

***Coleus barbatus* as a Potent Antimicrobial Agent Against Some Gastro-Intestinal Pathogens**

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ABSTRACT Plants have been used as traditional medicines since a long time against microbial diseases. Nowadays there is much stress to identify and isolate antimicrobial agents which could be found fruitful against microbial diseases. In the present study, *Coleus barbatus* extracts in different solvents were tested against some gastrointestinal pathogens. In the case of *Escherichia coli*, the inhibition was recorded by the treatment of all the extracts. *Staphylococcus aureus* was found to be resistant against hexane extract while ethanol extract inhibited the growth of *S. aureus*. Water extract showed no activity against *Pseudomonas aeruginosa* in comparison with control. In the case of *Salmonella typhimurium*, all extracts showed good inhibitory effects with respect to the control, maximum inhibition was found in case of water extract. In the case of *Staphylococcus epidermidis* in comparison to control, water extract was showing more inhibition than other extracts.

INTRODUCTION

Plants are the best friends of human being dedicating to humanity without selfishness. They are a good source of medicines. The natural plant products could be potential alternatives for controlling the pathogen associated with diseases. Natural products and their derivatives represent more than 50 % of the drugs in clinical use in the world (Cowan 1999). One of the paramount reasons for pursuing natural products chemistry resides in the actual or potential pharmacological activity to be found in alkaloids, terpenoids, coumarins, flavanoids, lignans, glycosides etc. Antimicrobial, antioxidant and anti-inflammatory activities of some plants were investigated (Mathur et al. 2010 a, b; 2011). *C. barbatus* belongs to the family Labiatae. Its leaves are ovate or obovate and glandular hairy. The flowers are purple or pale blue in colour. The flowers appear in spike like terminal racemes. The flowering season is from August to September. In Ayurveda, the stem and roots are shown to be used as a cure for gastric problems. They help in digestive system (Kirtikar and Basu

1991). Hypertension, glaucoma, inflammation and piles can be treated with this herb (Han et al. 2005). *Coleus* species have been used to treat heart disease, convulsions, spasmodic pain and painful urination (Pande et al. 2007). Hepato-protective effect of water soluble extract of *Coleus barbatus* on cholestasis on young rats was investigated (Joy et al. 1998). Antibacterial activities of some *Coleus* species were reported (Lans 2007; Senthilkumar et al. 2010; Singh et al. 2010). Diterpenes and Forskolol are obtained from *Coleus barbatus* (Leamon et al. 1981; De Souza et al. 1983).

MATERIAL AND METHODS

Chemicals and Glass Wares

All the chemicals and reagents used were of analytical grade and were procured from Ranchem and CDH, India. The medium used for the growth of bacterial cultures was obtained from Hi-Media Pvt. Ltd., India. The glass and plastic wares used were properly sterilized and were procured from Borosil, India.

Pathogenic Organisms Used for the Study

Skin and gastrointestinal pathogens used to study the antibacterial experiments were collected from National Chemical Laboratory (NCL), Pune and maintained under standard

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Table 1: Results (OD) of Broth dilution test at 100 µl concentration

Name of the organism	Control*	Hexane extract*	Chloroform extract	Ethanol extract*	Water extract*
<i>E. coli</i>	1.58	1.45	1.50	1.36	1.44
<i>P. aeruginosa</i>	1.81	1.62	1.56	1.38	1.81
<i>S. aureus</i>	1.87	1.59	1.81	1.75	1.69
<i>S. epidermidis</i>	1.67	1.41	1.58	1.34	1.40
<i>S. typhimurium</i>	1.73	1.73	1.70	1.51	1.52

laboratory conditions in nutrient agar. The organisms used for the study were: *Staphylococcus aureus* (NCIM-2079), *Staphylococcus epidermidis* (NCIM-2493), *Salmonella typhimurium* (NCIM-2501), *Escherichia coli* (NCIM-2065) and *Pseudomonas aeruginosa* (NCIM-2036).

Collection of Plant Material and Preparation of Solvent Extracts

The whole plant of *Coleus barbatus* was collected from the field of ICFRE, Dehradun (U.K), India and dried at room temperature for three days in the hot air oven. Dried plant material was grinded in the grinder for three times. Powdered dried plant material was soaked in different solvents (Alade and Irobi 1993).

Determination of Antimicrobial Activity

The antimicrobial activity of the extracts of *Coleus barbatus* were determined by Broth dilution method (Barry et al. 1999). Broth dilution test was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of antimicrobial extracts. Overnight cultures of test bacterial culture grown in nutrient broth were diluted 100 folds in Nutrient broth (100 µl bacterial culture in 10 ml NB). 100 µl extracts were added to tubes containing the bacterial cultures, to know the minimum inhibitory concentration in a particular tube, inhibiting the bacterial growth. The tubes were incubated at 37 °C for 18-24 hours. The tubes were examined for visible turbidity and optical densities of cultures were determined at 520 nm using Nutrient broth as control. The tubes with the lowest concentration of the extract and showing growth inhibition were taken as minimum inhibitory concentration of that particular extract while minimum concentration of the extracts that kills the bacterial growth were taken as Minimum Lethal Concentration (MLC).

RESULTS

Antibacterial activities of the plant *Coleus barbatus* was done by Broth Dilution Method. The results obtained were very interesting as shown in Table 1 and Figure 1. Varied results were obtained with plant extracts in different solvents. All the experiments were repeated thrice. In the case of *Escherichia coli*, (Table 1 and Fig. 1) the growth of this bacteria was inhibited by all extracts in comparison to control (O.D. - 1.58), but ethanol extract (O.D.-1.36) was found to show more inhibition than other extracts. Water extract (O.D.-1.44) and hexane extract (O.D.-1.45) showed nearly same inhibitory effect. Chloroform extract (O.D.-1.50) showed least inhibitory effect. According to Anova table (Table 2) due to treatment F. Calculated value is 32.59, whereas F. Table value 5% is 3.84. So, it showed significant results.

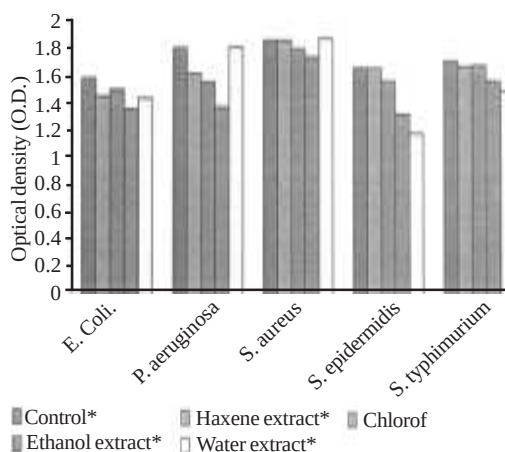


Fig. 1. Antibacterial properties of all extracts of *Coleus barbatus* by Broth dilution test

In case of *Staphylococcus aureus*, (Table 1 and Fig. 1), it was found to be resistant to hexane extract (O.D.-1.87) with respect to control (O.D.-1.87). Chloroform extract (O.D.-1.81) showed little inhibition. While ethanol extract

Table 2: Effect of all extract on *E. coli* by Broth dilution test

Source of variation	d.f.	S.S.	M.S.S.	F.Cal.	F.Tab 5%	Result
Due to treatment	4	0.06	0.01	32.59	3.84	S
Due to replication	2	0.0005	0.0002	0.62	4.46	NS
Due to error	8	0.003	0.0004			
Total	14	0.064	0.004			

(O.D.-1.75) was found to inhibit the growth of *S. aureus*. Water extract (O.D.-1.89) showed no effect on it. According to Anova table (Anova Table 3) due to treatment F. Calculated value was 815.01, whereas F. Table value 5% was 3.84. So, it showed significant results.

In case of *Pseudomonas aeruginosa* (Table 1 and Fig. 1), in comparison to control (O.D.-1.81) water extract (O.D.-1.82) showed resistance towards these bacteria. Ethanol extract (O.D.-1.38) showed more inhibition than other extracts. Chloroform extract (O.D.-1.56) showed more inhibition than hexane extract (O.D.-1.62). According to Anova table (Anova Table 4) due to treatment F. Calculated value was 62.43, whereas F. Table value 5% is 3.84. So, it showed significant results.

In case of *Salmonella typhimurium* (Table 1 and Fig. 1), all extract shows good inhibitory effect with respect to control (O.D.-1.73) Maximum inhibition was found was case of water extract (O.D.1.52). Ethanol extract (O.D.-1.59) also showed good inhibitory effect but shows

less inhibition than water extract. Chloroform extract (O.D.-1.70) and Hexane extract (O.D.-1.69) showed very less inhibition. According to Anova table (Anova Table 5) due to treatment F. Calculated value was 424.83, whereas F. Table value 5% is 3.84. So, it showed significant results.

In case of *Staphylococcus epidermidis* (Table 1 and Fig. 1) in comparison to control (O.D.-1.67), Water extract (O.D.-1.2) was showing more inhibition than other extract. Ethanol extract (O.D.-1.34) was also showing good inhibition. Chloroform extract (O.D.-1.58) was least effective and Hexane extract was showing resistance. According to Anova table (Anova Table 6) due to treatment F. Calculated value was 3212.08, whereas F. Table value 5% is 3.84. So, it showed significant results.

DISCUSSION

All the solvent extracts showed antibacterial activity with varying behaviour. Some extracts

Table 3: Effect of all extract on *P. aeruginosa* by Broth dilution test

Source of variation	d.f.	S.S.	M.S.S.	F.Cal.	F.Tab 5%	Result
Due to treatment	4	0.41	0.10	815.01	3.84	S
Due to replication	2	0.001	0.0007	5.73	4.46	NS
Due to error	8	0.001	0.0001			
Total	14	0.41				

Table 4: Effect of all extract on *S. aureus* by Broth dilution test

Source of variation	d.f.	S.S.	M.S.S.	F.Cal.	F.Tab 5%	Result
Due to treatment	4	0.03	0.009	62.43	3.84	S
Due to replication	2	0.001	0.0006	4.40	4.46	NS
Due to error	8	0.001	0.0001			
Total	14	0.039	0.002			

Table 5: Effect of all extract on *S. typhimurium* by Broth dilution test

Source of variation	d.f.	S.S.	M.S.S.	F.Cal.	F.Tab 5%	Result
Due to treatment	4	0.10	0.025	424.83	3.84	S
Due to replication	2	0.0002	0.0001	2.11	4.46	NS
Due to error	8	0.0004	0.00006			
Total	14	0.102				

Table 6: Effect of all extract on *S. epidermidis* by Broth dilution test

Source of variation	d.f.	S.S.	M.S.S.	F.Cal.	F.Tab 5%	Result
Due to treatment	4	0.51	0.128	3212.08	3.84	S
Due to replication	2	0.0001	0.00006	1.38	4.46	NS
Due to error	8	0.0003	0.00003			
Total	14	0.51				

showed antibacterial potential against particular bacteria but some extracts showed altered behavior. The methanol extract of *Coleus barbatus* showed antibacterial activity against microorganisms at a dose of 200 mg/ml. All the solvent extracts showed antibacterial properties. In Broth dilution only 100 ml concentration of all extracts were used to determine antimicrobial activity. In case of *E. coli*, ethanol extract showed maximum potency but in case of *S. aureus* hexane extract showed maximum potency. But only ethanolic leaf extract was giving good results against *S. typhimurium* and *S. epidermidis*. The whole plant except roots was used to prepare the extracts. In Broth dilution test we used only 100 ml concentration for these bacteria. In case of *S. epidermidis* ethanolic extracts showed maximum potency. Chloroform extracts showed resistance against most of the pathogens.

In case of *P. aeruginosa*, it was showing resistance against water extract. All other extracts were showing good antibacterial properties ethanol extracts was showing best results. These all bacteria are pathogenic so we can use *Coleus barbatus* to prepare the medicine for cure of these bacteria.

All observation and statistical data showed a clear-cut view about the activity of different extracts. All extracts were effective against all of these bacteria but only *S. aureus* was showing resistance against hexane extract. Ethanol extract was showing very good inhibitory effect against these bacteria. The results are in accordance with previous studies (Palombo and Semple 2001; Senthilkumar et al. 2010).

REFERENCES

- Alade PI, Irobi ON 1993. Antimicrobial activities of crude leaf extracts of *Acalypha wilkensisiana*. *Journal of Ethnopharmacology*, 39: 171-174.
- Barry AL, Craig WA, Nadler H, Reller LB, Sanders CC, Swenson JM 1999. Methods for determining bactericidal activity of antimicrobial agents: Approved guidelines. *NCCLS Standards and Guidelines*, 19(18): 1-29.
- Cowan M 1999. Plant products as antimicrobial agents. *Clinical Microbiol Rev*, 12(4): 564-582.
- De Souza NJ, Dohadwalla AN, Reden J 1983. Forskolol: A Labdane Diterpenoid with antihypertensive, positive inotropic, platelet aggregation inhibitory and adenylate cyclase activating properties. *Medicinal Res Rev*, 3(2): 201-219.
- Han LK, Morimoto C, Yu RH, Okuda H 2005. Effects of *Coleus forskohlii* on fat storage in ovariectomized rats. *Yakugaku Zasshi*, 125: 449-453.
- Joy PP, Thomas J, Mathew S, Skaria BP 2001. Medicinal plants. In: T K Bose, J Kabir, P Das, P P Joy (Eds.): *Tropical Horticulture*. Vol. 2. Calcutta: Naya Prakash, pp. 449-632.
- Kirtikar KR, Basu BD 1991. *Indian Medicinal Plants*. Vol.3. Dehra Dun: Singh B and Singh MP. Publishers, pp. 20-32.
- Lans C 2007. Ethnomedicines used in Trinidad and Tobago for reproductive problems. *Journal of Ethnobiology and Ethnomedicines*, 3(13): 1-12.
- Leamon KB, Padgett W, Daly JW 1981. Forskolol: Unique Diterpene activator of adenylate cyclase in membrane and intact cells. *Proc Nat Acad Sci USA*, 78: 3363-3367.
- Mathur A, Verma SK, Purohit R, Singh SK, Mathur D, Prasad GBKS, Dua VK 2010a. Pharmacological investigation of *Bacopa monnieri* on the basis of antimicrobial, antioxidant and anti-inflammatory properties. *Journal of Chemical and Pharmaceutical Research*, 2(6): 191-198.
- Mathur A, Prasad GBKS, Dua VK 2010 b. Screening of some Indian plants for their antibacterial and antifungal properties. *Flora and Fauna*, 6(2): 166-170.
- Mathur A, Prasad GBKS, Dua VK 2011. Anti-inflammatory activity of leaves extracts of *Murraya koenigii* L. *International Journal of Pharma and Biosciences*, 2(1): 541-544.
- Palombo EA, Semple SJ 2001. Antibacterial activity of traditional medicinal plants. *J Ethnopharmacology*, 77: 151-157.
- Pande PC, Tiwari L, Pande HC 2007. Ethoveterinary plants of Uttaranchal- A review. *Indian Journal of Traditional Knowledge*, 6(3): 444-458.
- Senthilkumar CS, Suresh Kumar M, Pandian MR 2010. *In vitro* antibacterial activity of crude leaf extracts from *Tecoma stans*, *Coleus forskohlii* and *Pogostemon patchouli* against human pathogenic bacteria. *International J Pharm Tech Res*, 2(1): 438-442.
- Singh A, Sharma PK, Garg G 2010. Natural products as preservatives. *International Journal of Pharma and Biosciences*, 1(4): 601-612.