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RESEARCH ARTICLE

Screening of *Callicarpa arborea* and *Hemigraphis alternata* for antibacterial activity

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Callicarpa arborea and *Hemigraphis alternata* are two medicinal plants claimed to have antimicrobial property in Indian traditional medicine. The methanol extracts of the stem bark of *C. arborea* and the leaves of *H. alternata* were prepared and tested for antibacterial activity using disk diffusion method. Four Gram-negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Salmonella typhimurium*; and two Gram-positive bacteria such as *Micrococcus luteus* and *Bacillus subtilis* were used. *C. arborea* extract was effective against all the bacteria tested, while *H. alternata* did not show any inhibitory activity. These findings suggest that *C. arborea* is a good source of antibacterial compound.

Keywords: *Callicarpa arborea, Hemigraphis alternata*, antibacterial activity, medicinal plant

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Introduction

Callicarpa arborea Roxb. (family Verbenaceae) is commonly known as "beauty berry" and is a small, moderate-sized tree, belonging to the family Verbenaceae. The tree is about 12 m tall and branch lets, inflorescences, and flower stalks are densely velvet hairy (**Figure 1**). It grows well in different environments and mainly found in Bangladesh, China, India, Indonesia, Nepal, Papua, New Guinea, Thailand, United States and Vietnam. The leaves and barks of *C. arborea* have been used in India to treat skin diseases, rheumatism and cutaneous diseases. The juice of fruit is known to relieve fever. Paste of the bark and juice has been used in Mizo traditional medicine to cure cuts and wounds.[1]

The ointment formulation of *C. arborea* is showed to have equipotent wound healing activities as that of the standard drug Framycetin ointment.[2] Ethnomedicinal survey documented the traditional usefulness of *C. arborea* stem bark in the management of diabetes mellitus.[3] The methanolic extract of the leaves exhibits significant antioxidant activity, which justifies that *C. arborea* leaves can play a pivotal role in preventing the degenerative diseases and other infections. Further, it can be used as a source of the natural antioxidants.[4] The leaves are also used as a traditional purpose for fermentation of soya bean, which is one of the most Mizo favourite dishes. *C. arborea* possesses potential antinociceptive, antioxidant, antimicrobial and thrombolytic activities, which further warrant its systematic chemical investigation in order to isolate the biologically active molecules.[1]

Hemigraphis alternata (Burm. f.) T. Anders. (synonym *Hemigraphis colorata*), known as "waffle plant" or "red-ivy", is member of the family Acanthaceae. It is an exotic plant adapted to India, it is a versatile tropical low-creeping perennial herb that reaches a height of 15 to 30 cm (**Figure 2**). It is

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a tropical perennial herb, chiefly grown as an ornamental indoor and outdoor plant due to its attractive and vivid foliage. Literally, *Hemigraphis* means "half writing" because the filament of the outer stamen bear brushes. The leaf has metallic purple lustre on upper surface and a solid dark purple on ventral side. It blooms irregularly throughout the year in the tropics. The seeds are small, flat and white in colour. The leaves are hairy and opposite about 2 to 8 cm long and 4 to 6 cm wide, bearing well-defined veins. The flowers are small (1 to 1.5 cm in diameter), five lobed, bell shaped with imbricate bracts and these flowers are white in colour with faint purple marks within and appear in terminal 2 to 10 cm long spikes.[5-7]

H. alternata is widely used in Indian traditional medicine for its wound healing activity.[8,9] The antibacterial activity is attributed to the wound healing effect.[10] In addition, that the methanol and ethyl acetate extracts of *Hemigraphis alternate* leaves were documented as having effective anti-inflammatory, antinociceptive, antidiarrhoeal,[11] as well as anticancer activity.[12] Therefore, the present study is to prepare the plant extracts from *C. arborea* stem bark and *H. alternata* leaves and test their effects on the growth of important bacteria.

Materials and Methods

Preparation of plant extract

The stem bark of *C. arborea* were collected from the forest in Aizawl. The leaves of *H. alternata* were collected from a home plant in Aizawl, India. The specimens were dried in shade at 23 to 26°C. The dried samples were crushed to powder using mortar and pestle. Extraction was done in a 5-l Soxhlet apparatus and serially in three solvents, i.e. from petroleum ether to chloroform to methanol. The extracts so obtained were concentrated in a vacuum rotary evaporator. The final semi-solid extracts were used for experiments.

Antibacterial test

The antibacterial activity of the plant extracts was determined using disk diffusion method. Six bacteria were used including four Gram-negative bacteria such as *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 1705), *Pseudomonas aeruginosa* (ATCC 15442), and *Salmonella typhimurium* (ATCC 14028); and two Gram-positive bacteria such as *Micrococcus luteus* (ATCC 4698) and *Bacillus subtilis*

Figure 1 | *Callicarpa arborea*.

Figure 2 | *Hemigraphis alternata*.

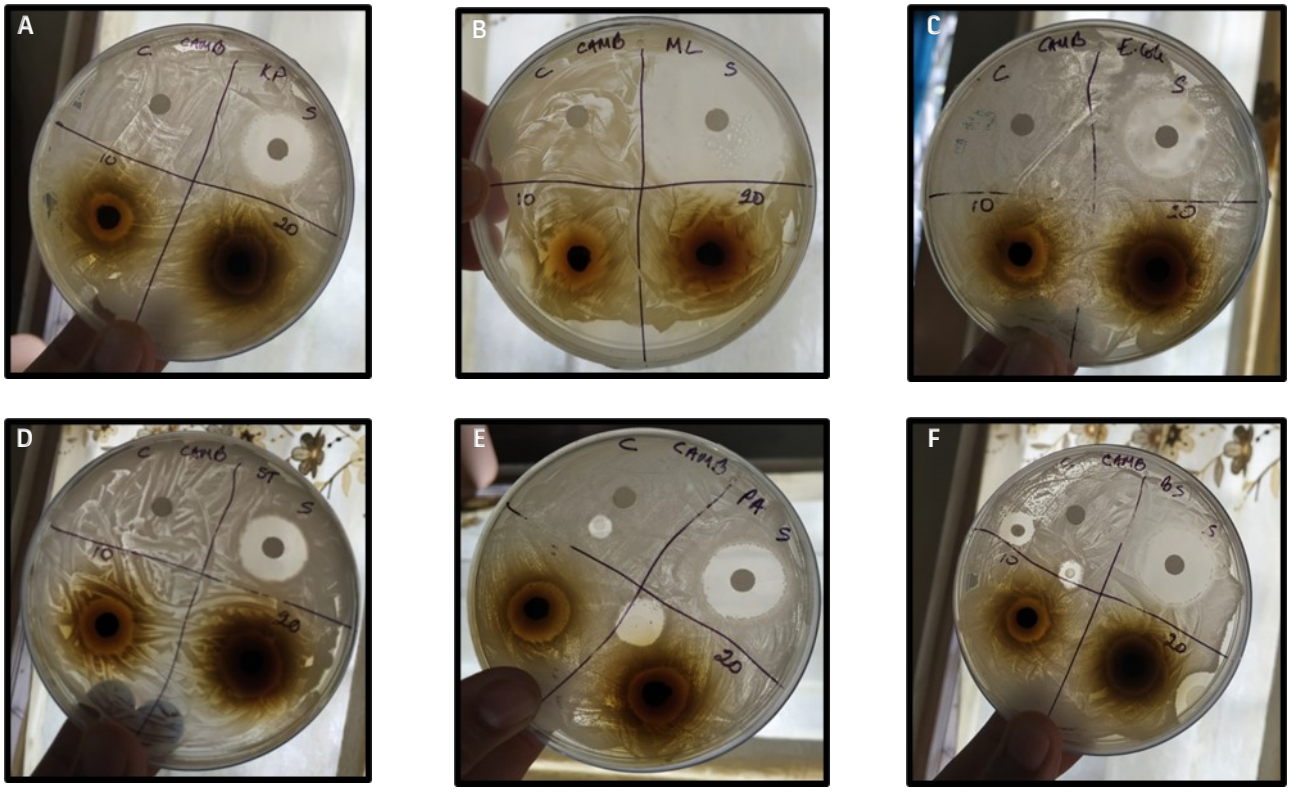

Figure 3 | Bacterial cultures treated with *C. arborea* extract (10 and 20) and ceftriaxone (S) against the control (C). A - *Klebsiella pneumoniae*, B - *Micrococcus luteus*, C - *Escherichia coli*, D - *Salmonella typhimurium*, E - *Pseudomonas aeruginosa* and F - *Bacillus subtilis*.

(ATCC 6051). The bacteria were cultured in sterile Petri culture plates containing Mueller-Hinton agar. Ceftriaxone (10 µg) disk was used as standard antibiotic. Two concentrations, i.e. 10 and 20 mg, were prepared for each extract. The extracts and the drug were inoculated on sterile disks and were placed on the culture disk. The plates were incubated at 37±1°C for 20 h and the corresponding zones of inhibitions were measured.

Result

The methanol extract of stem bark of *C. arborea* showed antimicrobial activity by inhibiting the growth of all the bacteria tested (**Figure 3**). The treatment data are shown in **Table 1**. The zones of inhibition of ceftriaxone and extract at 10 mg and 20 mg for *E. coli* were 1.4 cm and 1.6 cm respectively. For *B. Subtilis* the zones of inhibition of ceftriaxone and extract at 10 mg and 20 mg were 1.3 cm and 1.5 cm. 1.2 cm and 1.6 cm zone of inhibition was observed upon testing with ceftriaxone and extract at 10 mg and 20 mg for *K. pneumonia*. Testing with ceftriaxone and extract at 10 mg and 20 mg for *P. aeruginosa* the zone of inhibition were observed to be 1.5 cm and 1.6 cm. Zone of inhibition of extract and ceftriaxone at 10 mg and 20 mg for *M. Luteus* were 1.5 cm and 1.6 cm. *S. typhimurium* upon testing with ceftriaxone and extract at 10 mg and 20 mg, the zone of inhibition were 1.4 cm and 1.5 cm.

The methanol extract of *H. alternate* leaf did not show any inhibition on all the six bacteria (**Table 2**).

Discussion

Callicarpa arborea is traditionally known for the treatment of bacterial infection and experiments have supported this property. The present study is further evidence of the antibacterial activity as inhibitory effect was seen in all bacteria tested. In another study, an ethanol extract of stem bark of *C. arborea* was shown to be effective against Gram-positive bacteria such as *Bacillus cereus*, *B. megaterium, B. subtilis, Sarcina lutea, S. aureus;* and Gram-negative bacteria including *E. coli*, *P. aeruginosa*, *S. typhi*, *S. paratyphi*, *Shigella boydii, Sh. dysenteriae, Vibrio mimicus, V. parahemolyticus*; as well as fungal species such as *Aspergillus niger, Candida albicans* and *Sacharomyces cerevisiae.*[1]

Umachandur *et al.* also showed that the hydro-alcoholic extract (70% alcohol in water) of the leaves showed similar broad-spectrum antimicrobial activity.[13] The water (100 ml), 60% methanol (100 ml) and 60% ethanol extracts of the leaves were also demonstrated to be effective against bacteria such as *S. aureus*, *E. coli* and plant pathogenic fungus,

Table 1 | Antibacterial activity of *C. arborea* extract (CAE) and ceftriaxone (*n* = 3)

Sl. No.	Bacteria	Treatment	Zone of inhibition (cm)
1.	*Escherichia coli*	CAE 10 mg	1.4
		CAE 20 mg	1.6
		Ceftriaxone 10 µg	2
2.	*Bacillus subtilis*	CAE 10 mg	1.3
		CAE 20 mg	1.5
		Ceftriaxone 10 µg	2.2
3.	*Klebsiella pneumoniae*	CAE 10 mg	1.2
		CAE 20 mg	1.6
		Ceftriaxone 10 µg	1.9
4.	*Pseudomonas aeruginosa*	CAE 10 mg	1.5
		CAE 20 mg	1.6
		Ceftriaxone 10 µg	2
5.	*Micrococcus luteus*	CAE 10 mg	1.5
		CAE 20 mg	1.6
		Ceftriaxone 10 µg	3.4
6.	*Salmonella typhimurium*	CAE 10 mg	1.4
		CAE 20 mg	1.5
		Ceftriaxone 10 µg	1.7

Table 2 | Antibacterial activity of *H. alternata* extract (HAE) and ceftriaxone (*n* = 3)

Table 2 | Antibacterial activity of *H. alternata* extract (HAE) and ceftriaxone (*n* = 3)

Sl. No.	Bacteria	Treatment	Zone of inhibition (cm)
1.	*Escherichia coli*	HAE 10 mg	0.0
		HAE 20 mg	0.0
		Ceftriaxone 10 µg	2
2.	*Bacillus subtilis*	HAE 10 mg	0.0
		HAE 20 mg	0.0
		Ceftriaxone 10 µg	2.2
3.	*Klebsiella pneumoniae*	HAE 10 mg	0.0
		HAE 20 mg	0.0
		Ceftriaxone 10 µg	1.9
4.	*Pseudomonas aeruginosa*	HAE 10 mg	0.0
		HAE 20 mg	0.0
		Ceftriaxone 10 µg	2
5.	*Micrococcus luteus*	HAE 10 mg	0.0
		HAE 20 mg	0.0
		Ceftriaxone 10 µg	3.4
6.	*Salmonella typhimurium*	HAE 10 mg	0.0
		HAE 20 mg	0.0
		Ceftriaxone 10 µg	1.7

Fusarium oxysoporum f. sp. *ciceri* and *Fusarium graminarum*.[14]

In contrast to the traditional claim, the present data reveal that *Hemigraphis alternata* does not have antibacterial activity. This contradicts the report of Anitha *et al.* which showed that the aqueous, acetone, benzene, chloroform, ethanol, and petroleum ether extracts of the leaves and stem were all effective against a wide range of bacteria including *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus*, *Bacillus cereus*, *Salmonella typhi*, *Serratia marcescens*, *Acinetobacter* sp., *Enterobacter* sp., *Proteus mirabilis*, *Enterococcus faecalis* and *Streptococcus pyogenes*.[15] Jayaprakasan *et al.* reported that an Ayurvedic preparation consisting of *Terminalia chebula, Azadirachta indica* and *Hemigraphis colorata* extracts were effective against *E. coli*, *P. aeruginosa* and *S. aureus*.[16] But the biological activity can be due to the other two plants.

It is therefore clear that from the present study, *C. arborea* bark extract has antimicrobial activity that effectively inhibit the growth of different Gram-positive and Gram-negative bacteria tested. Thus, it may be concluded that it contains natural compound (s) that could be a potential source of antibiotic. However, contradicting the traditional usage and other experimental findings, *H. alternata* did not show any antimicrobial activity on all the bacteria tested.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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