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EVALUATION OF ANTIMICROBIAL ACTIVITY OF LEAVES OF *ALOCASIA INDICA* LINN

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ABSTRACT: Antimicrobial activity of different extracts prepared with petroleum ether, chloroform, acetone, ethanol and water from leaves of *Alocasia indica* Linn. was evaluated using agar well diffusion method against gram positive, gram negative bacterial and fungal strains. Extracts were subjected for minimum inhibitory concentration assay by two-fold dilutions method, bacterial and fungal growth rate assay. Gentamicin (5 µg/ml) and Fluconazole (5 µg/ml) were used as standard for antibacterial and antifungal assay respectively. Extracts showed significant *in-vitro* antibacterial and antifungal activity. Ethanol extract was found to possess potent antimicrobial activity.
KEYWORDS: *Alocasia indica* Linn., antimicrobial activity, agar well diffusion, minimum inhibitory concentration.

INTRODUCTION

Development of numerous defense mechanisms and multidrug-resistance in pathogenic microbes against antimicrobial agents has led a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action [1-4]. Several medicinal plants can represent alternative system of medicine in management of microbial infections and can be a good source for new potent antibiotics to which pathogen strains are not resistant[5-8]. The plant *Alocasia indica* (family-*Araceae*) is used in inflammation and in diseases of abdomen and spleen [9]. The juice of the leaves of the plant is used as digestive, anthelmintic, laxative, diuretic, astringent[10]. This plant contains flavonoids, cynogenetic glycosides, ascorbic acid, gallic acid, mallic acid, oxalic acid, alocasin, amino acids, succinic acid and β-lectines [11]. Sufficient scientific data is not available to justify the traditional antimicrobial potential of the plant; the present study is aimed to validate the therapeutic use of this plant as an antimicrobial agent.

MATERIALS AND METHODS

Plant Material

Fresh leaves of *Alocasia indica* Linn. were collected from different places at Karad, Dist – Satara (Maharashtra). Leaves of *Alocasia indica* Linn. were authenticated and a voucher specimen was deposited at Botany Dept., Yashwantrao Chavan College of Science, Karad Dist- Satara (Maharashtra). The fresh leaves of *Alocasia indica* Linn.– [AI] were separated from plant, washed under running tap water and then with isopropyl alcohol (5 %) followed by distilled water. Leaves were allowed to shed dry at 30 °C and 45 % RH for 15 days and homogenized to get a coarse powder. This powder was stored in an air tight container for successive extraction.

Preparation of Extracts

Aqueous extract (by cold maceration method)

250 gm of the powder was extracted with distilled water at room temperature by cold maceration method [12]. The filtrate was collected and concentrated on heating mantle at 45 °C till a syrupy mass was

obtained. Extract was successively dried by using rotary evaporator. The extract was preserved at – 4 ˚C. The yield of the extract was found to be 6.75%.

Solvent Extraction

The petroleum ether (40˚ C- 60˚ C), chloroform, acetone, and ethanol (95 %) extracts of leaves of *Alocasia indica* Linn. were prepared by soxhletion. The powdered plant material (250 g) was repeatedly extracted in a 1000 ml round bottomed flask with 500 ml solvents of increasing polarity starting with petroleum ether. The reflux time for each solvent was 40 cycles for complete extraction. The extracts were cooled at room temperature, filtered and evaporated to dryness under reduced pressure in a rotary evaporator. The yield of different extracts prepared with petroleum ether, chloroform, acetone, and ethanol were found to be 3.45%, 2.12%, 0.79 %and 0. 53% respectively.

Phytochemical screening

A preliminary phytochemical screening of all these extracts of AI was carried out for detection of phytoconstituents[13].

Determination of antimicrobial activity

Microorganisms

The test organisms *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumonia, Escherichia coli*, *Aspergillus niger*, *Candida albicans*, *Saccharomyces cerevisiae* were procured from National Collection of Industrial Microorganisms, NCL, Pune, India.

Culture media

The antibacterial and antifungal studies were carried out using nutrient agar medium and sabouraud medium was procured from HiMedia Chemicals, Mumbai.

Evaluation of Antibacterial and Antifungal Activity [14-16]

The *in vitro* antimicrobial activity of different extracts of AI was studied by agar well diffusion method. The antibacterial studies were carried out against *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumonia, Escherichia coli*, in nutrient agar medium. Antifungal studies were carried out against *Aspergillus niger*, *Candida albicans*, *Saccharomyces cerevisiae* in sabouraud medium. The medium was sterilized by autoclaving at 120°C (15 lb/in ^{2}). 30 ml of nutrient agar medium inoculated with the respective strains of bacteria and fungi and transferred aseptically into each sterilized Petri plate. The plates were allowed to solidify at room temperature. Bacterial concentration of 1×10^8 CFU/ml was used for antibacterial activity and fungal suspension of 1×10^6 CFU/ml for antifungal activity. In each plate wells of 8 mm diameter were made using a sterile borer.

The extracts were freshly reconstituted with dimethyl sulphoxide to 5 mg/ml and 10 mg/ml concentrations. The test samples and the control (0.2 ml) were placed in 8 mm diameter well. Antibacterial assay plates were incubated at 37 ± 1°C for 24 h, whereas antifungal assay plates were incubated at 28 ± 1°C 48 h. Dimethyl sulphoxide (DMSO) was used as solvent control and maintained at the same experimental conditions. Standard antibiotics Gentamicin (500 µg/ml) and Fluconazole (500 µg/ml) were used as positive antibacterial and antifungal control respectively. Diameter of the zone of inhibition surrounding each well was recorded. The extracts that showed antimicrobial activity were subjected to minimum inhibitory concentration (MIC) assay by using serial two-fold dilution method. MIC was interpreted as the lowest concentration of the sample, which showed clear fluid without development of turbidity. All experiments were performed in triplicate.

Bacterial and Fungal Growth rate assay [17-19]

3 ml of bacterial cultures of *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli, Bacillus subtilis* and fungal cultures of *Aspergillus niger*, *Candida albicans* and *Saccharomyces cerevisiae* were added to 27 ml nutrient broth containing 0.5 ml ethanolic extract of AI (10 mg/ml). The tubes containing bacterial and fungal cultures were incubated at 30 ^{o}C and 37°C respectively for approximately 24 h with gentle shaking. The optical density tubes were measured at 550 nm and 700 nm respectively after 0, 1, 2, 4 and 8 h incubations. Dimethyl sulphoxide (DMSO) was used as solvent control and maintained at the same experimental conditions. All assays were performed in triplicate.

RESULTS

All extracts at 5 and 10 mg/ml concentration exhibited significant antimicrobial activity against Gram-positive, Gram-negative bacterial and fungal strains under test. Results are comparable with standards as shown in [Table1]. The MIC of different extracts was evaluated across a concentration range of 5 mg/ml to 20 mg/ml is as shown in [Table 2]. The lowest MIC values were observed for ethanol extract in the range of 10.23 to 13.18 mg/ml. MIC values of acetone extract 12.14 to 14.32 mg/ml, chloroform extract 11.47 to 15.46 mg/ml, petroleum ether extract 11.45 to 17.24 mg/ml and aqueous extract 12.45 to 14.16 mg/ml were observed against the microorganism under test. The results reveal that various extracts of leaves of *Alocasia indica* were effective against both Gram-positive, Gram-negative bacteria and fungi which are associated with microbial disorders.

Selected ethanolic extract with significant activity was assayed by bacterial and fungal growth rate assays for its pattern of activity. Ethanolic extract of leaves of AI at concentration of 10 mg/ml significantly inhibited growth of *Bacillus subtilis* [graph no. 1], and *Escherichia coli* [graph no. 4] within 1 h indicating a rapid antimicrobial action. Ethanolic extract of leaves of AI also found to be effective in inhibiting growth of *Staphylococcus aureus* [graph no. 2] and *Klebsiella pneumonia* [graph no. 3] profoundly in range of 2-8 h. The AI leaf extract also inhibited growth of *Aspergillus niger* [graph no. 5], *Candida albicans*

[graph no. 6] *and Saccharomyces cerevisiae* [graph no. 7]. Ethanolic extract of leaves of AI inhibited growth of *Aspergillus niger* and *Saccharomyces cerevisiae* till the end of the 4 h incubation period but growth was observed after 4 h incubation period.

DISCUSSION

Preliminary phytochemical screening of the extracts showed presence of flavonoids, cynogenetic glycosides, tannins and polyphenolic compounds. All the extracts showed antimicrobial activity on the microorganisms under test but ethanolic extract found to be more effective. It has been reported that tannins inhibit many microbial enzymes in raw culture filtrates or in purified forms [20]. The astringent property of the tannins is reported to be due to its complexation with enzymes or substrates and metal ions [21, 22]. Polyphenolic compounds are known to have antimicrobial activity possibly due to enzyme inhibition in the oxidized forms or through more non-specific interactions with the proteins. Also various secondary metabolites of plant origin are known to possess antimicrobial activity [23]. From the present investigations, we can conclude that leaves of *Alocasia indica* Linn. possess significant antimicrobial activity due to presence of various phytoconstituents and it could be a source of new antibiotic compounds. Isolation, purification and characterization of the phytochemicals responsible for the above mentioned activity are in progress.

Table 1: Antibacterial activity of *Alocasia indica* extracts.

Extracts	Conc. mg/ml	Zone of inhibition (mm)* Mean±SE						
		B.s.	**S.a.**	**K.p.**	**E.c.**	**A.n.**	**C.a.**	**S.c.**
Aqueous	5	09.15± 0.06	12.32± 0.09	12.11± 0.09	08.42± 0.19	10.23± 0.12	11.35± 0.15	11.27± 0.32
	10	11.18± 0.09	13.22± 0.12	13.33± 0.15	11.38± 0.23	12.15± 0.16	12.15± 0.14	14.14± 0.10
PE	5	12.07± 0.07	11.22± 0.08	10.21± 0.04	09.22± 0.09	14.24± 0.15	10.65± 0.22	12.27± 0.22
	10	13.21± 0.15	12.34± 0.16	12.13± 0.10	12.18± 0.13	15.15± 0.11	13.27± 0.24	14.34± 0.12
Chloroform	5	11.17± 0.09	10.12± 0.05	12.10± 0.09	10.22± 0.09	10.34± 0.12	13.15± 0.12	15.27± 0.32
	10	12.11± 0.12	13.24± 0.10	14.13± 0.12	13.18± 0.13	11.85± 0.17	15.27± 0.17	17.14± 0.22
Acetone	5	15.24± 0.15	11.47± 0.15	12.23± 0.11	13.12± 0.12	11.2± 0.09	12.37± 0.15	14.17± 0.13
	10	17.21± 0.11	12.34± 0.10	16.24± 0.16	15.13± 0.16	13.7± 0.15	14.37± 0.21	15.24± 0.21
Ethanol	5	16.47± 0.14	12.57± 0.12	14.30± 0.21	16.23± 0.08	13.4± 0.11	15.37± 0.14	16.37± 0.06
	10	22.15± 0.24	16.23± 0.12	17.14± 0.16	19.31± 0.09	15.21± 0.14	16.23± 0.12	18.11± 0.10
Gentamicin	0.5	27.21± 0.11	24.11± 0.12	20.21± 0.08	22.15± 0.24	NT	NT	NT
Fluconazole	0.5	NT	NT	NT	NT	20.16± 0.09	23.21± 0.08	24.31± 0.08

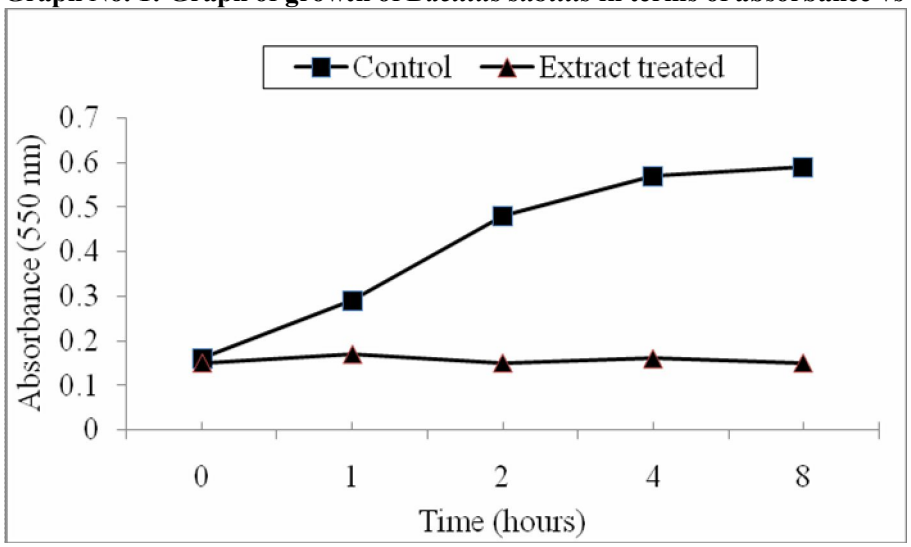
*Each value is Mean±SE of 3 assays, value is significantly different when $p<0.05$. NT- not tested. PE-Petroleum ether extract. B.s.-*Bacillus subtilis*; S.a.-*Staphylococcus aureus*; K.p.-*Klebsiella pneumonia,* E.c.-*Escherichia coli*; A.n.-*Aspergillus niger*; C.a.-*Candida albicans*; S.c.-*Saccharomyces cerevisiae*

Table 2: Minimum inhibitory concentrations (mg/ml) of *Alocasia indica* extracts

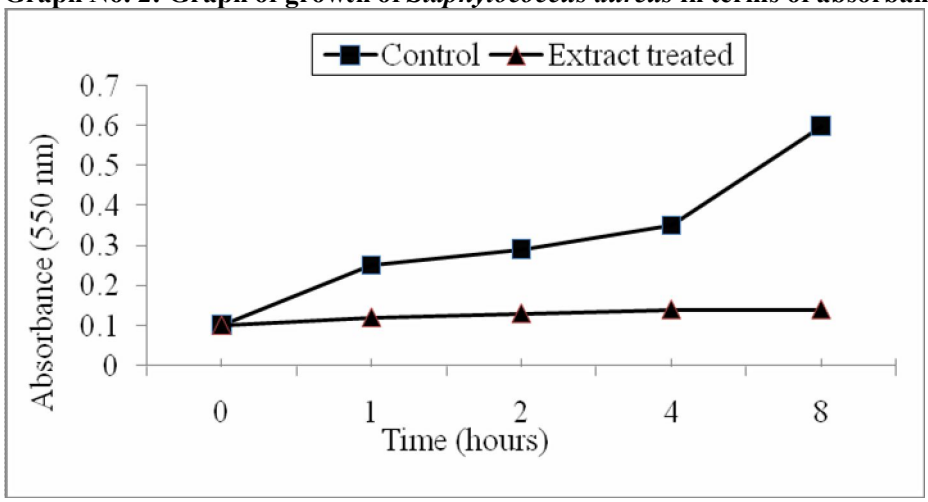
Microorganisms	MIC (mg/ml) Extracts				
	Aqueous	PE	Chloroform	Acetone	Ethanol
B.s.	NT	11.45	14.30	13.67	10.23
S.a.	13.25	11.56	NT	12.45	14.23
K.p.	13.56	13.23	11.47	12.65	13.18
E.c.	NT	13.47	14.54	14.32	11.23
A.n.	13.45	16.25	15.46	12.14	12.23
C.a.	14.16	17.24	12.32	13.26	12.63
S.c.	12.45	13.18	15.42	12.117	13.18

Each value is mean of 3 assays. NT- not tested. PE- Petroleum ether extract. B.s.-*Bacillus subtilis*; S.a.-*Staphylococcus aureus*; K.p.-*Klebsiella pneumonia,* E.c.-*Escherichia coli*; A.n.-*Aspergillus niger*; C.a.-*Candida albicans*; S.c.-*Saccharomyces cerevisiae*

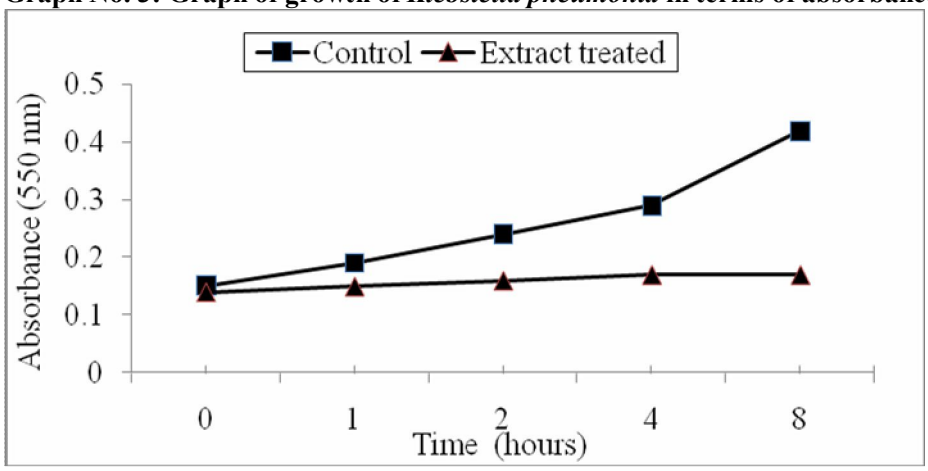
Graph No. 1: Graph of growth of *Bacillus subtilis* in terms of absorbance vs time (hours).



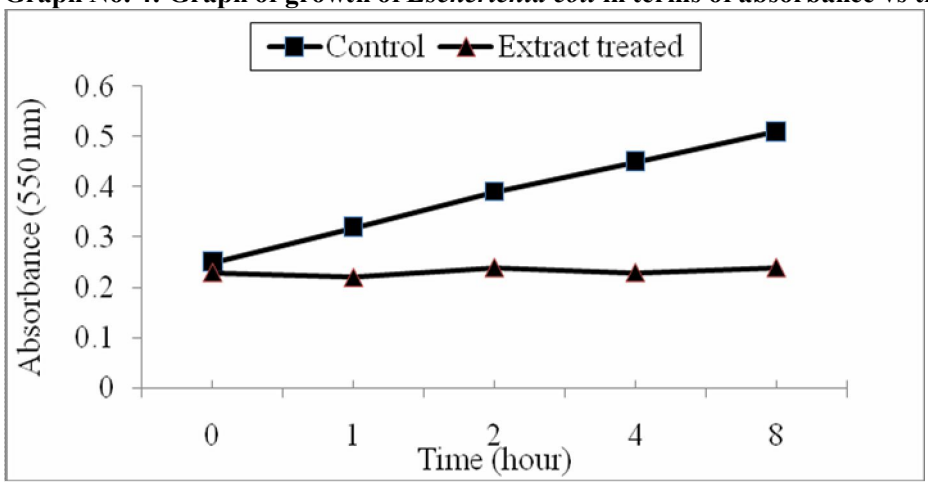
Graph No. 2: Graph of growth of *Staphylococcus aureus* in terms of absorbance vs time (hours).



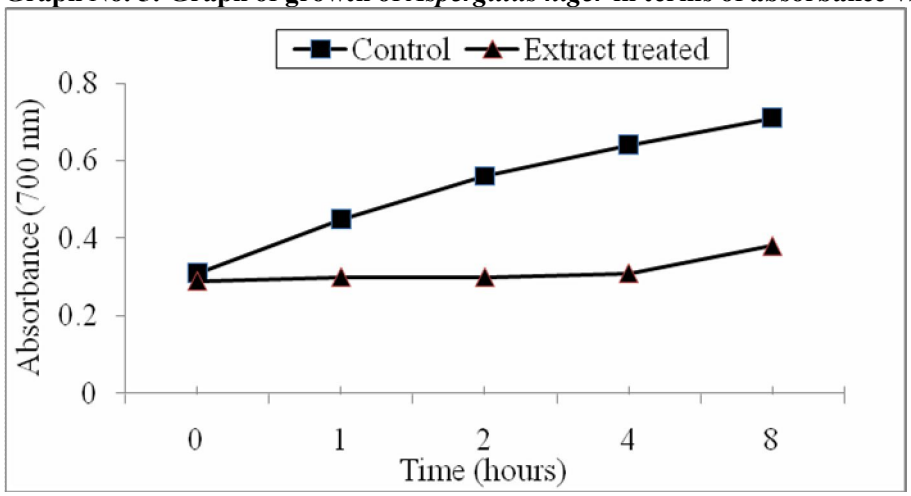
Graph No. 3: Graph of growth of *Klebsiella pneumonia* in terms of absorbance vs time (hours).



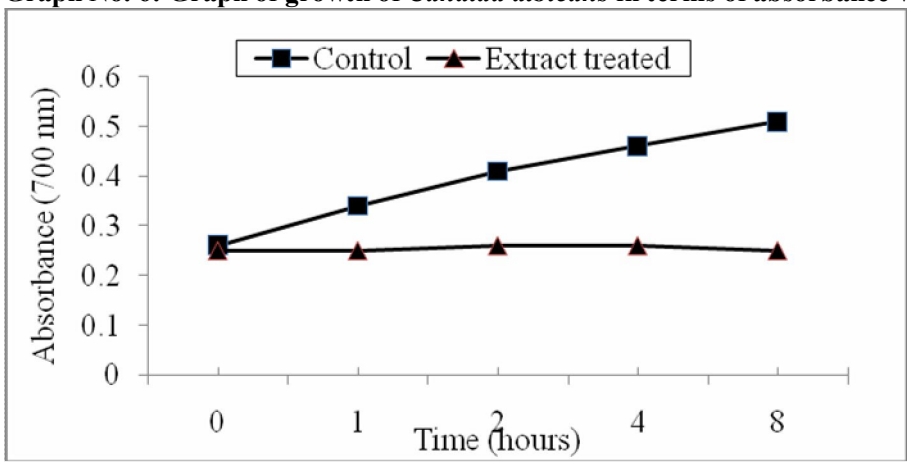
Graph No. 4: Graph of growth of *Escherichia coli* in terms of absorbance vs time (hours).



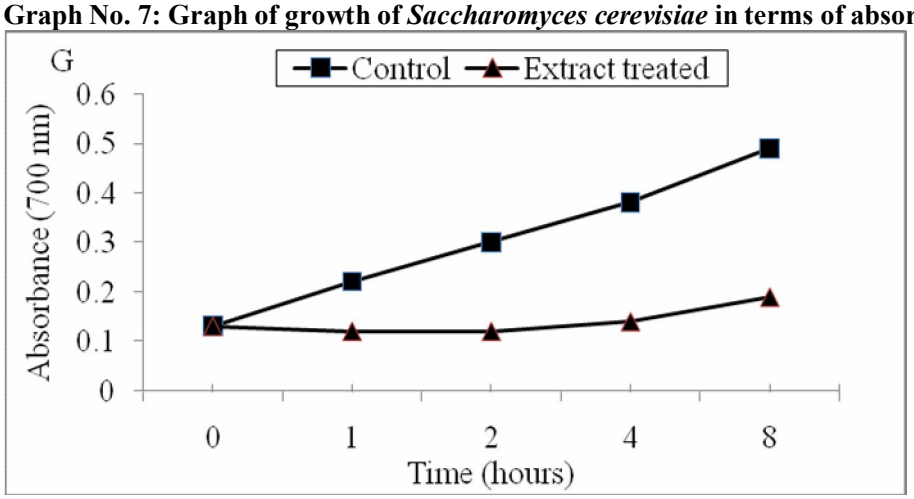
Graph No. 5: Graph of growth of *Aspergillus niger* in terms of absorbance vs time (hours).



Graph No. 6: Graph of growth of *Candida albicans* in terms of absorbance vs time (hours).



Graph No. 7: Graph of growth of *Saccharomyces cerevisiae* in terms of absorbance vs time (hours).



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REFERENCES

1. Rojas R., Bustamante B., Bauer J., Fernandez I., Alban J., Lock O., Antimicrobial activity of selected Peruvian medicinal plants, J. Ethnopharmacol., 2003, 88,199-204.
2. Hamburger M., Hostettmann K., Bioactivity in plants: the link between phytochemistry and medicine, Phytochemistry, 1991, 30, 3864-74.
3. Springfield E.P., Amabeoku G., Weitz F., Mabusela W., Johnson Q., An assessment of two Carpobrotus species extracts as potential antimicrobial agents, Phytomed., 20, 434-9.
4. Kloucek P., Polesny Z., Svobodova B., Vlkova E., Kokoska L., Antibacterial screening of some Peruvian medicinal plants used in Calleria District, J. Ethnopharmacol., 2005, 99, 309-12.
5. El-Seedi H.R., Ohara T., Sata N., Nishiyama S., Antimicrobial terpenoids from *Eupatorium glutinosum* (Asteraceae), J. Ethnopharmacol., 2002, 81, 293-6.
6. Duraipandiyan V., Ayyanar M., Ignacimuthu S., Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India, BMC Complement. Altern. Med., 2006, 6, 35-41.
7. Parekh J., Chanda S., *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plants, Turk. J. Biol., 2007a, 31, 53-8.
8. Wilson B., Abraham G., Manju V.S., Mathew M., Vimala B., Sundaresan S., Antimicrobial activity of *Curcuma zedoaria* and *Curcuma malabarica* tubers, J. Ethnopharmacol., 2005, 99, 147-51.

9. Kirtikar K.R., Basu B.D., *Alocasia indica* in Indian medicinal plants, vol.4, 2nd edition, (M/s Bishen Singh Mahendrapal Singh, New Cannaught place, Dehradun), 2617.
10. Nadkarni K.M., Basu B.D., *Alocasia indica* in Indian Material Medica (Popular prakashan Ltd, Mumbai, India). 72.
11. Prajapati N.D., *Alocasia indica* in A Handbook of Medicinal plants (Updesh Purohit for Agrobois India) sec 2, 32.
12. Organization Mondiale De La Sante, Quality control methods for medicinal plants materials, World Health Organization, Geneva, 1998, 32.
13. Khandelwal K.R., Practical Pharmacognosy Techniques and Experiments, 9th ed. Nirali Prakashan, Pune, 2000.
14. Mandal S.M., Mondal K.C., Dey S., Antimicrobial activity of the leaf extracts of *Hyptis suaveolens* (L.) poit., Ind. J. Pharm. Sci., 2007, 4, 568-569.
15. Mukherjee P.K., Balasubramanian R., Saha K., Saha B.P. and Pal M., Antibacterial efficiency of *Nelumbo nucifera* (Nymphaeaceae) rhizomes extract, Indian Drugs, 1995, 32**,** 274–276.
16. Jones R.N., Barry A.L., Gavan T.L., Microdilution and macrodilution broth procedures. In: Lennette E.H., Balows A., Hausler W.J., Editors, Manual of Clinical Microbiology, American Society for Microbiology, Washington, DC, 1985, 972–977.
17. Cock I.E., Antimicrobial activity of *Eucalyptus major* and *Eucalyptus baileyana* methanolic extracts, The Internet Journal of Microbiology, 2009, 6, 1.
18. Ciara Ignacio, Dorothy Thai, Comparative analysis of antifungal activity of natural remedies versus miconazole nitrate salt against *Candida albicans,* California Polytechnic State University, San Luis Obispo, 2005.
19. Timothy C., Michael F., and William M., Monitoring of Filamentous Fungal Growth by In Situ Microspectrophotometry, Fragmented Mycelium Absorbance Density, and 14C Incorporation: Alternatives to Mycelial Dry Weight, Applied and Environmental Microbiology, 1985, 101-108.
20. Jones G.A., McAllister T.A., Muir A.D., Cheng K.J., Effects of sainfoin (*Onobrychis viciifolia* scop.) condensed tannins on growth and proteolysis by four strains of ruminal bacteria, *Appl. Environ. Microbiol.,* 1994, 60,1374–8.
21. Mason T.L., Wasserman B.P., Inactivation of red beet betaglucan synthase by native and oxidized phenolic compounds, *Phytochemistry* 1987, 26, 2197–202.
22. Chung K.T., Lu Z., Chou M.W., Mechanism of inhibition of tannic acid and related compounds on the growth of intestinal bacteria., *Food. Chem. Toxicol.* 1998, 36, 1053–60.
23. Marjorie M.C., Plant products as antimicrobial agents, *Clin. Microbiol. Rev.*1999, *1*2, 564–83.

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