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Original Research Paper

Qualitative Phytochemical Analysis and *in vitro* Antibacterial Activity of *Acmella Ciliata* (H.B.K) Cassini and *Ichnocarpus Frutescens* (Linn.) R.Br. Against Two Pathogenic Bacteria

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ABSTRACT

In the present work, qualitative phytochemical analysis and *in vitro* antibacterial activity of the different component extracts of *Acmella ciliata* and *Ichnocarpus furtescens* against *Escherichia coli* and *Bacillus subtilis* were studied. Extracts were prepared in methanol and water. Antibacterial activity was compared with control and standard antibiotic ampicillin. Both the plant species exhibited antibacterial activity against the test pathogenic bacteria. However, methanolic leaf extracts of *Acmella ciliata* was found to have maximum number of bioactive components and highest zone of inhibition against both the test bacteria and therefore as per the present study, methanolic leaf extract of *A. ciliata* is indeed the potential antibacterial agent against *B. subtilis* and *E.* coli.

INTRODUCTION

Ayurveda, the science of life, prevention and longevity is believed to be the oldest and the most holistic medical system available. The Ayurvedic treatment is basically dependent on medicinal herbs. The search for potential antimicrobial bioactive components from plants is a thrust area of research. Despite the remarkable progress in synthetic organic chemistry in the 20th century, over 25% of prescribed medicines in industrialized countries are derived directly or indirectly from plants (Newman et al. 2000). Herbal products are capable of modulating the activity of enzymes and affecting the behaviour of many cell systems suggesting that extracts of different medicinal plants possess significant antioxidant, antibacterial and antifungal activity. However, plants used in traditional medicine are still understudied, particularly in clinical microbiology (Kirby et al. 1996).

In the last three decades, the pharmacological industries have produced a number of new antibiotics but resistance to these drugs by microorganisms has increased. Medicinal plants represent a rich source of antimicrobial agents and plants are used medicinally in different countries as a source of many potent and powerful drugs. Herbal medicines are safer than modern synthetic drugs because they are naturally existing, mild in action and lack many side effects at normal dosage, relatively inexpensive and locally available compared to most of the synthetic drugs. The aim of the present study was to analyse the phytochemical principles and to determine the *in vitro* antibacterial activity of methanol and aqueous extracts of two selected plant species *Acmella ciliata* (H.B.K) Cassini belonging to the family Asteraceae and *Ichnocarpus frutescens* (Linn.) R.Br. belonging to the family Apocynaceae against two pathogenic bacteria namely *Escherichia coli* and *Bacillus subtilis*.

MATERIALS AND METHODS

The plants, *Acmella ciliata* and *Ichnocarpus frutescens* were collected from the regions of Nattika Panchayath, Thrissur district, Kerala. They were dried under shade to avoid decomposition. After this, the plant parts such as leaf, stem, root and flower head were coarsely powdered and subjected to successive solvent extraction using Soxhlet apparatus. Each time about 3g of dried powder was subjected to solvent extraction with water and methanol.

The microorganisms used in the study were *Escherichia coli* and *Bacillus subtilis*. The two bacterial cultures were clinical isolates, obtained from Amala Ayurvedic Hospital and Research Centre, Amalanagar, Thrissur. The bacterial cultures which underwent subculturing, were maintained on nutrient agar and stored at 4°C.

The aqueous and methanol extract of leaf, stem and flower head of *A. ciliata* were subjected to preliminary phytochemical testing for the detection and identification of major phytoconstituents. In the case of *I. frutescens*, phytochemical analysis was performed in the aqueous and methanol extract of leaf, stem and root. Phytoconstituents analysed included alkaloids, tannins, flavanoids, steroids, phenols,

glycosides, terpenoids, anthroquinone, saponins and cardiac glycosides.

Antibacterial activity tests were performed by agar-well diffusion method (Cole 1994, Okeke et al. 2001). Three wells (5mm diameter each) were bored in each solidified agar plate with an aseptic cork borer. The test bacterial strains obtained from overnight broth culture were seeded separately on sterile solidified agar medium by swab plate technique using sterile cotton swabs. Different plant extracts were prepared and reconstituted in specific solvent system and 200 micro litre of each specific extract was dispensed into each of the wells with the aid of Pasteur pipette. After holding the plates at room temperature for about 2 hours to allow diffusion of the extracts into the agar, they were incubated for 24 hours at 37°C . The test were performed in triplicate for each microorganism and the average values were tabulated. Pure methanol and water were taken as control. Antibiotic ampicillin was taken as reference.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis of methanol and water extracts of *A. ciliata* and *I. frutescens* revealed the presence or absence of some phytoconstituents which are screened for the study and are presented in Tables 1 and 2. The data show the presence of steroids, glycosides, alkaloids, tannins, flavanoids, anthraquinones, saponins and cardiac glycosides in methanol leaf extracts of *A. ciliata,* whereas in *I. frutescens* only steroids, tannins and alkaloids were identified. However, in the methanol extracts of stem component, steroids, glycosides and terpenoids in *A. ciliata* and glycosides, terpenoids, steroids and flavanoids in *I. frutescens* were identified. The methanol root extracts of *I. frutescens* reveal the presence of flavanoids, saponins, steroids, glycosides and terpenoids, however, only flavanoids and saponins were detected in the methanolic flower head extracts of *A. ciliata*.

The screening of water extracts of *A. ciliata* detected the presence of constituents like flavanoids, steroids, terpenoids, anthraquinones and saponins in flower head; glycosides, saponins and steroids in the stem component and only saponin in the leaf component. However, the phytochemical screening of water extracts of *I. frutescens* revealed the presence of glycosides, saponins and terpenoids in root component; flavanoids, steroids, glycosides, terpenoids and saponins in stem component and tannin, flavanoids, saponins, glycosides and terpenoids in leaf component.

Qualitative phytochemical analysis of *A. ciliata* reveals that methanol leaf extracts contain maximum number of phytoconstituents compared to stem and flower head extracts in both water and methanol. This observation may be due to the compartmentalization and higher concentration of the phytoconstituents in the leaf components together with higher solubility property of methanol for different phytoconstituents present in the plant species (Stainer et al. 1986, Majorie 1999, Doughari et al. 2008). However, with respect to *I. frutescens,* the phytoconstituents detected in the methanol stem and water leaf extracts showed not much variation from other extracts but comparatively higher antimicrobial activity was obtained. This may be attributed to the higher concentration of specific constituents in the specific extracts (Majorie 1999). It is clear from the present study that the isolation of antimicrobial principles present in the plant material is largely dependent on the type of solvent and the component parts used in the extraction procedure.

With the exception of phenols in *A. ciliata* and phenols, anthraquinones and cardiac glycosides in *I. frutescens,* the presence of all the phytoconstituents screened and studied were detected. It has been widely observed and accepted that the medicinal value of plants lie in the bioactive phytocomponents present in the plants (Veeramuthu et al. 2008). The bioactive components identified in the extracts of the

Table 1: Phytochemical analysis of leaves, stem and flower head extracts of *Acmella ciliata.*

S. No.	Compounds	Water extract			Methanol extract		
		Leaf	Stem	Head	Leaf	Stem	Head
1	Alkaloids	-	-	-	+	-	-
2	Tannin	-	-	-	+	-	-
3	Flavanoids	-	-	+	+	-	+
4	Steroids	-	+	+	+	+	-
5	Phenols	-	-	-	-	-	-
6	Glycosides	-	+	-	+	+	-
7	Terpenoids	-	-	+	-	+	-
8	Anthraquinones	-	-	+	+	-	-
9	Saponins	+	+	+	+	-	+
10	Cardiac glycosides	-	-	-	+	-	-

+ indicates presence and – indicates absence

Table 2: Phytochemical analysis of leaves, stem and root extracts of *Ichnocarpus frutescens.*

S. No.	Compounds	Water extract			Methanol extract		
		Leaf	Stem	Head	Leaf	Stem	Head
1	Alkaloids	-	-	-	+	-	-
2	Tannin	+	-	-	+	-	-
3	Flavanoids	+	+	-	-	+	+
4	Steroids	-	+	-	+	+	+
5	Phenols	-	-	-	-	-	-
6	Glycosides	+	+	+	-	+	+
7	Terpenoids	+	+	+	-	+	+
8	Anthraquinones	-	-	-	-	-	-
9	Saponins	+	+	+	-	-	+
10	Cardiac glycosides	-	-	-	-	-	-

+ indicates presence and – indicates absence

tested plants are known to be bactericidal, pesticidal or fungicidal in nature, thus conferring the antibacterial property to the plants (Lutterodt et al. 1999, Pretorius et al. 2001, El astal et al. 2005).

Two pathogenic bacteria have been selected in the present investigation namely *Escherichia coli* and *Bacillus subtilis*. The data given in Table 3 and Fig. 1 have clearly revealed the methanol leaf extracts of *A. ciliata* demonstrated the highest ZOI against both the pathogenic bacteria, which were 9.8mm diameter and 17.3mm diameter respectively for *E. coli* and *B. subtilis*. Methanol extracts of flower head against both the test bacteria and water extracts of leaf and stem component against *B. subtilis* and *E. coli* respectively did not exhibit any ZOI. With respect to the plant *I. frutescens*, the data presented in Table 4 and Fig. 1 have revealed, the water extracts of leaf demonstrated the highest ZOI against *E. coli* whereas the methanol extracts of stem demonstrated highest ZOI against *B. subtilis* which were 9.5mm diameter and 14.3mm diameter respectively for *E. coli* and *B. subtilis*. Methanol extracts of leaf and water extracts of root against both the test bacteria and methanol extracts of stem component against *E. coli*, did not exhibit any ZOI.

The present study result indicates that there are differences in the antibacterial effects of different component parts in different solvent extracts of the same plant as well as between plants against the test pathogens (Subin & Navya 2012). Among the various component extracts of *A. ciliata* and *I. frutescens* tried in the present study for antibacterial activity, the highest ZOI was induced by the methanol leaf extracts of *A. ciliata* against both the test bacteria with an average of 17.3mm diameter against *B. subtilis* and an average of 9.8mm diameter against *E. coli*. The highest antimicrobial activity exhibited by the methanol leaf extracts of *A. ciliata* compared to other extracts may be attributed to the presence of more and higher concentrations of specific potent phytochemicals (Prusti et al. 2008, Majorie 1999, Subin & Navya 2012). The present observation suggests that the methanol solvent extraction is suitable to verify the antibacterial activity and the same trend is supported by many investigators (Krishna et al. 1997, Natarajan et al. 2005). The absence or lower zone of inhibition exhibited by certain extracts in the present study may be due to the absence or insufficient concentration of antimicrobial principles so as to be effective or it may be due to the lack of antibacterial properties of specific constituents towards the test bacteria (Stainer et al. 1986).

The study shows that the pure solvent methanol and water used in the investigation as control, did not produce zone of inhibition against *E. coli* and *B. subtilis*, however, the inhibition zone induced by the reference ampicillin was higher against both the test bacteria (Table 5). The zone of inhibition recorded by different extracts in the present investigation was in the range of 0 to 9.8mm diameter against *E. coli* and 0 to 17.3mm diameter against *B. subtilis* which were considerably lower than the ZOI produced by the reference ampicillin, and it was 32mm and 26mm diameters respectively against *E. coli* and *B. subtilis*. But at the same time the present study results indicate the scope and importance of present plant extracts in controlling the test pathogens. This is because the present antibacterial activity noticed in the study is in response to treatment with crude plant extracts, which may contain different compounds including both specific and non specific antibacterial phytoconstituents. Further refining and purification of the crude extracts may be useful in getting specific antibacterial principles in pure form and may enhance the antibacterial properties as in the case of antibiotic ampicillin in pure form (Jayalakshmi et al. 2011).

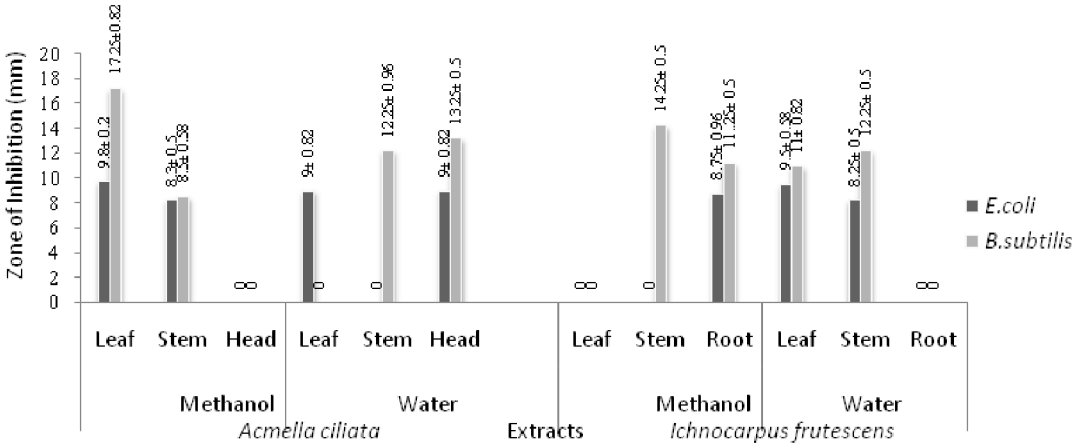

Fig. 1. A comparative evaluation of antibacterial properties exhibited by different component extracts of *Acmella ciliata* and *Ichnocarpus frutescens* against *Escherichia coli* and *Bacillus subtilis*.

Table 3; Zone of inhibition produced by different extracts of *Acmella ciliata* against *Escherichia coli* and *Bacillus subtilis.*

Plant	Bacteria	Zone of Inhibition in methanol extract (mm)			Zone of Inhibition in water extract (mm)		
		Leaf	Stem	Head	Leaf	Stem	Head
Acmella ciliate	*E. coli*	9.8 ± 0.2	8.3 ± 0.5	0.0 ± 0.0	9.0 ± 0.82	0.0 ± 0.0	9.0 ± 0.82
	B. subtilis	17.25 ± 0.82	8.5 ± 0.58	0.0 ± 0.0	0.0 ± 0.0	12.25 ± 0.96	13.25 ± 0.5

Table 4: Zone of inhibition produced by different extracts of *Ichnocarpus frutescens* against *Escherichia coli* and *Bacillus subtilis.*

Plant	Bacteria	Zone of inhibition in methanol extract (mm)			Zone of inhibition in water extract (mm)		
		Leaf	Stem	Root	Leaf	Stem	Root
Ichnocarpus frutescens	*E. coli*	0.0 ± 0.0	0.0 ± 0.0	8.75 ± 0.96	9.5 ± 0.58	8.25 ± 0.5	0.0 ± 0.0
	B. subtilis	0.0 ± 0.0	14.25 ± 0.5	11.25 ± 0.5	11.0 ± 0.82	12.25 ± 0.5	0.0 ± 0.0

Table 5: Zone of inhibition produced by control (methanol and water) and reference (Ampicillin) against *Escherichia coli* and *Bacillus subtilis.*

Bacteria	Zone of inhibition in methanol (mm)	Zone of inhibition in water (mm)	Zone of inhibition in Ampicillin (mm)
E. coli	0.0 ± 0.0	0.0 ± 0.0	32 ± 1.73
B. subtilis	0.0 ± 0.0	0.0 ± 0.0	26 ± 1.73

All data presented in the table are average of three replicates.

CONCLUSION

The present study revealed that both the plants selected, *Acmella ciliata* and *Ichnocarpus frutescens,* have antibacterial activity against the tested pathogenic bacteria. The results obtained from the phytochemical analysis and antibacterial activity efficiency test recommend the application of *A. ciliata* leaf component extracts in human protection against the test pathogenic bacteria *E. coli* and *B. subtilis,* as it may contain better concentrations of specific potential bioactive components than others. The investigation is also suggesting that the preparation of the leaf extracts should be done using the solvent methanol, as it is observed to be capable of dissolving and accommodating phytoconstituents in more number and in higher concentrations. The bioactive substances from these extracts can therefore, be employed in the formulation of antibacterial agents for the control of test pathogens. Further research is necessary for the isolation and purification of the bioactive substances and for the determination of their respective antibacterial potencies with the view to formulating novel microbicidal agents.

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