ANTIBACTERIAL ACTIVITY OF THREE MEDICINAL PLANTS OF KUMAUN HIMALAYA AGAINST SOME PATHOGENIC BACTERIA

S. C. SATI, POONAM TAKULI, P. KUMAR* AND K. KHULBE

Department of Botany, D.S.B. Campus, Kumaun University, Nainital-263002, (UK), India
Email: pk2461989@gmail.com

Abstract

The antibacterial property of methanol, ethanol and hexane extracts of *Berberis aristata*, *Chenopodium ambrosioides* and *Tinospora cordifolia* grown in Kumaun Himalayan were investigated against some pathogenic gram positive and gram negative bacterial strains (*Bacillus subtilis*, *Agrobacterium tumefaciens*, *Escherichia coli*, *Xanthomonas phaseoli* and *Erwinia chrysanthemi*) using disc diffusion method. Methanol extract of *B. aristata* was found with highest inhibitory activity against *E. chrysanthemi* (ZOI, 11±0.3mm). Whereas lowest inhibition was recorded in ethanolic extract of *B. aristata* against *E. coli*. The hexane extract of *B. aristata* and methanolic extract of *C. ambrosioides* were found totally inactive against all the pathogens tested.

Key words: *Berberis aristata*, *Chenopodium ambrosioides*, *Tinospora cordifolia*, Antibacterial activity, Disc diffusion

INTRODUCTION

The medicinal properties of plant species have made an outstanding contribution in the origin and evaluation of many traditional herbal therapies. The plant based, traditional medicine system placed an important role in health care with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary healthcare (Owolabi et al., 2007). Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against different pathologies (Sati et al., 2014).

Antimicrobials of plant origin are efficient in the treatment of infectious diseases mitigating simultaneously many of the side effects that are often associated with synthetic antimicrobials. Several studies indicated the presence of many compounds with antimicrobial properties in plants or plant parts (Darokar et al., 1998; Joshi and Sati, 2012). To develop alternative antimicrobial drug one approach is to investigate the local medicinal plants, which represent rich source of antimicrobial agents (Khulbe and Sati, 2009; Sati and Kumar, 2015). In the present investigation three ethnobotanically well known medicinal plants namely, *Berberis aristata*, *Chenopodium ambrosioides* and *Tinospora cordifolia* growing in Nainital, Kumaun Himalaya were analyzed for their antibacterial activity against diseases causing bacteria.

2. MATERIAL AND METHODS

Ethnobotany of selected plants and collection

Berberis aristata DC, commonly known as barberry and locally "Kirmoda" belongs to the family Berberidaceae (Fig. 1). This plant is well known for its medicinal property in traditional as well as modern medicine system as plant parts are used in the treatment of skin diseases, eye diseases, wound healing, ulcers, malarial fever, diabetes, dysentery, uterine, and vaginal disorders (Kirtikar & Basu, 1933). *B. aristata* root contains alkaloids which are berbamine, berberine, oxycanthine, epiberberine, palmatine, dehydrocaroline, jatrorhizine and columbamine (Chatterjee, 1951 and Saied et al., 2007). The fruits of *B. aristata* are given as a cooling laxative to children. The dried extract of the roots is used as an application in ophthalmia. A very important ayurvedic preparation "rasaut" is from this plant. This is used to treat skin diseases, menorrhagia, diarrhea, cholera, jaundice, eye and ear infections, as well as urinary tract infections.

Chenopodium ambrosioides Linn. (maxican tea , Indian worm seed) commonly known as "Banbathu" belongs to family Chenopodiacae (Fig. 2). *C. ambrosioides* is commonly available weed, having significant traditional and pharmacological activities. The juice of the plant is taken as vermifuse (Kirtikar et al., 1987). Various workers reported antipruritic, antinociceptive, antimicrobial, anthelmintic and other pharmacological activities of the plant (Lall et al., 1999; Chandler, 1961; Olajide et al., 1997; Kiuchi et al., 2002).

Tinospora cordifolia (Guduchi), a medicinal plant of the family Menispermaceae is a deciduous climbing shrub (Fig. 3). It is popularly known as 'Giloe' in the Indian medicine system and has traditional use form centuries in the treatment of jaundice, diabetes, skin diseases and anemia (Chadha, 1976). The plant is widely used in the Ayurvedic system of medicine as general tonic, anti-inflammatory, antiarthritic, antiallergic, anti malarial, antidiabetic, and aphrodisiac (Rao et al., 2008). Its stem is also used in general debility, dyspepsia, fever, and urinary diseases (Singh et al., 2003; Gupta et al., 1967).

Plants and plant parts were collected from suburbs of Nainital, Kumaun Himalaya, India and authenticated by the Department of Botany, Kumaun University, Nainital. Voucher specimens of all used plants/parts were deposited in the herbarium.

Fig.1 Berberis aristata

Fig.2 Chenopodium. ambrosioides

Fig. 3 Tinospora cordifolia

Extraction procedure

Collected plant materials (Bark and leaves) were thoroughly washed and dried at room temperature. The dried materials were powdered in an electric grinder. To prepare a stock solution, 25 g of plant powder was subjected to 100 ml of different solvents (methanol, ethanol, and hexane) and kept in a shaker for 6 to 10 h. The prepared extracts were filtered through Whatman filter paper no. 1. The filtrates were concentrated on a rotary evaporator under vacuum at 20°C and utilized for antibacterial assessment (Mohanta et al., 2007). The concentrated extracts were kept in separate cap tubes and stored at 4 °C for their further antimicrobial assays.

Test microorganisms

To evaluate the antibacterial potential of test plants five pathogenic (gram positive and gram negative) bacterial strains were selected. *Bacillus subtilis* MTCC No. 121, *Agrobacterium tumefaciens* MTCC No. 609 and *Escherichia coli* were obtained from IMTECH, Chandigarh, India, whereas *Xanthomonas phaseoli* and *Erwinia chrysanthemi* were obtained from Plant Pathology Department, G. B. Pant University of Agriculture and Technology, Pantnagar, India.

Antibacterial assay

Antibacterial assays were performed by using disc diffusion method (Bauer et al., 1966). A small sterile cotton swab was dipped into the 24-hour-old culture of bacteria and was inoculated by streaking the swab over the entire agar surface. After inoculation the plates were allowed to dry at room temperature in laminar

chamber. The filter paper discs (5 mm) loaded with 40µl of extract were placed on the surface of the agar plates. After 5 min the plates were incubated at 37 ± 2°C for 24 h. Gentamycin was used as positive controls and the respective solvent were taken as negative control. After 24 h of incubation, the dishes were observed for bioactivity (Plate 1) and the diameter was observed for zone of inhibition (ZOI). All tests were performed in triplicate and observed values of ZOI are expressed as mean value with standard error of means (SEM).

RESULTS

The antibacterial activity results of *Berberis aristata*, *Chenopodium ambrosioides*, and *Tinospora cordifolia*, using different solvent extracts (methanol, ethanol and hexane) are summarized in table 1, 2 and 3. All the extracts of tested plants showed variable activity against all the tested bacterial strains. Methanol extract of *B. aristata* showed highest antibacterial activity (ZOI 11±0.3mm) recorded against *E. chrysanthemi*. Whereas ZOI of 9±0.3mm of ethanol extract of *B. aristata* was recorded against *B. subtilis*, *X. phaseoli*, and *E. chrysanthemi*. Both ethanol and methanol extracts were found totally inactive against *A. tumefaciens*. While hexane extract was found totally inactive against all the tested strains (Fig.4).

Table 1. Antibacterial activity of different extracts of *B. aristata* bark

	Diameter of inhibition zone (mm)*			
Microorganisms	**E**	**M**	**H**	**G**
A. tumefaciens	na	na	na	21±1.0
B. subtilis	9±0.3	10±0.0	na	20±0.3
E. chrysanthemi	9±0.5	11±0.3	na	19±0.0
E. coli	6±0.3	9±0.6	na	20±0.8
X. phaseoli	9±0.3	10±0.3	na	16 ±0.8

*All the values are mean ± Standard Error of Mean (SEM) of three determinations H, M, E-Hexane, Methanol and Ethanol extract. G-Gentamycin (+ control), na- not active

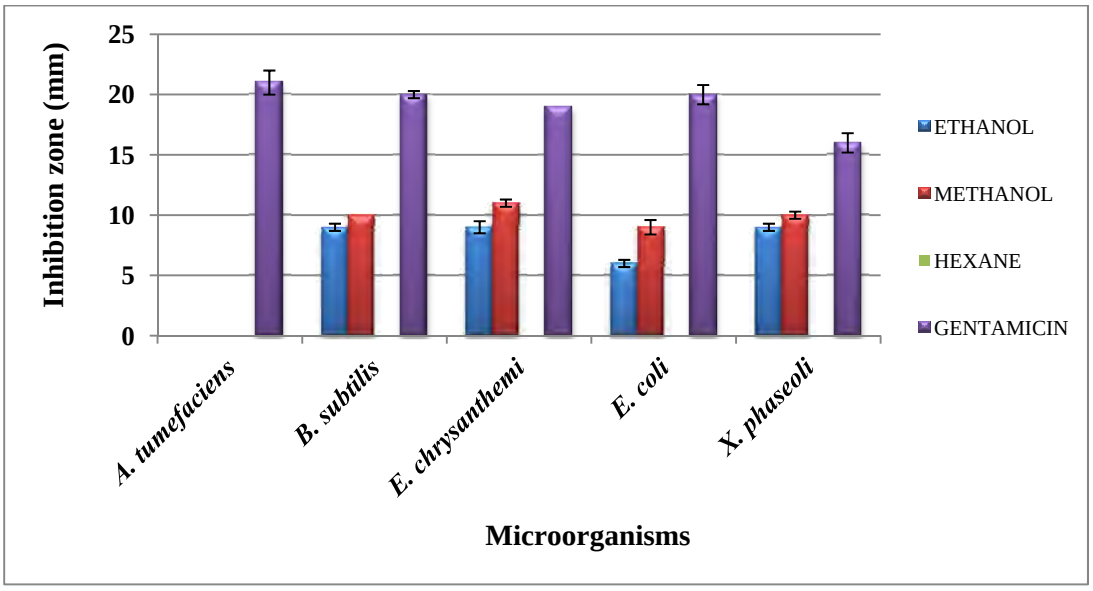

Fig.4. Inhibition zones of different bark extracts of *B. aristata* against five bacterial strains

Highest activity of *C. ambrosioides* leaves was observed in hexane and ethanolic extracts showing up to ZOI of 9.3mm against different tested strains, with hexane extract showing activity against all the tested strains (Table 2). Methanolic extract of the plant did show no activity against bacterial strains tested in the present study (Fig.5).

Table 2. Antibacterial activity of different leave extracts of *C. ambrosioides*

	Diameter of inhibition zone (mm)			
Microorganisms	**E**	**M**	**H**	**G**
A. tumefaciens	7.5±0.3	na	7±1.3	21±1.0
B. subtilis	7±0.3	na	9±0.3	20±0.3
E. chrysanthemi	9.3±0.7	na	9±0.3	19±0.0
E. coli	na	na	9.3±2.3	20±0.8
X. phaseoli	7±0.3	na	7.3±0.3	16±0.8

*All the values are mean ± Standard Error of Mean (SEM) of three determinations **H, M, E**-Hexane, Methanol and Ethanol extract. G-Gentamycin (+ control), na- not active

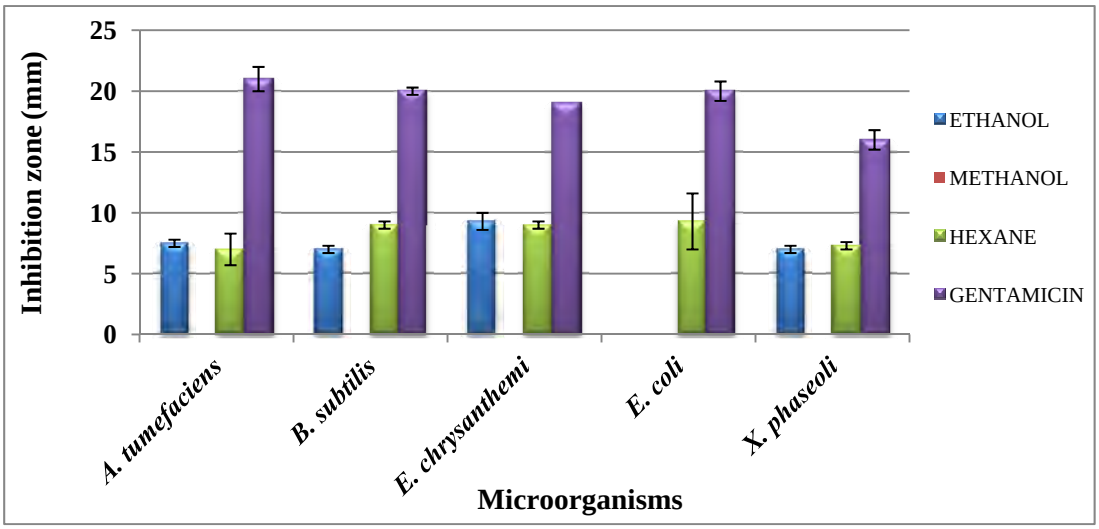

Fig.5. Inhibition zones of different leaves extracts of *C. ambrosioides* against five bacterial strains

While testing different extracts of *T. cordifolia* leaves, highest activity was observed for ethanolic extract with ZOI 9 mm against *X. phaseoli* and ZOI 8 mm against *B. subtilis*, *E. chrysanthemi* and *E. coli*. Slightly low activity (ZOI 7.5 mm) was found against *A. tumefaciens* (Table. 3). Methanolic extract of the plant showed activity only against *B. subtilis* and *X. phaseoli* (ZOI, 7 mm), whereas no activity was observed against *E. chrysanthemi* and *E. coli*. Hexane extract did not show any activity against any of the tested strains (Fig. 5).

Table 3. Antibacterial activity of different extracts of *T. cordifolia* leave

	Diameter of inhibition zone (mm)			
Microorganisms	**E**	**M**	**H**	**G**
A. tumefaciens	7.5±0.3	na	na	21±1.0
B. subtilis	8±1.1	7±0.3	na	20±0.3
E. chrysanthemi	8±0.6	na	na	19±0.0
E. coli	8±0.3	na	na	20±0.8
X. phaseoli	9±1.0	7±0.6	na	16 ±0.8

*All the values are mean ± Standard Error of Mean (SEM) of three determinations **H, M, E**-Hexane, Methanol and Ethanol extract. G-Gentamycin (+ control), na- not active

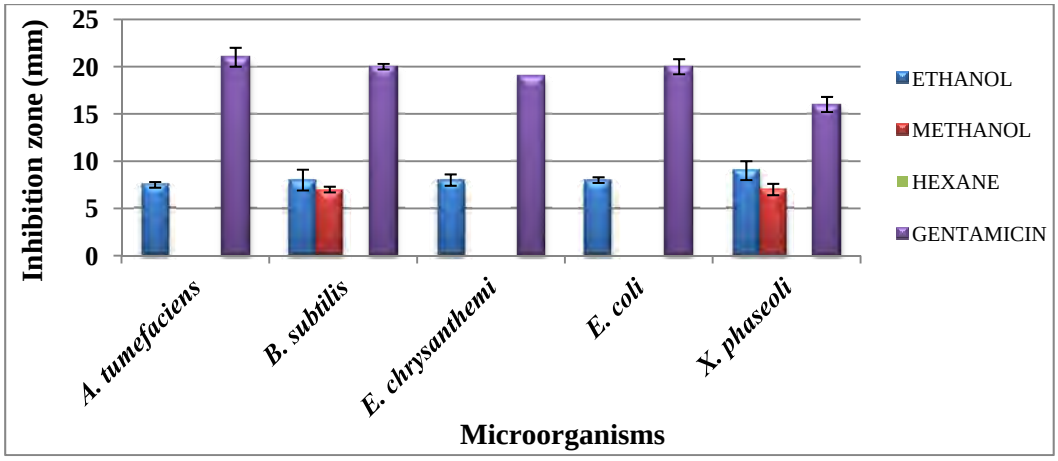

Fig.6. Inhibition zones of different leaves extracts of *T. cordifolia* against five bacterial strains

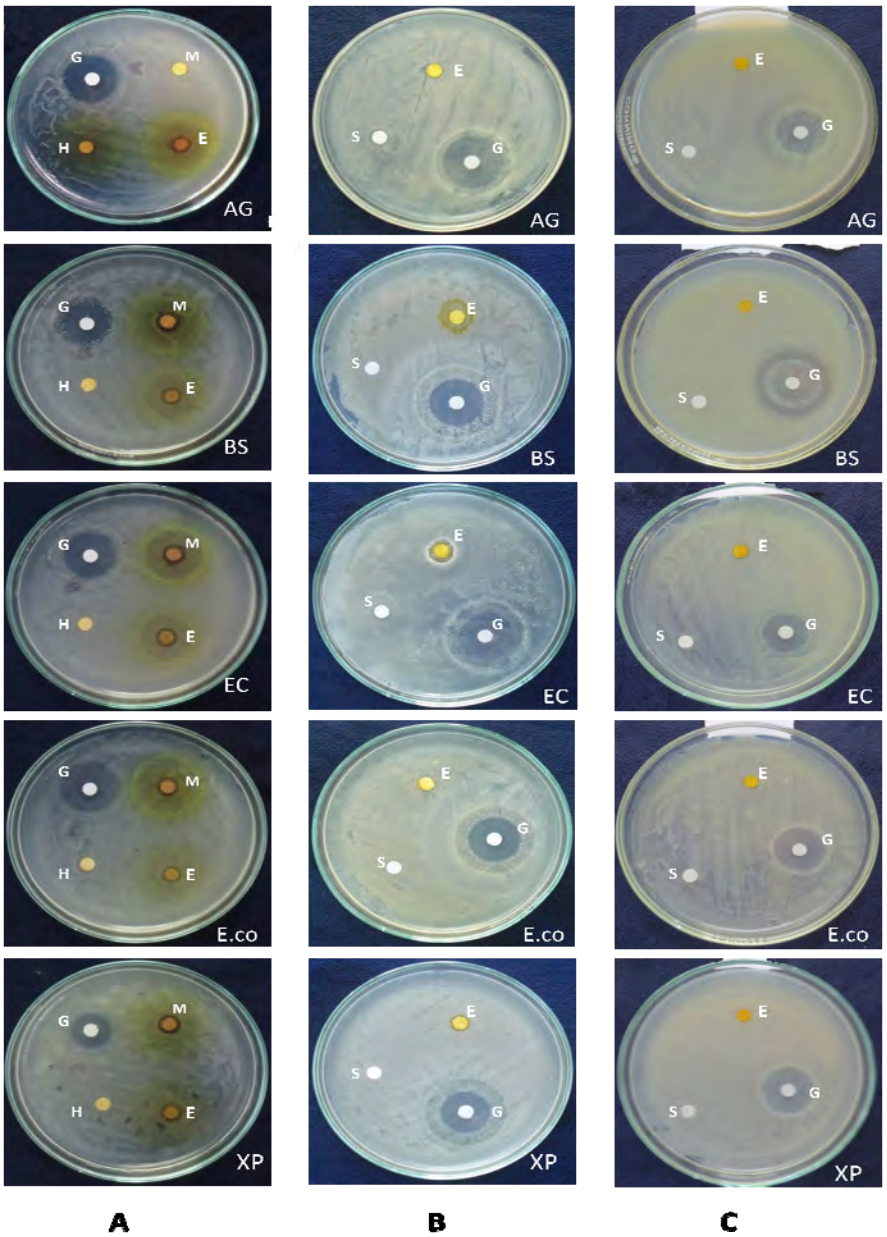

Fig.7. Antibacterial activity of different extracts against pathogenic bacteria. A- *B. aristata*, B- *C. ambrosioides*, C- *T. cordifolia*; H- Hexane extract, E- Ethanol extract, M- Methanol extract; XP- *Xanthomonas phaseoli*, BS- *Bacillus* subtilis, AG- *Agrobacterium tumefaciens*, E.co- *Escherichia coli*, EC- *Erwinia chrysanthemi*; G- Gentamycine (positive control) and S- Solvent (negative control).

DISCUSSION

In the present investigation three highly important medicinal plants (*B. aristata, C. ambrosoides* and *T. cordifolia*) were analyzed to find out their inhibitory effects against some plant and animal pathogenic bacteria, using three different organic solvents (methanol, ethanol and hexane). Multiple factors affect the outcome of antimicrobial efficacy of plant products. The results obtained from antimicrobial efficacy of plant extract is often difficult to compare with published results due to the influence of several factors, such as, environment, climatic conditions during plant growth, choice of plant extracts, choice of extraction methods, antimicrobial test employed, and on test microorganisms (Nostro et al., 2000; Hammer et al., 1999).

Highest inhibitory potential was observed in methanolic extract of *B. aristata* against *E. chrysanthami* (11mm), followed by *B. subtilis* and *X. phaseoli* (10 mm) and *E. coli* (9 mm). A very less activity was observed in case of methanolic extract of *T. cordifolia* as it showed only 7 mm inhibition zone against *B. subtilis*, and *X. phaseoli*, while in the case of *C. ambrosioides*, methanolic extract was found totally inactive against all the test microorganisms.

Likewise hexane extracts of *B. aristata* and *T. cordifolia* were totally inactive while hexane extract of *C. ambrosioides* revealed inhibitory effect. These variable inhibitory effects of tested plants using same organic solvent may be due to variability among the phytoconstituents of these plants and their variable properties to get dissolved in different solvents. Ethanol extract of all the tested plants showed a significance antibacterial activity against all the test bacterial strains and suggest the use of ethanol for extraction of bioactive molecules.

In the existing literature, berberine has been reported to be produced by numerous *Berberis* spp. including, *B. nepalensis*, *B. asiatica*, *B. vulgaris* and *B. lycium* (Chandra and Purohit, 1980), *B. aetnensis* (Iauk et al., 2007), *B. stolonifera* (Stadler et al., 1988), *B. chitria* (Hussaini and Shoeb, 1985). The most active ingredient of *B. aristata* is berberine, a quaternary isoquinoline alkaloid and the content of berberine is used as biomarker of the plant. It is mostly found in the roots, rhizomes and stem bark (Pasrija et al., 2011). Native berberine has already been reported to possess antimicrobial activities against a wide variety of microorganisms including Gram-positive and Gram-negative bacteria, fungi, and protozoa (Amin et al., 1969; Birdsall and Kelly, 1997; Park et al., 1999; Park et al., 2001; Iauk et al., 2007; Pasrija et al., 2011; Wagh and Vedhale, 2010). Apart from antimicrobial activities, berberine has also been reported to possess anti-inflammatory, analgesic, and antipyretic potentials (Kupeli et al., 2002; Yesilada and Kupeli, 2007*)*. Our findings supports these previous results, as different extract of *B. aristata* revealed inhibitory effect against test microbes and this activity may be due to the presence of berberine.

C. ambrosioides, is well known in traditional uses as a worm repellent. Its oil is particularly rich in monoterpenes (Kokanova-Nedialkova et al., 2009; Chekem et al., 2010; El-Seedi et al., 2012) and monoterpenes are able to affect bacterial cellular integrity resulting in inhibition of respiration and alteration in permeability (Helander et al., 1998). Previous findings concluded that the antimicrobial activity of *C. ambrosioides* could be attributed due to the presence of monoterpene hydrocarbons (Sokmen et al., 2003; Deba et al., 2008; Okoh et al., 2010; Kumar et al., 2007; Jardim et al., 2008). This essential oil is also known to inhibit the growth of dermatophytes (Kishore, 1999) and other filamentous fungi such as *Aspergillus*, *Fusarium* and *Colletotrichum* (Jardim et al., 2008). It also possesses antiaflatoxigenic, antimalarial and antioxidant properties (Kumar et al., 2007) as well as antihelmintic and worm expelling activities (Potawale et al., 2008).

Mahesh and Satish (2008) and Nascimento, et al. (2000), reported that *T. cordifolia* displayed antimicrobial activity. Samy and Igaanacimuthu (2000) also reported good antimicrobial activity of various extracts of leaves against *B. subtlis, E. coli, P. vulgaris* and *S. aureus*. Samy (2005) found antibacterial activity of methanolic extract of stem against *E. aerogenes, P. vulgaris,* and *P. mirobilis*. Various other workers (Gangan et al., 1994 &1995, Bhatt and Sabata, 1989, Hanuman et al., 1986, Gopi et al., 2004) have shown the presence of some phytochemicals (alkaloids, diterpenoids, lactones, glycosides, steroids, sesquiterpenoids, phenolics, aliphatic compounds and polysaccharides) in these plants. Alkaloids like berberine (Padhya, 1986; Rao et al., 2008; Srinivasan et al., 2008), palmatine, tembetarine, magnoflorine, choline, tinosporine, columbin, isocolumbin, tetrahydropalamatine have been isolated from extracts of stem and roots of the plants (Bisset and Nwaiwu, 1983; Sharma et al., 1998; Gupta et al., 2003).

In single plant many active secondary metabolites are present and medicinal effect can be attributed to either to a single compound or synergistic effect of many compounds. Thus the present antibacterial activity of tested plants can either be due to presence of some specific bioactive molecule or due to the synergistic effect of different phytoconstituents. Our findings in addition to support earlier works generated new data for their effectiveness against plant pathogenic bacteria. Thus previous results regarding to antimicrobial activity of these plants not only support the present *in vitro* antimicrobial activity but also highlight the alternative source of natural or plant based antibiotics as good phytotherapy sources.

CONCLUSION

The investigation on antibacterial activity against some pathogenic bacteria revealed that the different plant/parts extracts of *B. aristata*, *C. ambrosioides* and *T. cordifolia* exhibited significant bioactivity and support folkloric use of these plants as broad spectrum antimicrobial agents. This study not only substantiates the utilization of these plants as an antibacterial in future but also warrants further studies to isolate the active principles, elucidate their structure and their pharmacological activities.

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