

STUDIES ON PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITIES OF *BOERHAAVIA DIFFUSA*, L.; AND *CENTELLA ASIATICA*, (L.) URBAN

Roopasree K.*¹ and Dr. Prathyusha P.²

¹Department of Botany, Nirmala College for Women, Red fields, Coimbatore – 641018.

²Assistant Professor Department of Botany, Nirmala College for Women, Red fields,
Coimbatore – 641018.

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*Corresponding Author

Dr. Prathyusha P.

Assistant Professor

Department of Botany,

Nirmala College for

Women, Red fields,

Coimbatore – 641018.

ABSTRACT

The present study was to find out the phytochemical and antibacterial activity of water and ethanolic extract of *Boerhaavia diffusa* and *Centella asiatica* leaves. Both the plants are used in Ayurvedic and traditional medicines and are also the wild edible plants. Preliminary phytochemical analysis of carbohydrate, protein, starch, amino acid, steroid, glycosides, flavonoids, alkaloids, tannins, saponins, terpenoids and gum tested. Moisture and total ash content were also done. Antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* were determined by using disc diffusion method at the concentration of 25mg/ml, 50mg/ml, 75mg/ml and 100mg/ml.

KEYWORDS: Antibacterial activity, *Boerhaavia diffusa*, *Centella asiatica*, *Staphylococcus aureus* and *Escherichia coli*.

INTRODUCTION

Human beings have depended on nature for their simple requirements. For the large proportions of world's population medicinal plants continue to show a dominant role in the healthcare system and this is mainly true in developing countries, where herbal medicine has continuous history of long use. Medicinal plants are the nature's gift to human being to make disease free healthy life. Herbal medicine is still the mainstay of about 75-80% of the whole population, mainly in developing countries, for primary health care because, better compatibility with the human body and fewer side effects (Barnet, 1992).

Phytochemicals are primary and secondary metabolites contained in plants. The medicinal properties of phytochemicals play a vital role in many treatments for diseases. The phytochemicals do not have any side effects like the other pharmaceutical medicines have. So this can cure the human diseases without causing any harm to human. They can be considered as “man friendly medicines” (Sahira banu and Cathrine, 2015).

An antimicrobial agent is that kills microorganisms or stops their growth. Antimicrobial medicines are different types according to the microorganisms they act against like bacteria, fungi, virus etc. Pharmaceutical companies spending lot of time and money to make effective medicinal extract from plants in cost effective manner. The resistance against drug by pathogenic microbes increases the necessity of finding new antibiotic sources.

C. asiatica is a popularly known as *Centella*, Pennywort or Indian pennywort, Brahmi or Gotu kola. It belongs to the family Apiaceae. It used in Ayurvedic, traditional African and Chinese medicinal system. Stem, leaves, and aerial parts are used to the traditional drug formulas to decrease blood pressure, cure the fresh wound, heal bruised and diuretic (Ullah, *et al.*, 2009).

B. diffusa used in Ayurveda, Unani and other traditional medicinal system. It has a long history of uses by the indigenous and tribal medicinal people and in Ayurveda and Unani medicines. It belongs to the family Nyctaginaceae). The plant helps to cure epilepsy, dysentery, pneumonia, jaundice, anemia, gonorrhoea, enlargement of spleen etc. and also used against poison of scolopendrids. Root extract strengthen, tones and balance of liver (Rawat *et al.*, 1997).

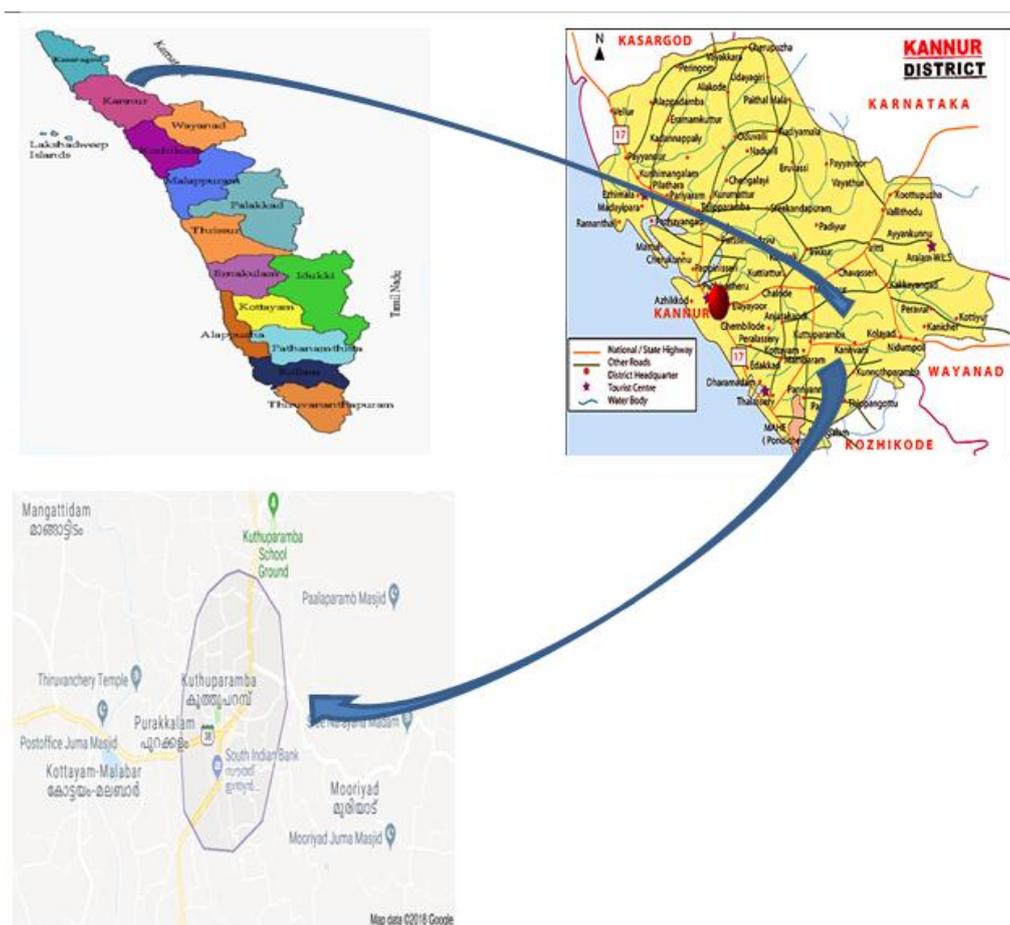
Present study deals with phytochemical screening and antimicrobial activities of *C. asiatica* and *B. diffusa* against gram positive bacteria *S. aureus* and gram negative bacteria *E. coli*.

MATERIALS AND METHODS

Study area

B.diffusa and *C.asiatica* leaves were collected from Kuthuparamba. It is one of the main town and a municipality in the Kannur district, Kerala. Kuthuparamba is a main town, even though the village behavior persists. It is the place having a total area of 16.76sq.km. There are region comprising hills, agricultural lands, valleys, plain lands, and small streams. Kuthuparamba has an average elevation of 76 m (Figure-1).

Figure 1: Study area it is below.



Collection and preparation of plant extracts of plant material

Fresh plant leaves are collected randomly. The leaves are washed thoroughly under running tap water. Shade dried homogenize to fine power and stored in air tight bottle for future use.

Dried powered leaves were extracted with two different solvent water and ethanol. The 150 grams of powered leaves are mixed with 300ml of distilled water and ethanol, and each solvent separately in an orbital shaker incubator for about 48hrs at room temperature. Extracts are filtered, concentrated, dried and stored in the refrigerator at 4°C for further use.

Phytochemical analysis

Phytochemicals analyses of various extract of plants were carried out and their bioactive compounds were determined by the method of Raman, 2006; Karpagam *et al.*, 2008; Kokate *et al.*, 2001.

Physicochemical analysis

Physicochemical analyses were done by testing moisture content and total ash content.

Antimicrobial activity

Antibacterial studies were carried out by Disc diffusion method of Bauer *et al.*, 1996. The ethanol and water extract were used to screen the antibacterial activity.

RESULT AND DISCUSSION

1. Phytochemical analysis

In *B. diffusa* carbohydrate, amino acid, glycoside, flavonoid, and terpinoid were present in both ethanolic and water extract. Starch and protein were present only in water extract and alkaloids and tannins were only present in ethanolic extract. Steroids, saponins and gum were absent in both extract (Table-1). Similar result was reported by Deepti Malhotra *et al.*, (2013) and Thakur and Sulekha, (2015). Phytochemical analysis of *C. asiatica* shows the presence of carbohydrate, protein, starch, amino acid, glycoside and alkaloids were observed in both ethanol and water extract. Steroids and flavonoids were only present in ethanolic extract and terpinoid was only present in water extract of *C. asiatica*. Saponins, tannins and gum were completely absent in both extract of *C. asiatica* (Figure -2). Similar work has been done by Rashmi Saxena Pal and Yogendra Pal, (2016), and Mariappan Senthilkumar, (2018).

Table 1: Phytochemical Screening of Water and Ethanolic Extract of *B. Diffusa* and *C. Asiatica*.

TEST FOR	ETHANOL		WATER	
	<i>Boerhaavia diffusa</i>	<i>Centella asiatica</i>	<i>Boerhaavia diffusa</i>	<i>Centella asiatica</i>
Carbohydrate	+++	++	++	+
Protein	-	++	++	++
Starch	-	++	+	+
Amino acid	+++	++	++	+
Steroid	-	++	-	-
Glycosides	+++	++	++	+
Flavonoids	+	+	++	-
Alkaloids	++	++	-	+
Tannins	+	+	-	-
Saponins	-	-	-	-
Terpinoides	+	-	++	++
Gum	-	-	-	-

+++ : More amount, ++ : Marginal amount, + : Minimum amount, - : Absent
Number of + value indicate the intensity of compound present

Figure 2: Phytochemical screening of *B. diffusa* and *C. asiatica*.



Phytochemical screening of *C. asiatica*



2. Physicochemical analysis

In my physicochemical analysis the moisture content of dried leaf of *B. diffusa* is 9.02% and total ash content is 5.78%. The moisture content of dried leaf of *C. asiatica* is 8.49% and ash content is 5.12% (Table-2). The similar results were reported in Thakur and Sulekha, (2016) and Seneviratne *et al.*, (2016).

Table 2: Physicochemical analysis of powered dry leaves of *B. diffusa* and *C. asiatica*.

Sl.No	Parameter analysed	<i>Boerhaavia diffusa</i>	<i>Centella asiatica</i>
1.	Moisture content (%)	9.02	8.49
2.	Total ash (%)	5.78	5.12

3. Antimicrobial activity

Ethanol extract of *B. diffusa* showed more antimicrobial activity when compared to water extract. In ethanolic extracts zone of inhibition are present only in 75% and 100% mg/ml of concentration against *E. coli* and *S. aureus* respectively. In 100mg/ml of concentration it shows maximum activity against *E. coli* (14mm) (Table-3). The water extract of *B. diffusa*

showed activity only in 100mg/ml concentration against *E. coli* (10mm) and *S. aureus* (12mm) (Table-4 & Figure-3). Similar results were reported by Shilpa and Vidhale, (2010) and Umamaheswari, (2010).

The ethanolic extract of *C. asiatica* showed no activity in 25mg/ml and 50mg/ml of the concentrations against all the two bacteria studied. It shows maximum activity against *E. coli* (13mm) and minimum activity against *S. aureus* (12mm) (Table-5). Antimicrobial activity of the water extract of *C. asiatica* was presented in the Table – 6. The zone of inhibition was present only in 100mg/ml against the bacteria *E. coli* (7mm) and *S. aureus* (10mm). The antibacterial activity of both the plants showed considerable variation in both ethanolic and water extracts (Figure-4). Similar work has been reported by Lalitha *et al.*, (2013) and Thangavel Arumugam *et al.*, (2011).

Table 3: Antibacterial activity of ethanolic extract of *B. diffusa*.

Sl.No	Name of the bacterial species	Zone of inhibition (mm)				
		C(Gentamycin) (mm)	25% mg/ml	50% mg/ml	75% mg/ml	100% mg/ml
1.	<i>E.coli</i>	15	-	-	10	14
2.	<i>S.aureus</i>	16	-	-	12	13

Table 4: Antibacterial activity of water extract of *B. diffusa*.

Sl.No	Name of the bacterial species	Zone of inhibition (mm)				
		C(Gentamycin) (mm)	25% mg/ml	50% mg/ml	75% mg/ml	100% mg/ml
1.	<i>E.coli</i>	13	-	-	-	10
2.	<i>S.aureus</i>	16	-	-	-	12

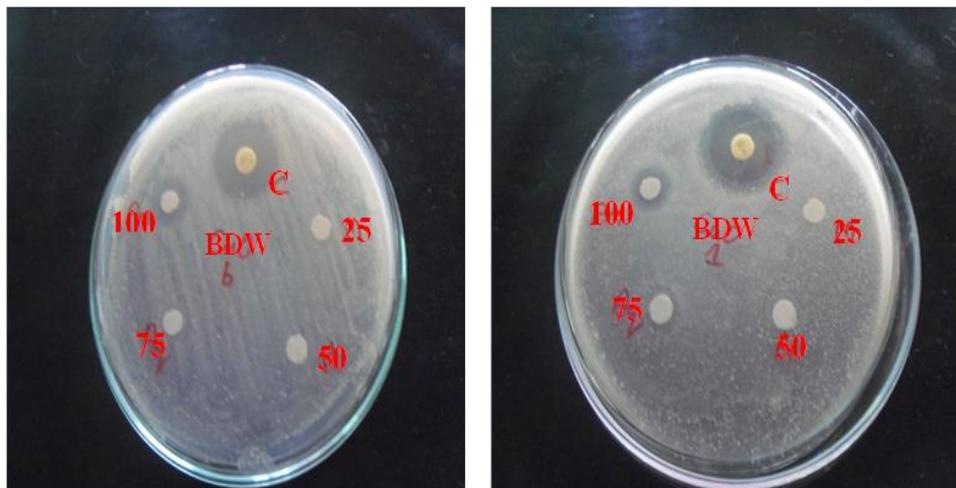
Table 5: Antibacterial activity of ethanolic extract of *C. asiatica*.

Sl.No	Name of the bacterial species	Zone of inhibition (mm)				
		C(Gentamycin) (mm)	25% mg/ml	50% mg/ml	75% mg/ml	100% mg/ml
1.	<i>E.coli</i>	15	-	-	12	13
2.	<i>S.aureus</i>	14	-	-	10	12

Table 6: Antibacterial activity of water extract of *C. asiatica*.

Sl.No	Name of the bacterial species	Zone of inhibition (mm)				
		C(Gentamycin) (mm)	25% mg/ml	50% mg/ml	75% mg/ml	100% mg/ml
1.	<i>E.coli</i>	12	-	-	-	7
2.	<i>S.aureus</i>	14	-	-	-	10

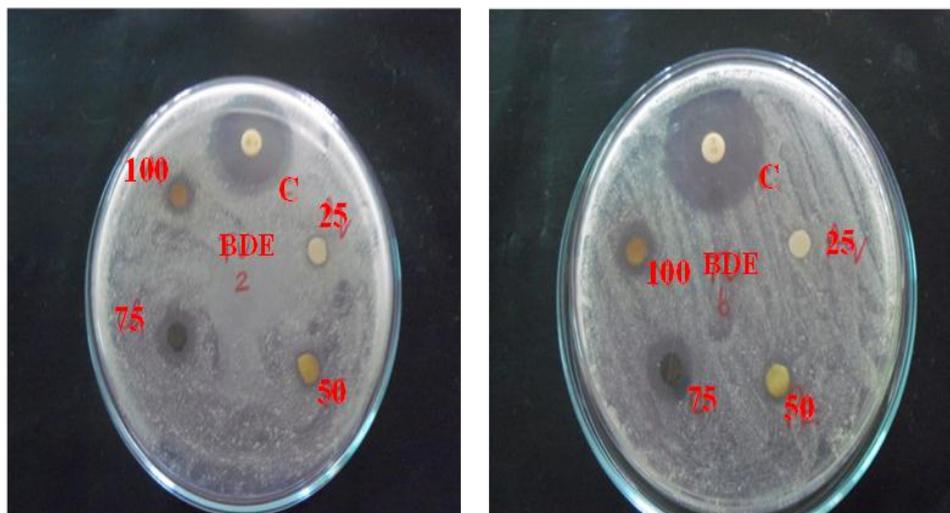
Antibacterial activity of water extract of *B. diffusa*



S. aureus

E. coli

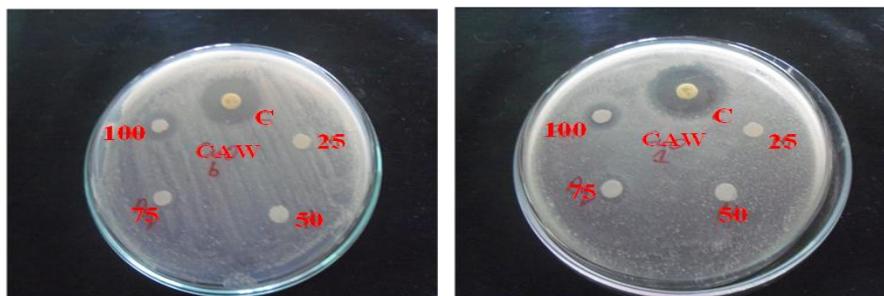
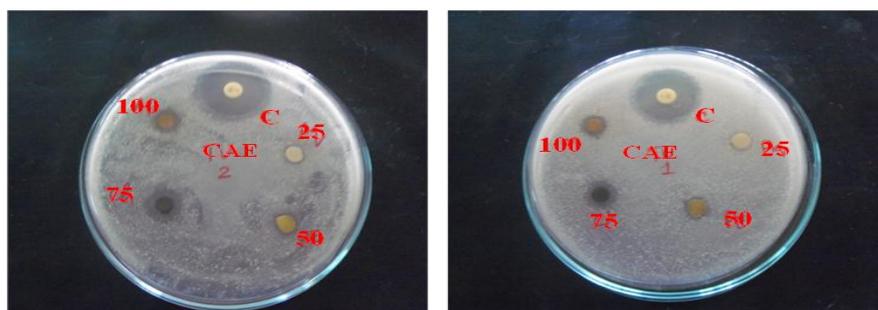
Antibacterial activity of ethanolic extract of *B. diffusa*



S. aureus

E. coli

FIGURE - 4

Antibacterial activity of water extract of *C. asiatica**S. aureus**E. coli***Antibacterial activity of ethanolic extract of *C. asiatica****S. aureus**E. coli***CONCLUSION**

In the present study leaf extracts of *B. diffusa* and *C. asiatica* contain many phytochemicals. In *B. diffusa* carbohydrate, amino acid, glycoside, flavonoid, and terpinoid were present in both ethanol and water extract. Starch and protein were present only in water extract and alkaloids and tannins were only present in ethanolic extract. Steroids, saponins and gum were absent in both extract. In *C. asiatica* carbohydrate, protein, starch, amino acid, glycosides and alkaloids were observed in both ethanol and water extract. Steroids, tannins and flavonoids were only present in ethanolic extract and terpinoid was only present in water extract. Saponins and gum were completely absent in both extract of *C. asiatica*.

Both the plants show antibacterial activity in water and ethanolic extract. The ethanolic extract was more active when compared with water extract. The inhibiting activity may be pathogen specific or due to the phytochemical properties of respective plant species and solvent used for the extraction of secondary metabolites. The chemical constituent present in

the plant are responsible for the antibacterial activity. In the present study the maximum activity is showed in ethanolic extracts.

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