 1983.

12. Inoculums Standardization in Antimicrobial Susceptibility Tests. Evaluation of overnight age culture. J. Chin. Microbial. 17: 140-145.
13. Balandrin MF, Klocke JA, Wurtele ES, Bollinger WH. Natural plant chemicals: sources of industrial and medicinal materials. Science, 1985; 228: 1154-1160.
14. Beloin N, Gbeassor M, Akpagana K, Hudson J, de Soussa K, Koumaglo K, Arnason JT. Ethnomedicinal uses of Momordica charantia (Cucurbitaceae) in Togo and relation to its phytochemistry and biological activity. J. Ethnopharmacol, 2005; 96: 49-55.
15. Chopra RN, Nayar SI, Chopra IC. Glossary of Indian Medicinal Plants. Published by CSIR, New Delhi, 1956.
16. Ocimum sanctum. The Indian home remedy. In: Current Medical Scene, March- April 1992 [Edited and published by Rajeshwari S, Cipla Ltd., Bombay. Central, Bombay].
17. Sen P. Drugs News & Views, 1993; 1(20): 15–21.
18. Senguttuvan Jamuna et al. Asian Pac J Trop Biomed 2014; 4(1): S359-S367.
19. Khan Imran et al. Arch. Appl. Sci. Res., 2010; 2(2): 246-250.