Analysis of phytoconstituents and antimicrobial properties of leaf extract of *Commenila benghalensis* L, against selected microbes.

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DOI: 10.29322/IJSRP.9.04.2019.p8841
http://dx.doi.org/10.29322/IJSRP.9.04.2019.p8841

Abstract: *The leaf extracts of Commelina benghalensis L were subjected to phytochemical and antimicrobial analysis. Chloroform extract showed the best results. Presence of tannin, favonoid, phenol, carbohydrate and volatile oil were observed in the chloroform extract which showed the maximum activity against the pathogenic bacterial strains Escherechia coli, Staphylococcus aureus, Salmonella typhi and fungal strain Candida albicans with 7mm, 1mm, 3mm and 4mm of inhibition zone respectively compared to hexane and methanolic extract which showed the least activity. The dilution susceptibility test method was used to determine the Minimum Inhibition Concentration (MIC) of the chloroform extract. MIC value was determined as 22.5mg/ml. The present data provides the basis that leaf extract of Commelina benghalensis L can also be used for therapeutic purposes against common pathogenic microbes.*

Keywords: Antimicrobial activity, *Commelina benghalensis* L, MIC, zone of inhibition.

Introduction: *Commelina benghalensis L.* is a medicinal plant and native in Southeast Asia. It is used in the Indian subcontinent as a folk medicine for the treatment of variety of ailments. It is also known as tropical spiderwort, is an herbaceous perennial and a troublesome weed, native to Africa and tropical Asia. They belong to this family Commenliaceae and genus Commenlina (Santosh et al. 2013). Whole part of the plant is medicinally useful. Previous studies have shown that the leaves possess antioxidant, diuretic, antihypertensive, and anti inflammatory effects. It is also used for conjunctivitis, cataracts, night blindness, pain (headaches and toothaches), skin diseases (eczema, abscesses, acne, scabies, and warts), respiratory tract and mental disorders and insomnia. These beneficial effects have been attributed to the presence of primary and secondary metabolites such as polyphenols, amino acids, alkaloids and flavonoids. These phyotocompounds have therapeutic importance). Literature search revealed not many reports on antibacterial activity of C. benghalensis L except a few (Sumithra and Purushothaman 2017). Therefore, the present study was designed to investigate the antibacterial activity of leaf extract of C. benghalensis L. in order to examine the pharmacological basis of the use of the plant in folk medicine for the treatment of infectious diseases.

Materials and Method: The plant of *Commelina benghalensis* was collected from the Nursery near Gandhi Maidan Patna. The fresh leaves were cleaned and then were dried in hot air oven at 45^0C. Dried leaves were ground to a course powder in a blender. To 200mg of coarse powder 500ml of each Hexane, Chloroform and Methanol were added and kept for 24 hours. The solvent from each extract was filtered for phytochemical and anti microbial screening.

Prelimnary screening of phytocomponds: Freshly prepared extracts of the leaves were subjected to phytochemical analysis to find out the presence of the following phyto constituent like sterols, tannins, sugar, alkaloids, flavonoids, saponins, terpenoids and glycosides by standard methods (Jemilat et al. 2010)

Test for Alkaloid: Three ml of extracts were stirred with 3 ml of 1% HCl on steam bath. Mayer and Wagner's reagent was then added to the mixture. Brown red precipitate was taken as an evidence for the presence of alkaloid.

Test for Tannin: Two ml of extracts were stirred with 2 ml of $FeCl_3$ solution. Formation of green colour indicated the presence of tannin.

Test for Saponin: Five ml of extracts were shaken vigorously with 5 ml of distilled water in a test tube and warmed. The formation of stable foam was taken as an indication of the presence of saponin.

Test for Flavonoid: Two ml of extracts were treated with 2 ml of 10% lead acetate solution. Formation of a yellow precipitate was taken as a positive test for flavonoid.

Test for Terpenoid: Two ml of extracts were treated with 2 ml chloroform and evaporated to dryness. 2 ml of concentrated H_2SO_4 was added. Development of a greyish colour indicated the presence of terpenoid.

Test for Glycoside: Two ml of extracts were treated with 2 ml of acetic acid. The solutions were cooled in ice. H_2SO_4 was then added carefully. A colour change from violet to blue to green indicated the presence of glycoside.

Test for Steroid: Two ml of leaf extracts were dissolved in 2 ml of chloroform. 2 ml concentrated H_2SO_4 was added in it. The upper layer turned red and H_2SO_4 layer showed green colour. This indicated the presence of steroid.

Test for Phenol: Few drops of 5% ferric chloride were added to 2ml of extracts. Appearance of black colour indicated the presence of phenol.

Antibmicrobial sensitivity assay by disc diffusion method: Pure culture of pathogenic strains of *Staphylococcus aureus, Salmonella typhi, Escherichia coli and Candida albicans* were obtained from the Microbiology department, PMCH, Patna. Disc diffusion method was used to assess the antimicrobial effect of the leaf extract against the selected test microorganisms. Tests were performed in triplets against each selected strains of bacteria and fungus. Chloramphenicol and Fluconazole were used as control for bacterial and fungal strains respectively.

Baybei et al (2004), method was followed.Concentration of the sample ranging from 2**.5, 5, 7.5,** 10, 12.5, 15, 17.5, 20, 22.5 and 25mg/ml were used to measure the Minimum Inhibition Concentration (MIC) by dilution susceptibility test

Resuts and Discussion: The result of the investigation indicates the presence of five active phytochemicals in the Chloroform extract. Methanol extract contains three whereas the Hexane extract contained only two secondary metabolites as shown in Table 1. All the extracts showed variable degree of antimicrobial activity against selected test microorganisms. Chloroform extract was observed as most effective crude sample against the selected bacterial strain *E.coli*. Hexane extract was found to be more effective against the fungal strain *Candida albicans.* The results of the antimicrobial analysis indicate that chloroform extract had more inhibitory effect on *Escherichia coli* than *Staphylococcus aureus* which showed inhibition zone of 7mm and 1mm respectively. Methanol extract was ineffective against any test microorganism. The results are tabulated in Table 2. The Minimum Inhibition Concentration (MIC) value indicated that high concentration of extract was effective against the pathogenic microorganisms.

Table 1. Phytochemical screening of leaf extracts of in different solvents

Phytochemical tested	**Different Leaf extract in different solvent**		
	Chloroform	**Hexane**	**Methanol**
Tannin	+	-	+
Flavanoid	+	+	+
Saponin	-	-	-
Steroid	-	-	-
Terpinoid	-	-	-
Glycoside	-	-	-
Phenol	+	-	-

(Present +, absent -)

Table 2. Antimicrobial screening of leaf exracts of *Commelina benghalensis L*

Test Microorganisms	**Diameter of zone of inhibition(mm)**			**Control (+ve)**
	Chloroform	**Hexane**	**Methanol**	
Bacteria				
Staphylococcus aureus	1	-	-	5
Escherichia coli	7	-	-	4
Salmonella typhi	3	4	-	10
Fungi				
Candida albicans	4	5	-	7

Chloramphenicol was used as standard antibacterial agent and Fluconazole was used as standard antifungal agent.

Extraction and phytochemical screening of bioactive agents from medicinal plants permits the demonstration of their physiological activities. The present study on leaf extract of *Commelina benghalensis L* shows the presence of tannins, flavonoids, carbohydrates and phenol. The results are similar to the earlier work of Sumithra and Purushothaman (2017) which also showed the presence of these phytocompounds.. According to Misra et al (2016) tannins and phenol compounds have been

found to inhibit bacterial and fungal growth and also capable of protecting certain plants against infection. The present study showed that Chloroform extract was most effective against the bacterial strains and it contained most of the phytochemicals. *Staphylococcus aureus* shows minimum inhibition zone and *E.coli* shows maximum inhibition(Kunle and Egharevba 2009). The Hexane extract showed the maximum inhibitory effect against the fungal strain. The isolation of volatile oils in *Commelina benghalensis L* confirmed the activity showed against the test organisms by this plant and also in part confirmed the report (Jarald *et al*., 2009) of the oils isolated from same plant by distillation to exhibit great antibacterial activity.

Conclusion:

The results of this study suggests that the leaves of *Commelina benghalensis L* are rich in phytochemicals which have intermediate antimicrobial activity against the pathogenic microbes *Staphylococcus aureus, Salmonella typhi* and *Escherichia.coli. Candida albicans* is markedly resistant to Lemongrass. The MIC of leaf extracts of *Commelina benghalensis L* reveals that a higher dose of the plant extract is required to bring about a significant activity in the body. Four active ingredients were identified in the plant leaf which include flavonoids, tannins, carbohydrates and volatile oils. Further studies can be made to identify the chemical nature of the antimicrobial properties present in the leaf extract of *Commelina benghalensis L*.

Acknowledgement:

I am grateful to the principal of the college, Dr. Sister M. Rashmi AC and the Central Research Laboratory, Patna Women's College, Patna for the cooperation and assistance to carry out my research work.

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