EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 6
309-313
Year: 2017

PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF LEAF EXTRACT OF *COMMELINA BENGHALENSIS* L

***D. Sumithra[1] and Dr. Sumithra Purushothaman[2]**

[1]Research Scholar, Department of Biotechnology, Marudupandiyar College, Thanjavur.
[2]Assistant Professor, Department of Botany & Biotechnology, Bon Secours College for Women, Thanjavur.

***Corresponding Author: D. Sumithra**
Research Scholar, Department of Biotechnology, Marudupandiyar College, Thanjavur.

Article Received on 21/03/2017 **Article Revised on 12/04/2017** **Article Accepted on 04/05/2017**

ABSTRACT
Plant secondary metabolites provide a vital role for the medicinal activity of plant species. The present investigation deals with qualitative screening of secondary metabolite and antimicrobial activity of *Commelina benghalensis* L. leaves extract belongs to family of Commelinaceae. Plant metabolites screenings were performed by using various solvents systems of varying polarity of acetone, ethanol and aqueous extracts. In this examination the crude extracts showed the presence of flavonoids, carbohydrates, saponins, phlobatannins, and volatile oil while Phenol, steroids and terpenoids were absent in all the solvents. Alkaloids present in acetone and ethanolic extracts. On the other hand tannins were absent only in acetone extract. Anthroquinone was present in ethanolic and acetone extract. The ethanolic leaf extract was tested against Gram positive and Gram negative bacterial pathogens. Plant extract showed antibacterial activity against *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Enterococcus faecalis* while no inhibitory activity against *Klepseilla pneumoniae.* The result of this proposed study enlightened the evidence that, *Commelina benghalensis* L. contains various bioactive compounds that may be useful in the synthesis of therapeutic drugs and also suggest the herbal medicine.

KEY WORDS: *Medicinal plants, Commelina benghalensis L., secondary metabolites.*

INTRODUCTION
Nature as a source of medicine has been inherited and is an important component of the health care system. Modern medicine has evolved from folk medicine and traditional system only after through chemical and pharmaceutical screening (Boopathi and Sivakumar, 2011). Almost 20% of the plants found in the world have been submitted to pharmaceutical or biological tests for various diseases (Suffredini *et al*., 2004). Screening of bioactive compounds from plants has led to the invention of new medicinal drugs which have efficient protection and treatment roles against various diseases, including cancer (Sheeja and Kuttan, 2007) and Alzhemir's diseases (Mukherjee and Kumar, 2007).

Natural products has a vital role in pharmacological and commercial industries, produce a lot of health care and medicinal products such as antimicrobial, anti-tumour agent, anti-hepatotoxic, cardiotonic, CNS stimulant, nutraceuticals, sweeteners, food additives and animal feed (Gortzi *et al.*,2008; Verma *et al.*, 2009). In addition, plants contain important bioactive clusters such as alkaloids, flavonoids, saponins, steroids, terpenoids, polysaccharides and tannins that are largely contributing to various biological activities in traditional and modern therapeutic principles (Doughari *et al*., 2012; Mahboubi *et al*., 2013).

Commelina benghalensis L. (family Commelinaceae) is a perennial herb native to tropical Asia and Africa, used in the Indian subcontinent as a folk medicine for the treatment of leprosy, headache, fever, constipation, jaundice and snake bite (Kirtikar and Basu 1980*;*Yusuf *et al.*, 1994*;* Hasan *et al.*, 2008). The plant is also used for mouth thrush (Ssenyonga and Brehony 1993), inflammation of the conjunctiva, psychosis (Adjanohoun, 1993), epilepsy, nose blockage in children (Okello and Segawa, 2007), insanity (Tabuti *et al.*, 2003) and exophthalmia. *C. benghalensis*is L. used therapeutically as a diuretic, febrifuge and anti-inflammatory (Deyuan and Robert 2000, Hong and DeFillipps 2000 and Upadhyayay, Mishra 1965). It is used as an animal fodder, eaten by humans as a vegetable in Pakistan, also used there medicinally, but with different purported effects, including as a laxative and to cure inflammations of the skin as well as leprosy (Qaiser and Jafri, 1975). The plant is also reported to have antitumor, anticancer and antioxidant activity (Mbazima *et al.*, 2008*;* Hasan 2008). Previous phytochemical investigations of the Commelina genus were reported on *C. undulata* R.Br.,*C. benghalensis* L. and *C. communis* L. from which several

types of compounds such as alkaloids, steroids,terpenoids, iridoids, flavonoids, lignans, aliphatic alcohols, polyols, and phenolic acids were obtained (Sharma and Tandon 1982 and Tang 1994). Additionaly, the whole plant of *C. benghalensis* L. was reported to contain alkaloid, volatile oil, wax, vitamin-C and higher levels of both lutein and β-carotene (Raju *et al.,* 2007; Lakshminarayana *et al.,* 2007).

A broad range of phytochemicals present in plants are known to inhibit bacterial pathogens (Cowan, 1999; Medina *et al*., 2005). The determination of such biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Organic solvents such as ethanol, acetone, and methanol are often used to extract bioactive compounds (Eloff, 1998).

Ethanol is the most commonly used organic solvent by herbal medicine manufacturers because the whole products can be safely used internally by consumers of herbal extracts (Low Dog, 2009).Furthermore, the bioactivity of plant extracts depends on the water and ethanol concentration used in the extraction process (Ganora, 2008).However a great amount of research has been performed to determine the antibacterial activity of medicinal plants, optimal extraction of bioactive compounds has not been well known for most plants.

To maximize up take the recovery of plant antimicrobials for human consumption, establishing optimal and specific extraction condition using various solvent system. Hence, the objective of this study was to determine qualitative investigation was carried out to evaluate the presence of phytochemicals. In this report a comparative study on three different solvents used to screening of high yield of bioactive clusters. Furthermore, the ethanolic leaf extract as a good source for the determination of the antimicrobial activity against various human pathogens.

MATERIALS AND METHODS

Extraction: The leaves were washed thoroughly with running tap water, leaf material was then air dried under shade after complete shade drying the plant material was grounded in mixer, the powder was kept in small plastic bags with paper labelling. 10 g of plant powder was extracted in 250 ml of solvents, (acetone, ethanol and aqueous) by soxhlet extraction technique overnight at respective solvent temperatures. The obtained extracts were subjected to vacuum evaporator to evaporate excess solvents. After that, the dried crude extract yields were weighed and used for further experimental studies.

Phytochemical studies: Preliminary characterization of phytochemical constituents crude plant extract samples were dissolved in respective solvents used for qualitative confirmation of major phytochemical constituents such as alkaloids, flavonoids, phenolics, saponins, steroids, tannins, carbohydrates and volatile oils (Trease and Evans, 2002).

Microorganisms: Bacterial strains were obtained from Department of Biotechnology, Marudhupandiyar College, Thanjavur, Tamil Nadu and were used for assay of antibacterial activity. Microorganisms were maintained at 4°C on nutrient agar slants. The studied bacterial strains comprised: *Bacillus subtilis, Staphylococcus aureus, enterococcus faecalis, Escherichia coli, Klebsiella pneumonia.*

Antibacterial Assay: The antibacterial assay was performed by agar well diffusion method (Perez et al., 1990) for solvent extract. The Muller Hinton Agar media was inoculated with the 100 µl of the inoculum ($1x10^8$ Cfu) and poured in to petriplates. In this method a well was prepared in the plate using a cork-borer (0.85) 50,100µg of test sample was introduced in to the well. The plates were incubated overnight at 37^0C and microbial growth was determined by measuring the diameter of zone of inhibition. The controls were maintained where pure solvent was used instead of the extract for each strain. The result was obtained by measuring zone of diameter (table 2).

RESULTS AND DISCUSSION

The result obtained for qualitative screening of phytochemicals in leaves of *Commelina benghalensis* L. are presented in Table 1. The preliminary phytochemical screening tests may be helpful in the screening of the bioactive compounds and finally may help in the detection and development of new drugs. Further, these tests make easy their qualitative separation and quantitative assessment of pharmacologically active chemical compounds (Bhandary *et al.,* 2012) In this present work the screening of phytochemical constituents were carried out on select solvents such us aqueous, ethanol and acetone. Further, the presence of different phytochemicals in the three different organic solvent extracts may be responsible for the therapeutic properties of *C.benghalensis* L. In this examination the crude extracts shows the positive result of flavonoids, carbohydrates, saponins, phlobatannins, and volatile oil compound in ethanolic extract while in acetone extract alkaloids and terpenoids were absent and also in aqueous extract shows the negative response of alkaloids. On the other hand tannins were absent only in acetone extract. Many phytochemicals are non-nutritive plant chemicals that have disease preventive values (Suresh *et al.,* 2011). It is proof from result studied plant extract shows negative response for non-nutritive plant chemicals like phenol, steroid and terpenoids. Saponin and phlobatannins were present in all the solvent extracts. Anthraquinone was present in both ethanol and acetone extract.

The presence of some of these secondary metabolites suggests that the plant might be of medicinal significance and supports the origin for some of the ethno-uses. For

instance, the presence of flavonoids suggest that the plant have been reported to exert multiple biological effects including, anti-allergic, anti-inflammatory, anti-microbial antioxidant, anti- cancer activity (Kunle and Egharevba, 2009) It also suggests that the plant might have diuretic properties (Jayvir *et al*., 2002). The presence of tannins shows that the plant is astringent as documented and suggests that it might have antiviral and anti-bacterial activities and can relief in wound healing and burns (Haslem, 1989). Saponins and glycoside are also very important classes of secondary metabolites as some are cardio-active and used in treatment of heart conditions (Oloyode, 2005). Some researchers have also investigated that some saponins have anti-cancer and immune modulatory properties (Kunle and Egharevba, 2009; Evan, 2002). Volatile oils are used in the industries for various purposes, both as a pharmaceutical/cosmetic raw material for production of emollients and active ingredient for the respiratory tract infections. They are also used as flavouring agents, in aromatherapy, perfumery etc. egs are eucalyptus oil, lemon oil and peppermint.

In the present study plant extract of *C. benghalensis* showed higher antibacterial activity against *Escherichia coli* than *Bacillus subtilis*, *Staphylococcus aureus Enterococcus faecalis* while no inhibitory activity against *Klebsiella pneumonia* (Fig.1). In this study also shows the presence of different phytochemicals with biological activity that can be valuable therapeutic index (Jigna parekh and Sumithra chanda, 2007). From the result, it is concluded that *C. benghalensis* have great potential use as phytomedicine and have pharmacological activities. Development of phytomedicine is inexpensive and less time consuming and suitable to our economic conditions.

Table 1: Phytochemical Analysis of *Commelina benghalensis* L.

Secondary metabolites	Aqueous	Ethanol	Acetone
Carbohydrates Fehling's test	+	+	+
Phlobatannins	+	+	+
Alkaloids wagner's test	_	+	+
Flavonoids	+	+	+
Saponin Forth test	+	+	+
Steroids Liebermann-Burchardt test	_	_	_
Terpenoids Liebermann-Burchardt test	_	_	_
Tanins Braemer's test	+	+	_
Volatile oil	+	+	+
Phenol Ferric chloride test	_	_	_
Anthraquinone Borntrager's test	_	+	+

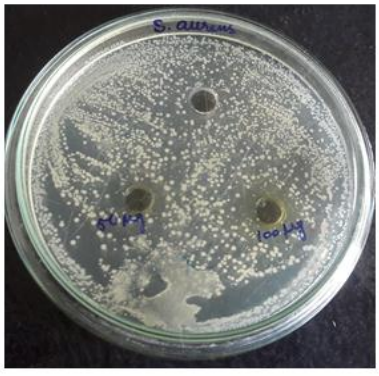
Keys: + = present/ positive - = absent/negative

Table: 2 Antibacterial activity of leaf extract of *Commelina benghalensis L.*

Microorganisms	50 µg/mL	100 µg/mL
	Diameter of inhibition zone(mm)	
Gram positive bacetria		
Bacillus subtilis	3	5
Staphylococcus aureus	5	8
Enterococcus faecalis	4	5
Gram negative bacteria		
E.coli	7	10
Klebsiella pneumoniae	N	N

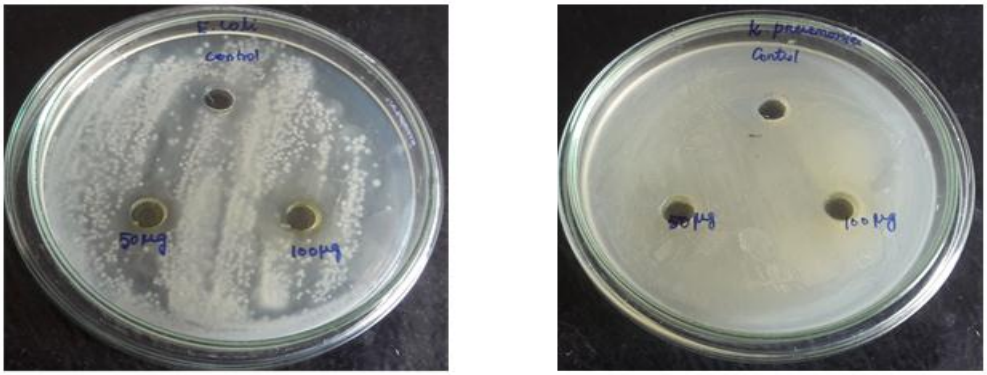
N=No inhibition

Bacillus subtilis



Staphylococcus aureus

Enterococcus faecalis



E.coli ***Klebsiella pneumoniae***

Fig: 1 Photograph of Antibacterial activity

CONCLUSION

Medicinal plants have the great therapeutic and economic values in all over the world. The present results offer a scientific basis for traditional use of *Commelina benghalensis* L against various ailments. Further studies are required for this plant to validate their medicinal importance. In addition, isolation, characterization and elucidation of the structures of the bioactive compounds which may be responsible for their antimicrobial activity and other medicinal values of this widely available weed *C. benghalensis*.

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