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Comparative screening of antimicrobial activity using ethanolic extract of *Canscora decurrens* Dalz. obtained from soxhlet and microwave

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Abstract

The present study was designated to investigate antimicrobial activity by using whole plant ethanolic extract (100mg/ml) of *C. decurrens*. The extracts were screened for antibacterial and antifungal activity by disc diffusion method and extraction was done with the help of modern method (microwave) and conventional method (soxhlet) against bacterial strains *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and antifungal strains *Aspergillus niger*, *Rhizoctonia solani* selected for antifungal activity. In microwave extract observed maximum antibacterial activity against bacterial strain *E. coli* (10.07), *P. aeruginosa* (10.65) and *S. aureus* (10.37) as compared to soxhlet extract similarly in case of fungi, *Rhizoctonia solani* (10.32) and *Aspergillus niger* (10.93) screening maximum inhibition zone in microwave extract as compared to soxhlet extract. The result clearly prove that microwave extracts of *C. decurrens* was found to have more potential than soxhlet extract in the tested microorganisms.

Keywords: Antimicrobial, *C. decurrens*, soxhlet, microwave, disc diffusion method

1. Introduction

C. decurrens belongs to family Gentianaceae commonly known as “shankhpushpi” it is a potential medicinal plant known to cure large number of disorders of central nervous system. Although its medicinal uses are well documented in old literature [1] it is not reported to have been prescribed or used in commercial formulation used for nerve activity. The alcoholic extract of *C. decurrens* is able to cure a number of disorders of CNS particularly concentration problem, learning disorders, weak school performance, refusal of school, dyslexia, mental retardation development retardation and delayed speech development, aberration, mental dementia and age related dementia, Alzheimer’s diseases, dysmnnesia, fragile x syndrome, depression, schizophrenia, affective psychosis, mania, states of anxiety and panic, hyperkinetic behavioral disorder among children and adults, hallucination, compulsive washing behavior abnormalities, anorexia nervosa, lack of motivation, borderline syndrome autism, bipolar affective disorder, mental impairment, stage fright, nightmare, tic disorder and lack of self confidence [2]. In *C. decurrens* is rich in different phytochemical like major phenolic compounds namely anthracene, coumarin, flavonoids, xanthenes and phenolic acids and terpenoids as well as alkaloids etc in *C. decurrens* signify its high medicinal potential as each of these groups show a variety of pharmacological activities in human beings [6, 9].

Medicinal plants with antimicrobial activity are known to offer protection against various bacterial, viral and other diseases and find industrial application [10]. In the past few years a number of investigations have been conducted worldwide to prove antimicrobial activities from medicinal plants. Medicinal plants would be the best source to obtain a variety of drugs. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. The use of plant extracts and phytochemicals with known antimicrobial properties is of great significance. 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 [13].

One of the objectives of this study was to determine comparative antimicrobial activity against ethanolic extracts extracted from soxhlet and microwave of *C. decurrens* against some bacteria and fungus like, *Escherichia coli* and *Staphylococcus aureus* and *Pseudomonas aeruginosa* and *Aspergillus niger*, *Rhizoctonia solani*.

2. Materials and method

Antimicrobial activity was done by using ethanolic extract of *C. decurrens* obtained from soxhlet and microwave against bacterial strains (*Escherichia coli* (NCIM 2685) *Pseudomonas aeruginosa* (NCIM 2036), *Staphylococcus aureus* (NCIM 5021) bacterial strains were

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obtained from National chemical laboratory, Pune and antifungal strains *Aspergillus niger*, *Rhizotonia solani* selected for antifungal activity.

2.1 Extraction

Two types of extraction methods were used (1) By soxhlet extractor for 12hrs (2) By microwave oven for 4 minutes.

- Soxhlet extraction: 5 gram powder was taken and extracted with 150 ml of ethanol at (60 °C) for 12 hrs. The extract was filtered with whattmanno.1 filter paper and evaporated at room temperature and residue was weighed and dissolved suitably in alcohol so that stock of 0.5gm\ml concentration was obtained. It was stored in amber coloured bottle in refrigerator for detection of different phytochemical groups [5].
- Microwave extraction: 500 mg powder was taken in and extracted 15 ml ethanol, mixed well and placed in the water bath in microwave (Kenstar3D power and set at 60%) in 8 cycles of 30sec for 4 min [7]. The extract was filtered with cotton and evaporated at room temperature. The residue was weighed, stored in labeled amber coloured bottles in the refrigerator and used for the detection of different phytochemical groups.

2.2 Composition for solid media

(a) Muller Hinton agar medium (Himedia) was used for bacterial culture, containing following constituents.

Beef infusion solids- 4.0 g\ lit

Starch- 1.5 g\ lit

Casein hydrolysate- 17.5 g\ lit

Agar- 15.0 g\ lit

Final pH 7.4 +/- 0.2 at 37 °C

38 gm powder of above medium was suspended in 1 liter distilled water and was boiled to dissolve the medium completely. It was autoclaved at 121 °C and 15 lbs pressure for 15 min.

(b) Composition of PDA agar medium for fungal culture (Himedia) was as follows:

Potatoes, infusion from 200. g\ lit

Dextrose 20. g\ lit

Agar 15. g\ lit

Final pH (at 25 °C) 5.6±0.2

39 grams in medium was suspended 1000 ml distilled water. Solution was heated to boiling to dissolve the medium completely and sterilized by autoclaving at 15 lbs pressure (121 °C) for 15 minutes.

2.3 Application for test sample

Mullar Hinton agar and potato agar media were poured in sterilized petriplate and allowed to solidify under aseptic condition. Antimicrobial susceptibility was studied by the Kirby Bauer disc diffusion technique. Discs of 6mm diameter were cut out from WhattmanNo.1 filter paper. and were saturated with 100mg/ml of ethanolic extract (obtained from soxhlet and microwave).For negative control filter paper disc were saturated with absolute ethanol.100µl cultures were spread on the agar plate then impregnated disc were placed and kept for incubation, at 37 °C for 24hr in case of bacteria and 45hr for fungi Ciprofloxacin(5mcg/disc), and bavistin (10 mg/ml)(filter paper disc) were used as positive control to compare activity of standard antibiotics and ethanolic extract against test organisms. After incubation the antimicrobial activity of test sample was determined by measuring the diameter of zone of inhibition (mm).

2.4 Statistical Analysis

The mean and ANOVA was calculated by using SPSS version 21 with one-way analysis followed by Tukey's multiple comparison test and significance was determined at $p < 0.05$.

3. Results

It was interestingly to that when the plants are routinely grown on MS media, occasional contamination of medium by bacteria or fungi resulted in luxurious growth of certain bacteria or fungi on the medium however, growth of seedling growing in the infected medium was not affected at all (Fig. 1). *In vitro* antimicrobial assay, ethanolic extract of whole plant was tested in different bacteria and fungi.

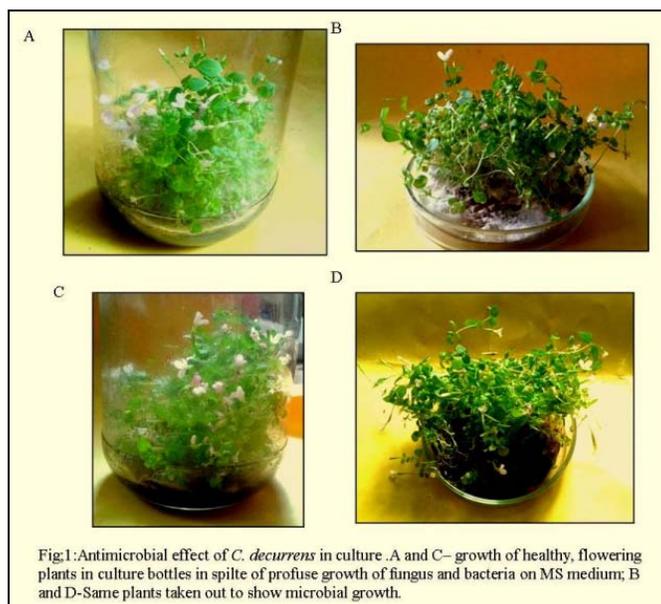


Fig 1: Antimicrobial effect of *c. decurrens* in culture. A and C- growth of healthy, flowering plants in culture bottles in spite of profuse growth of fungus and bacteria on M S medium; B and D- Same plants taken out to show microbial growth.

Antimicrobial activity of ethanolic extracts obtained from microwave (modern) and soxhlet extraction (conventional) methods was compared against 3 bacterial strains namely *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and 2 fungi viz. *Rhizactonia solani* and *Aspergillus niger*. All The test strains of bacteria were

sensitive to ciprofloxacin (standard disc 5mcg\disc) and fungal strains were sensitive to bavistin (10mg\ml filter paper disc). Hence they were used as positive control. Disc saturated with Ethanol was used as a negative control and showed no effect against bacterial and fungal growth.



Fig 2: In vitro antimicrobial activity of ethanolic extract obtained by soxhlet and microwave. A, (Ciprofloxacin) B (soxhlet), C (microwave) in *Escherichia coli*; D (ciprofloxacin), E (soxhlet) F (microwave) - in *Staphylococcus aureus*; G (ciprofloxacin) H (soxhlet) I - (microwave) in *Pseudomonas aeruginosa* J - (bavistin) K (soxhlet) L (microwave) in *Rhizactonia solani*; M (bavistin) N (soxhlet) O and P - (microwave) in *Aspergillus niger*

Fig 2: In vitro antimicrobial activity of ethanolic extract obtained by soxhlet and microwave a, (ciprofloxacin) B (soxhlet) C (Microwave) In *Escherichia coli*; D (ciprofloxacin); E (soxhlet) F (microwave) - in *staphylococcus aureus*; G (ciprofloxacin) H (soxhlet) I - (microwave) in *pseudomonas aeruginosa* j - (bavistin) k (soxhlet) L (microwave) in *Rhizactonia solani*; M (bavistin) N (soxhlet) o and P - (microwave) In *Aspergillus niger*

Results of the antimicrobial activity of *C. decurrens* whole plant extract (100mg/ml) as shown in table.1 exhibited inhibitory activity against *Escherichia coli* (fig.C) in microwave extract showed inhibition zone of 10.07mm and soxhlet extract no zone was found (fig.B). Similarly *Staphylococcus aureus* exhibited inhibition zone 10.37mm in microwave extract (fig.F) which is slightly more as compared to soxhlet extract with 10.25mm (fig.E). In *pseudomonas aeruginosa* with inhibition zone 10.65mm in microwave

extract (fig.I) where as in soxhlet it was 10.27mm (fig.2H). In case of fungi, *Rhizactonia solani* showing maximum inhibition zone in microwave extract 10.32mm (fig.L) as compared to soxhlet extract (fig.K) with 10.22mm. Similarly *Aspergillus niger* was exhibited maximum zone in microwave extract (10.93mm) (fig O,P) as compared to soxhlet (0.97mm) (fig.N). In conclusion microwave extracts of *C. decurrens* was found to have more potential than soxhlet extract in the tested microorganisms.

Table 1: Antimicrobial activity of ethanolic extract of *C. decurrens*

Microbe/ Extract	Zone of inhibition (mm)			
	Negative control	Positive control	Soxhlet extract	Microwave extract
Bacteria	Ethanol	Ciprofloxacin		
<i>Escherichia coli</i>	-	30.72±0.04 ^b	-	10.07±0.06 ^a
<i>Staphylococcus aureus</i>	-	20.65±0.05 ^b	10.25±0.02 ^a	10.37±0.02 ^a
<i>Pseudomonas aeruginosa</i>	-	20.57±0.05 ^c	10.27±0.05 ^a	10.65±0.05 ^b
Fungi		Bavistin		
<i>Rhizactonia solani</i>	-	20.40±0.04 ^b	10.22±0.02 ^a	10.32±0.02 ^a
<i>Aspergillus niger</i>	-	20.03±0.02 ^b	0.96±0.02 ^a	10.93±0.01 ^b

The data shown are means±SD of three replicates. Mean with in row followed by same letter are not significantly different at p≤0.05. Different letter a, b, and c denote significant difference (p≤ 0.05) between different treatment

4. Discussion

In the present investigation, extract obtained from microwave assisted extraction and soxhlet extraction showed bactericidal activity against 3 bacteria namely *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and 2 fungi *Rhizactonia solani*, *Aspergillus niger*. The aqueous extract of garlic inhibited the growth of both Gram positive and gram negative tests bacterial cultures, but microwave extract showed better activity than soxhlet extract in *Glycyrrhiza glabra* ^[11, 12]. The activity may be the indicative of the presence of broad spectrum antibiotic compounds or simply general metabolic toxins ^[1]. Whole plant ethanolic extract contained alkaloid, bitter principle, anthracene, flavonoids, coumarin, triterpenoids, phenolic acids and xanthenes. In, *Sida cordifolia* leaves show high antimicrobial activity and contains a number of phytochemicals that are responsible for such activity ^[10] (Table.1) among them phenolic compound were known for antimicrobial potential against bacteria and fungi in wild mushroom ^[2]. The antimicrobial potential of plant extract may be due to presence of these phytochemicals like, flavonoids in *Crataegus Oxyacantha* ^[8]. As the search for new antimicrobial agents is in demand, plant extract may provide attractive alternate source of antimicrobial drug against various microbial diseases. The present studies provide the evidence of antimicrobial property of *C. decurrens* against bacteria and fungi.

5. Conclusion

The present investigation means that MAE is better over soxhlet extraction methods when proper microwave power and polar solvent is used for extraction. Ethanol extract by microwave assisted extraction shows good antibacterial and antifungal activity than soxhlet it is due to extracts active phytoconstituents.

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