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Phytochemical Screening of Selected Medicinal Plants and their Antibacterial Activities Against *Bacillus Substilis* and *Staphylococcus Aureus*

KEYWORDS: Phytochemicals, Medicinal plants, Secondary metabolites, Antibacterial activity

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ABSTRACT *The traditional medicine involves the use of different plant extracts or the bioactive constituents. This type of study provides the health application at affordable cost. Secondary metabolites are responsible for medicinal activity of plants. Hence in the present study phytochemical screening of some important medicinal plants was carried out. Qualitative phytochemical analysis of these plants confirms the presence of various phytochemicals like alkaloids, terpenoids, saponins, steroids, anthocyanin and tannins. The results suggest that the phytochemicals with medicinal properties can be used for curing various ailments and possess potential antioxidant and leads to the isolation of new and novel compounds. Also the study of antibacterial activity against Bacillus substilis and Staphylococcus aureus shows the resistivity power of the plants against them.*

INTRODUCTION

Humans, since ancient times, have been exploiting the nature, particularly plants in search of new drugs. This has resulting in the use of large number of medicinal plants with curative properties to treat various diseases. Nearly 80% of the world's population relies on traditional medicines for primary health care, most of which involve the use of plant extracts. In India, almost 95% of the prescriptions are plant based in the traditional systems of Unani, Ayurveda, Homeopathy and Siddha. The study of plants continues principally for the discovery of novel secondary metabolites. Around 80% of products are of plant origin.

Plant products have been part of phytomedicines since time immemorial. These can be derived from any part of the plant like bark, leaves, flowers, seeds, roots etc. which contains active components. Knowledge of the chemical constituents of plants is desirable because such information will be of value for the synthesis of complex chemical substances. Such phytochemical screening of various plants is reported by many workers. In the present work, qualitative phytochemical analysis of 25 medicinal plants is been carried.

MATERIALS AND METHODS

Plant materials

In August 2013 the plant materials was collected from the botanical garden of Padmashri Vikhe Patil College of Arts, Science and Commerce, Pravaranagar. These plants were identified with the help of the available literature and were authenticated by Dr. K. J. Salunke and Dr. A. S. Wabale, Department of Botany, Padmashri Vikhe Patil College of Arts, Science and Commerce, Pravaranagar. The collected parts of the medicinal plants were cleaned, air-dried in the shade and grinded into fine powder.

Extraction

The leaves were washed thoroughly 2-3 times with running tap water, then air dried under shade and the plant material was grinded in mixer. The powder was kept in small plastic bags with paper labeling. The grinded leaf material of 5gm was weighed using an electronic balance and were crushed in 25 ml of distilled water and kept on shaker for overnight and was filtered through Whatman No.1 filter paper. The filtrate was stored at room temperature.

Phytochemical Screening

Qualitative phytochemical screening was carried out with the following:-

- **Steroids:**
1 ml of the extract was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added to the test tube.

- **Terpenoids:**
2 ml of extract was added to 2 ml of acetic anhydride and concentration of H_2SO_4.

- **Tannins:**
2 ml of extract was added to few drops of 10% ferric chloride.

- **Saponins:**
5 ml of extract was mixed with 20 ml of distilled water and then agitated in a graduated cylinder for 15 minutes.

- **Alkaloids:**
2 ml of extract was mixed with 3ml of Dilute HCL and placed in steam bath for 5 min. Then add 1 ml of Dragendoff's reagent.

- **Phlobatannin:**
1 ml of extract was boiled in 2ml of 1% aq. HCL.

- **Quinone:**
1 ml of extract was mixed with 1 ml of concentrated sulphuric acid.

- **Glycosides:**
2 ml of extract was mixed with 3 ml of chloroform and 1 ml 10 % ammonia solution was added.

- **Flavanoids:**
3 ml of 1 % aluminium chloride solution was added to 5 ml of extract.

Testing for Antibacterial properties of plant extracts:
Agar Well Diffusion Method was used for testing the antibacterial properties of selected medicinal plants. Nutrient agar plates were inoculated with 100 µl of inoculums. Wells were created using cork borer with 6 mm diameter and filled with 100 µl of plant extracts. Plates seeded with bacteria were allowed to incubate at 37°C. Zones of inhibition were measured after 24 hours of incubation (J. Parekh et al, 2006, Dhia Hassawi et al, 2006). The contents of nutrient agar

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(1000ml.D/W) was

Peptone-5gm
Yeast extract-3gm
Nacl-5gm
Agar-15gm

Sr. No.	Bacteria used	Grown on media	Gram stain
1.	*Bacillus subtilis*	Nutrient agar	G+ve
2.	*Staphylococcus aureus*	Nutrient agar	G+ve

RESULTS AND DISCUSSIONS

▪ **Phytochemical Screening of Water Extract of Plants**

Sr. no	Name of plants	Alkaloi-ds	Saponin	Tannin	Flavon-oids	Terpen-oid	Quin-one	Glyco-side	Stero-id	Phloba-tannin
1	*Murraya koenigii*	+	-	+	+	+	+	-	+	-
2	*Terminalia arjuna*	-	+	+	+	+	+	-	+	-
3	*Clematis gauriana*	-	+	-	-	+	+	-	+	-
4	*Adathoda vasica*	+	+	+	+	+	+	-	+	-
5	*Abrus precatorius*	-	+	+	+	+	+	-	+	-
6	*Gymnema sylvestre*	-	+	-	-	+	+	-	+	-
7	*Mimosa pudica*	+	+	+	+	+	+	-	+	-
8	*Phyllanthus fraternus*	+	+	-	-	+	+	-	+	-
9	*Plumbago zeylanica*	-	+	+	-	+	+	-	+	-
10	*Asparagus racemosus*	-	+	-	-	+	+	-	+	-
11	*Barleria pronitis*	-	+	+	+	+	+	-	+	-
12	*Acacia catechu*	+	+	+	+	+	+	+	+	-
13	*Terminalia bellerica*	-	-	+	+	+	+	+	+	-
14	*Saraca indica*	-	+	+	+	+	+	+	+	-
15	*Butea monosperma*	-	+	+	+	+	+	+	+	-
16	*Santalum album*	+	+	+	+	+	+	+	+	-
17	*Aloe vera*	-	+	+	-	+	+	-	+	-
18	*Vitis spp.*	-	-	-	-	+	+	+	+	-
19	*Ficus racemosa*	-	+	+	+	+	+	+	+	-
20	*Annona squamosa*	+	+	+	+	+	+	-	+	-
21	*Withania somnifera*	+	-	-	-	+	+	-	+	-
22	*Bougainvellia spectabilis*	-	+	-	+	+	+	-	+	-
23	*Boswellia serrata*	+	+	-	+	+	+	-	+	-
24	*Clitoria ternatea*	-	+	+	+	+	+	-	+	-
25	*Mirabilis jalapa*	+	-	-	-	+	+	-	+	-
26	*Eucalyptus sp.*	+	+	+	-	+	+	-	+	-
27	*Aegle marmelos*	-	+	-	+	+	+	-	+	-
28	*Nyctanthes arbor- tritis*	-	+	-	+	+	+	-	+	-
29	*Nerium oleander*	+	+	+	+	+	+	-	+	-
30	*Annona reticulata*	+	+	+	-	+	+	-	+	-

*** '+' indicates the presence of Phytochemical**

• **Steroids:**
The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids.

• **Terpenoids:**
Formation of blue, green rings indicates the presence of terpenoids.

• **Tannins:**
A yellowish precipitate indicated the presence of tannins.

• **Saponins:**
Formation of foam indicates the presence of saponins.

• **Alkaloids:**
Cloudy orange, red slightly yellow or turbid brown colour indicates presence of alkaloids.

• **Phlobatannin:**
Red colour indicates presence of Phlobatannin.

• **Quinone:**
Red colour indicates presence of quinine.

• **Glycosides:**
Pink colour indicates presence of glycosides.

• **Flavonoids:**
Yellow colour indicates the presence of flavonoids.

▪ **Testing for Antibacterial (Gram positive) properties of plant extracts:**
Letter 'A' in the photograph indicates the antibiotic-Penicillin which was used as a standard. The plants like Terminalia arjuna, Asparagus racemosus, Acacia catechu, Terminalia bellerica, Saraca indica showed the highest zone of inhibition against Bacillus substilis gram positive bacteria.

Terminalia bellerica, Saraca indica, Butea monosperma, Aloe vera, Vitis spp., and Annona squamosa. Acacia catechu and Withania somnifera showed the highest zone of inhibition against Staphylococcus aureus gram positive bacteria.

Sr. No.	Name of plant species (Methanolic extract)	Zone of inhibition(mm)	
		Bacillus subtilis	Staphylococcus aureus
1	*Murraya koenigii*	8	17
2	*Terminalia arjuna*	10	10
3	*Clematis gauriana*	-	12
4	*Adathoda vasica*	-	7
5	*Abrus precatorius*	8	11
6	*Gymnema sylvestre*	-	12
7	*Mimosa pudica*	14	12
8	*Phyllanthus fraternus*	9	10
9	*Plumbago zeylanica*	-	11
10	*Asparagus racemosus*	12	9
11	*Barleria pronitis*	8	9
12	*Acacia catechu*	24	25
13	*Terminalia bellerica*	16	21
14	*Saraca indica*	1	11
15	*Butea monosperma*	8	12
16	*Santalum album*	9	8
17	*Aloe vera*	11	17
18	*Vitis spp.*	11	11
19	*Ficus racemosa*	9	8
20	*Annona squamosa*	9	9
21	*Withania somnifera*	18	19
22	*Bougainvellia spectabilis*	6	8
23	*Boswellia serrata*	19	10
24	*Clitoriea ternatea*	15	13
25	*Mirabilis jalapa*	10	12

+ indicates presence and – indicates absence of phytochemical.

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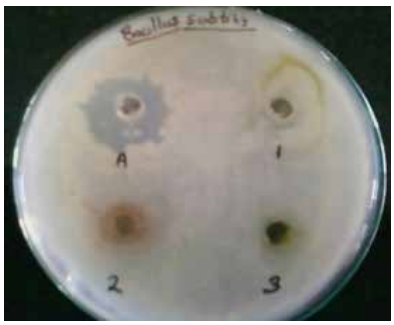
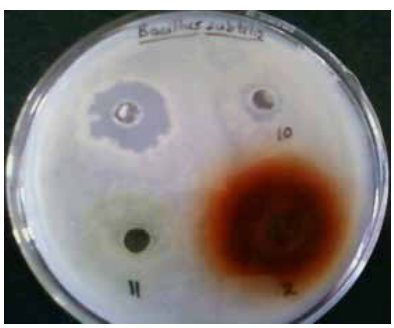
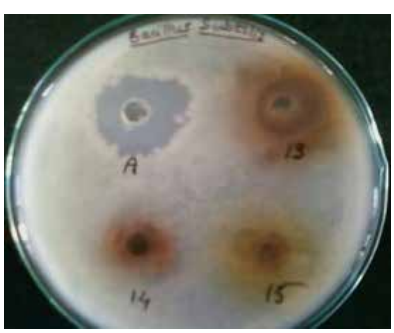
• **Photographs showing antibacterial properties of plant extracts against gram positive bacteria Bacillus substilis**

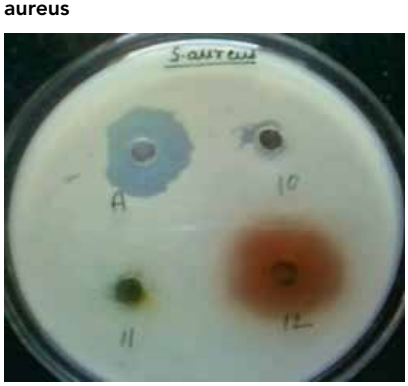


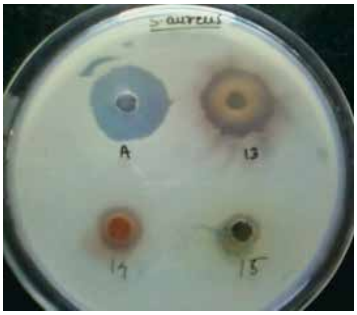


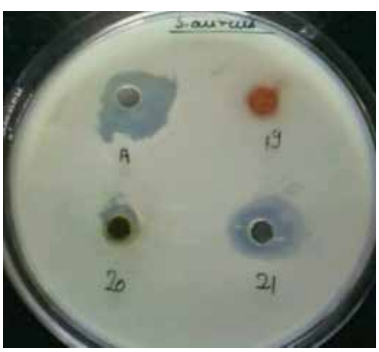


Letter 'A' in the photograph indicates the antibiotic-Penicillin which was used as a standard. The plants like Terminalia arjuna, Asparagus racemosus, Acacia catechu, Terminalia bellerica, Saraca indica showed the highest zone of inhibition against Bacillus substilis gram positive bacteria.

• **Photographs showing antibacterial properties of plant extracts against gram positive bacteria Staphylococcus aureus**







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Acacia catechu and Withania somnifera showed the highest zone of inhibition against Staphylococcus aureus gram positive bacteria.

CONCLUSION

Phytochemical screening of medicinal plants:-

Phytochemical screening of medicinal plant was done in distilled water. The results indicated that all the phytochemicals except Phlobatannin are soluble in water. Results reveal that Acacia catechu has more than 7 phytochemicals. Also Santalum album, Butea monospermous, Saraca indica, Terminalia bellerica, Barleria pronitis contain high amount of alkaloids, terpenoid, saponin, glycosides and tannins.

Acacia catechu showed 24mm and 25mm zone of inhibition in both the bacteria respectively. Terminalia bellerica, Aloe vera and Withania somnifera were found to be best in inhibiting the growth of both the bacteria.

It can be concluded that the above mentioned medicinal plant species can be used in manufacturing of antimicrobial drugs in future.

REFERENCE AJIBOYE B.O, IBUKUN E.O, EDOBOR G, OJO A.O, ONIKANNI, S.A. 2013, Qualitative and quantitative analysis of Phytochemicals in SENECIO BIAFRAE Leaf, Journal of Pharmacy and Pharmaceutical Sciences. 5: 428-432. | Akhtar, H., Virmani, O. P., Popli, S. P., Misra, L. N., Gupta, M. M., Srivastava, G. N., Abraham, Z. and Singh, A. K. 1992. Dictionary of Indian Medicinal Plants. CIMAP, Lucknow. | Edeoga, H.O. and Eriata, D.O. 2001. Alkaloid, tannin and saponin contents of some Nigeria medicinal plants. J. Med. Aromatic Plant Sci. 23:344-349. | Jain, S. K. (1991). Dictionary of Indian folk medicine & Ethnobotany, Deep Publications, New Delhi.Nadkarni, A. K. (1954). Dr. K. M. Nadkarni's Indian Material Medical, Bombay, Revised Ed. 2002. | Kakad Subhash, A. S. Wabale and M. N. Kharde., 2013. Phytochemical Screening and Antimicrobial Studies on Plumbago zeylanica L. Adv. Biores., 4 (3): 115-117. | Wabale A. S., 2007. Ethnomedicinal Studies of Selected Plants from Akole and Sangamner Talukas of Ahmednagar District. PhD Thesis, University of Pune. - 143. |