Phytochemical screening and antimicrobial activity of *Coccinia cordifolia* L. plant

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Abstract: The medicinal plant, *Coccinia cordifolia* L. was analyzed for its chemical composition[Dr. Farha1]. The antimicrobial activities of the methanol, water, ethanol and ethyl acetate extracts of *Coccinia cordifolia* L. plant were evaluated against some Gram positive bacteria (*Sarcina lutea, Bacillus subtilis* and *Staphylococcus aureus),* Gram negative bacteria (*Salmonella typhi, Shigella dysenteriae* and *Escherichia coli*) and fungi (*Candida albicans, Aspergillus niger* and *Penicillium notatum*). Chemical analysis showed that the plant is rich in nutrients, especially antioxidant compounds such as total phenol, vitamin C and β-carotene. Phytochemical screening showed that the methanolic extract contains the bioactive constituents such as tannins, saponins, phenols, flavonoids and terpenoids. In the methanolic extract of the plant, promising antimicrobial potential was observed against the tested microorganism. Methanolic extract showed highest activity against *Shigella dysenteriae*, *Escherichia coli*, *Staphylococcus aureus,* and *Candida albicans* compared to the other extracts. Water extract showed less antimicrobial activity as compared to other extractants.

Keywords: Medicinal plants, Antimicrobial activity, *Coccinia cordifolia,* phytochemical.

INTRODUCTION

In human and animals, serious diseases are caused by bacterial and fungal pathogens (Burik and Magee, 2001). Antimicrobial agents are components that hamper growth of microorganisms such as extracts of spices and herbs. Lin *et al.* (2005) reported that the plant extracts, such as spices and herbs have antimicrobial activity and are rich in phenolic secondary metabolites.

Coccinia cordifolia (Synonym: *Coccinia indica*, *Coccinia grandis*, Family: Cucurbitaceae) commonly known as "ivy gourd" is available in wild form and is native of Asia and Central Africa, and distributed in Australia, China, India, Bangladesh, Tropical Asia and Africa. It is one of the medicinal herbs in the traditional practice of Bangladesh as well as Indian medicine (Mollik *et al*., 2010; Chopra *et al.,* 1986)[Ali Mazha2]. This plant has hypolipidemic (Kumar *et al.*, 1993), antimutagenic (Kusamran *et al*., 1998), hypoglycemic (Eshrat, 2003) and anti-inflammatory (Juneja *et al.*, 2007) activities. This plant is also used in various skin diseases, bronchitis, small pox, ring worm, scabies (Perry and Metzger, 1980) and ulcers (Behl *et al*., 1993). Umamaheswari and Chatterjee (2008) reported that the leaf of *C. cordifolia* contains a potential natural antioxidants. Antimicrobial activities of *C. grandis* leaf and fruit extracts against several bacterial and fungal strains have also been reported (Dewanjee *et al.,* 2007; Farrukh *et al*., 2008). However, no scientific report on the antimicrobial activity of the aerial parts (stems and leaves) of *C. cordifolia* plant has been found thus far. This is the first report of *C. cordifolia* plant extract on the inhibitory effect on the growth of various bacterial and fungal pathogens.

Therefore, the aim of this study is to investigate antibacterial and antifungal properties of different extracts, phytochemical screening and chemical compositions of *C. cordifolia* plant.

MATERIALS AND METHODS

Collection and preparation of samples

Fresh parts (stems and leaves) of *C. cordifolia* plant were collected from Rajshahi University campus, Bangladesh. The plant parts were dried and powdered. For methanol, ethanol and ethyl acetate extraction, 20 g powdered plant samples were mixed by occasional stirring and shaking in a shaker for 3 days[Ali Mazha3] and filtered through Whatman No.1 paper. With a rotary evaporator at 40°C, the solvents were removed to obtain dry extract. The extracts were stored at -20°C until used. For water extract, 20 g powdered plant sample was mixed in boiling water and filtered and the extract was dried by freez drier and stored at -20°C until used.

Biochemical analysis

The pH[Ali Mazha4] of plant extract was determined by the conventional procedure using a pH meter. The total protein and water-soluble protein were determined by the

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micro-Kjeldahl method and spectrophotometrically (Lowry *et al*., 1951) respectively. Total phenol and lipid contents were determined colorimetrically (Bray and Thorpe, 1954; Bligh and Dyer, 1959) respectively. Soluble carbohydrate was determined with the Anthrone method (Sadasivam and Manickam, 1992). Calcium, sodium, iron, and potassium contents were determined by Atomic Absorption Spectroscopic method (Issac and Johnson, 1975). Phosphorus content was determined by the colorimetric means of Vermani and Narula, 1995. Vitamin C was determined by the method as described by Hassan and Hassan (2008) and β-carotene was determined by the method described by Jensen (1978). All samples were analyzed in triplicate and expressed as percent of dry matter (organic matter) or milligrams per 100 g dry mass (phenol, vitamins and minerals).

Phytochemical screening of methanol extract

To identify the phytochemicals of methanol extract, chemical tests were carried out by standard methods of analysis (Evans and Trease, 1989; Gibbs, 1974).

Test microorganisms

Six cultures of bacteria were used in the study, among these were three Gram positive (*Sarcina lutea, Bacillus subtilis,* and *Staphylococcus aureus*) and three Gram negative (*Shigella dysenteriae, Salmonella typhi* and *Escherichia coli*) bacteria and three fungi (*Candida albicans, Aspergillus niger* and *Penicillium notatum*).

Antimicrobial study

Antimicrobial activity of the four different samples; 1: Methanol extracts, 2: Water extracts, 3: Ethanol extracts and 4: Ethyl acetate extracts of dried leaves and stems were individually tested against studied microorganisms.

In vitro antimicrobial test was carried out by paper disc diffusion method, (Barry, 1980; Bauer *et al.,* 1966). MacConkey agar plates at 37°C were used to grow bacterial cultures and maintained on nutrient agar slants. Fungal cultures were grown at 30°C and maintained on Sabouraud glucose agar slants. An inoculums size, 10^6cfu/ml for bacteria was used in nutrient agar plates and on Sabouraud glucose agar plates 2×10^5 spores were used for fungi (Mandal *et al*., 2000). Extract inseminated disc was previously prepared at the concentrations of 200 µg/ml (6 mm in diameter) for bacteria and 2000 µg/ml for fungi were placed on sensitivity plates with controls. Streptomycin (200 µg/ml) and griseofulvin (2000 µg/ml) were used as positive controls for bacteria and fungi, respectively. Then, the sensitivity plates were incubated at 37°C for 24 h for bacteria and at 30°C for 3 days for fungal spores (Mandal *et al.*, 2000). By the measurement of the clear zone surrounding the disc on agar surfaces, the antimicrobial activity of the extract was recorded.

Minimum Inhibitory Concentration (MIC): Tube dilution method was used to determine MIC for each of the test organism (Doughari, 2006). Two milliliters of nutrient broth, a loopful of test organism (previously diluted to 0.5 McFarland turbidity standards for bacterial isolates), 10^6 cfu/ml for fungal strains was taken to the tubes containing extracts, 0.5 ml of different concentrations (0-200 and 0 - 2000 µg/ml for bacterial and fungal strains respectively). By using antibiotics streptomycin for bacteria and griseofulvin for fungi, the procedures were repeated on the test organisms. A tube containing nutrient broth seeded only by the test organisms served as negative control,. Tubes with cultures of bacteria were then incubated at 37°C for 24 h and for fungal spores at 30°C for 3 days. The tubes were examined by observing the turbidity for microbial growth after incubation.

Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC): From the test tubes for each set in the MIC determination not showing any growth, a loopful of broth was taken then streaked on sterile nutrient agar for bacteria and Sabouraud glucose agar for fungi. Inoculated plates were then incubated at 37°C for 24 h for bacteria and 30°C for 3 days for fungi. After incubation, concentration was noted as MBC and MFC at which no visible growth was seen.

RESULTS

Biochemical analysis

Chemical composition such as pH, carbohydrate, total protein, water soluble protein, lipid, total phenol, vitamin C, β-carotene, calcium, sodium, potassium, phosphorus and iron content of *C. cordifolia* L. plant is shown in table 1.

Table 1: Chemical composition of *Coccinia cordifolia* L. plant

Chemical composition	Amount
pH	5.60 ± 0.11
Carbohydrate (%)	12.62 ± 0.15
Total Protein (%)	14.55 ± 0.20
Water soluble Protein (%)	11.25 ± 0.21
Lipid (%)	4.00 ± 0.19
Total phenol (mg/100 g)	61.92 ± 0.08
Vitamin C (mg/100 g)	25.55 ± 0.10
β-carotene (mg/100 g)	70.05 ± 0.12
Potassium (mg/100 g)	3.38 ± 0.20
Phosphorus (mg/100 g)	1.15 ± 0.10
Sodium (mg/100 g)	0.95 ± 0.14
Iron (mg/100 g)	2.23 ± 0.11
Calcium (mg/100 g)	3.79 ± 0.15

Values are mean ± S.D. of triplicate analyses and expressed as percent dry matter.

The pH of the plant was on acidic (5.60) side of the scale. The nutrient contents such as carbohydrate, total protein,

water soluble protein and lipid were 12.62, 14.55, 11.25 and 4.00%, respectively. The antioxidant contents such as total phenol, vitamin C and β-carotene were 61.92, 25.55, 70.05 mg/100 g plant, respectively. Therefore, this plant is a good source for total phenol, vitamin C and β-carotene, and thus the plant can be consumed as a source of vegetable. Oboh, (2005), reported that phenols are stronger antioxidant than vitamin E and C. The mineral contents such as potassium, phosphorus, sodium, iron and calcium were 3.38, 1.15, 0.95, 2.23, 3.79 mg/100 g, respectively. The present value of this investigation was quite different to that of nutrient and mineral contents of *C. cordifolia* L. leaves (Ruby *et al*., 2000), this difference might be due to the different soil conditions[Dr. Farha5].

Phytochemical screening

The crude methanolic extract of the *C. cordifolia* L. plant revealed the presence of phytochemicals such as flavonoids, saponins, tannins and terpenoids (Table 2). The presence of these active ingredients of this plant was somewhat similar to the herbal tea containing, *Ficus deltoidea*, *Orthosiphon stamineus* and *Stevia rebaudian*. This shows that the *C. cordifolia* L. plant can be also used to produce herbal tea.

Table 2: Qualitative analysis of *Coccinia cordifolia* L. plant

Phytochemical	Methanolic extract
Flavonoids	+
Saponins	+
Tannins	+
Lignin	-
Terpenoids	+

+ indicates presence; - indicates absence

Antimicrobial activity of C. cordifolia* L. *plant

The *in vitro* antimicrobial activity of *C. cordifolia* L. plant extracts is shown in Table 3. The plant showed high activity against *Shigella dysenteriae*, *Escherichia coli* and *Staphylococcus aureus*, (13.0, 12.5, and 12.6 mm, respectively) from its methanol extract. It is equal in case of *S. dysenteriae* and very close in case of *E. coli* and *S. aureus* very close to the positive control streptomycin (table 3). These bacteria are food borne pathogens and therefore, methanolic extract of the plant can be useful for food preservation.

Methanolic extract of the plant showed moderate activity against *Bacillus subtilis*, *Sarcina lutea* and *Salmonella typhi* (8.1, 9.1 and 8.0 mm, respectively) whereas ethanol extract was moderately active against Gram positive and Gram negative bacteria which were used in this study. Ethyl acetate extract was also moderately active against all bacteria except *S. aureus*. Water extract showed moderate activity against *S. dysenteriae* and *S. typhi* (9.1 and 7.8 mm, respectively) and lowest activity against *S. lutea* (2.2 mm) and no activity against *E. coli, B. subtilis* and *S. aureus.*

Among fungi, the methanolic extract has the highest activity against *Candida albicans* (16.0 mm) than *Aspergillus niger* (10.1 mm) and *Penicillium notatum* (9.6 mm) whereas and water extract showed the lowest activity against *P. notatum* (7.0 mm) than *C. albicans* (9.0 mm) and *A. niger* (7.2 mm). Both ethanol and ethyl acetate showed more activity against *C. albicans* than *A. niger* and *P. notatum*. *C. albicans* is responsible for the disease candidiasis; therefore this plant can be potentially used as an active ingredient in skin lotions or cream preparations with antifungal activity.

Table 4 shows the test results of MIC and MBC. The result showed that methanol extract was highly sensitive against *S. aureus, E. coli* and *S. dysenteriae*, (high sensitivity of MIC and MBC, ranging from 10 to 25 µg/ml, moderately sensitive to *B. subtilis, S. lutea* and *S. typhi* (moderate sensitivity of MIC is 100 µg/ml and MBC, ranging from 125 to 200 µg/ml). Ethanol extract has shown moderate activity (MIC ranges from 75 to 125 µg/ml and MBC ranging from 100 to 150 µg/ml) against all Gram positive and Gram negative bacteria. Ethyl acetate extract was found moderately active (MIC ranges from 75-125 µg/ml and MBC ranges from 75 to 150 µg/ml) to *B. subtilis, S. lutea, E. coli, S. typhi* and *S. dysenteriae* whilst resistant to *S. aureus* (MIC and MBC >200 µg/ml). Water extract was moderately active (MIC ranges from 75 to 200 µg/ml and MBC ranges from 100 to 200 µg/ml) to *S. lutea, S. typhi* and *S. dysenteriae* whilst resistant to *S. aureus*, *B. subtilis* and *E. coli* (MIC and MBC >200 µg/ml). *C. albicans* with MIC and MFC values of 200 µg/ml and 400 µg/ml, respectively with methanol extract has shown highest sensitivity whereas *P. notatum* was least affected by water extract with MFC and MIC values of 2000 and 1800 µg/ml, respectively.

DISCUSSION

Chandarana *et al.* (2005) reported that most of the pathogenic organisms become resistance to antibiotic. So, the alternative natural treatment for microbial infection can give a pathway to develop new antimicrobial agents. From the results of this study it has been observed that methanol extract of *C. cordifolia* L. plant can potentially inhibit the growth of fungal and certain bacterial pathogens. This study also indicates that the plant contained total phenol, vitamin C and β-carotene and the methanol extract showed the presence of saponins, flavonoids, terpenoids and tannins by phytochemical screening. These compounds have been reported to possess antimicrobial activity (Aziz *et al*., 1998, El-Gammal and Mansour, 1986, Cowan, 1999).

Table 3: Antimicrobial activity of *Coccinia cordifolia* L. plant extracts

Microbial culture	Zone of inhibition (diameter in mm)				
	Methanol Extract (200 µg/ml)	Water Extract (200 µg/ml)	Ethanol Extract (200 µg/ml)	Ethyl acetate Extract (200 µg/ml)	Streptomycin (200 µg/ml)
Gram positive bacteria					
Sarcina lutea	9.1 ± 0.2	2.2 ± 0.2	6.1 ± 0.2	6.5 ± 0.2	10.0 ± 0.1
Bacillus subtilis	8.1 ± 0.2	-	5.0 ± 0.1	5.5 ± 0.1	12.0 ± 0.1
Staphylococcus aureus	12.6 ± 0.1	-	6.2 ± 0.2	-	13.0 ± 0.1
Gram negative bacteria					
Salmonella typhi	8.0 ± 0.2	7.8 ± 0.2	6.0 ± 0.2	7.2 ± 0.2	11.0 ± 0.7
Shigella dysenteriae	13.0 ± 0.1	9.1 ± 0.1	6.0 ± 0.1	7.5 ± 0.1	13.5 ± 0.1
Escherichia coli	12.5 ± 0.1	-	8.0 ± 0.1	8.5 ± 0.1	13.0 ± 0.1
Fungi	(2000 µg/ml)	(2000 µg/ml)	(2000 µg/ml)	(2000 µg/ml)	Griseofulvin (2000 µg/ml)
Candida albicans	16.0 ± 0.1	9.0 ± 0.1	10.1 ± 0.1	11.0 ± 0.1	18.0 ± 0.2
Aspergillus niger	10.1 ± 0.2	7.2 ± 0.1	8.0 ± 0.1	9.0 ± 0.1	14.2 ± 0.1
Penicillium notatum	9.6 ± 0.2	7.0 ± 0.1	8.3 ± 0.1	8.1 ± 0.1	11.2 ± 0.1

'-' indicates no measurable zone. Values are mean ± S.E.M. of 3 replicates.

Table 4: Minimum inhibitory and microcidal concentrations of *Coccinia cordifolia* L. plant extracts

Microbial culture	Methanol Extract (µg/ml)		Water Extract (µg/ml)		Ethanol Extract (µg/ml)		Ethyl acetate Extract (µg/ml)	
Bacteria	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram positive bacteria								
Sarcina lutea	100	150	200	200	125	150	100	150
Bacillus subtilis	100	150	>200	>200	100	100	100	150
Staphylococcus aureus	10	25	>200	>200	100	100	>200	>200
Gram negative bacteria								
Salmonella typhi	100	125	125	125	100	150	125	125
Shigella dysenteriae	10	25	75	100	75	100	75	100
Escherichia coli	15	25	>200	>200	75	100	75	75
Fungi	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Candida albicans	200	400	1000	1500	700	800	800	1000
Aspergillus niger	700	900	1500	2000	1500	1700	1000	1100
Penicillium notatum	1200	1200	1800	2000	1500	1500	1500	1800

Mean values from three replicates are recorded, MIC – Minimum Inhibitory Concentration, MBC – Minimum Bactericidal Concentration, MFC – Minimum Fungicidal Concentration

CONCLUSIONS

In search of novel plant bioactive materials encouraged us to study the activity of the mentioned plant extracts toward an array of microorganisms. Results showed the potential activity of *C. cordifolia* L. plant extracts against some bacteria and fungi for the first time. This investigation does not reveal the chemical compound that is responsible for the antimicrobial activity. Now we will direct our study to explore the main compound from this plant responsible for the aforesaid activity.

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