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Garima Tamta
Department of Chemistry, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Nisha Mehra
Department of Chemistry, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

S Tandon
Department of Chemistry, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Viveka Nand
Department of Chemistry, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Shiva Biswas
Department of Chemistry, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Corresponding Author:
Garima Tamta
Department of Chemistry, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

In vitro antimicrobial assay of leaves, bark and fruits of *Ficus auriculata* collected from two different regions of Uttarakhand

Garima Tamta, Nisha Mehra, S Tandon, Viveka Nand and Shiva Biswas

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Abstract
The present study is to evaluate the antimicrobial efficacy of *Ficus auriculata* fruits, leaves and bark collected from two different regions of Uttarakhand i.e, Almora and Haldwani. These parts of plants extracted in hexane, chloroform and methanol by successive soxhlet extraction technique. For antimicrobial assay, activity was done by agar well diffusion method against four human pathogenic bacteria (two gram positive and two gram negative bacteria).The results concluded that Almora leaves hexane extract possessed the higher antibacterial potential on increasing dose dependent manner at 800µg/ml (15.7±0.25 mm) Haldwani fruits hexane extract (15.5±0.25 mm) against *E. coli*. Almora bark chloroform extract inhibits the bacteria *E. coli* and *B. subtilis* with maximum zone (14±0.11 mm) and (12.4±0.15 mm). Haldwani leaves methanol extract showed the maximum inhibition zone (13±0.57 mm) against *E. coli* and Haldwani bark methanol extract showed the maximum inhibition zone (12.9±0.057 mm). These naturally occurring antimicrobials agents due to its safe and non toxic nature.

Keywords: Antimicrobial activity, *Ficus auriculata*, fruits, leaves and bark

1. Introduction
According to WHO 80% of world populations depends on medicinal plants and the rest of population health depends on commercial. About 21,000 species of plants used for their medicinal properties (Mahalakshmi M *et al*., 2014) [10]. India has the greatest resources of medicinal herbs endowed with a wide range of agroclimatic conditions and is known as the botanical backyard of the world. India is a biodiversity hotspot and a great variety of fruiting trees are indigenous to this region of the world as confirmed by various reports (Baliga MS *et al*., 2011) [2]. Over the centuries, Indian herbal drugs used as a major source of medicines for the treatment and prevention of many diseases. Ethnobotany embraces a complicated relationship between plants, people and way of life. This relationship between flora and human cultures is no longer confined to the use of vegetation for meals, clothing and shelters, but also includes their use for spiritual ceremonies, ornamentation and fitness care (Raghavendra MP *et al*., 2015) [15].

Ficus is a genus that consists of 750 species of medicinal plants primarily occurring in tropical and subtropical regions throughout the world. There is a large variation in the habitat of this species. Ficus genus belongs to the mulberry family (Moraceae) (Kunwar *et al*., 2006) [8]. Fig species are rich in nutrient, vitamins, mineral elements, water, and fats. Figs are rich source of calcium and fiber. According to USDA data for the Mission variety, dried figs are rich in fiber, vitamin K, copper, magnesium, manganese, calcium, potassium (Ahmad S *et al*., 2013) [1]. The literature survey reported that figs have been cultivated over 1100 years and these are one of the earliest cultivated plants for human use (Lansky EP *et al*., 2008) [9]. The genus can be gently reviewed by the very distinguishing syconium and lactory latex and are collectively known as "figs". Ficus plants are used by humans in different ways throughout the tropical and sub-tropical regions. Plants are origin of medication and nutrition and are used as decorative trees, devotional plants, lac hosts, fuel, fodder hedges or enclosures (Shi Y *et al*., 2018) [17].

Taxonomic classification: (Shilpakar A *et al*., 2009) [18]
Kingdom: Plantae (Plants)
Subkingdom: Tracheobionta (Vascular plants)

Superdivision: Spermatophyta (Seed plants)
Division: Magnoliophyta (Flowering plants)
Class: Magnoliopsida (Dicotyledons)
Order: Urticales
Family: Moraceae (Mulberry family)
Genus: Ficus L. (Fig)
Species: *F. auriculata* Lour.
Synonyms: *F. roxburghii* Wall

1.1 Common Indian names
Gular, Timbal, Timal, Timla, Tirmal, Tremal, Trimmal Habitat -It consists of over 800 species and is one of about 40 genera of the mulberry family. The plants are mainly distributed in temperate, tropical and subtropical regions of about 1800 – 2600 m altitude. It is native to Asia, especially in India, China, Nepal, Bhutan, Pakistan, Myanmar, Thailand, Vietnam, Malaysia etc.

1.2 Botanical description: *F. auriculata* is also known as Elephant earfig tree because of its large leaves, The tree is a very large and evergreen, 4-10 m tall, with huge spreading limbs supported by aerial roots which later form accessory trunks extending to a large area. The bark is greyish brown with rough texture. Leaves are simple, broad, oval, ovate or orbicular-ovate to oblong 5-8 cm long, 5-12.5 cm, petioles about 1.2-5 cm long, stipules are 1.5–2 cm. Fruits with fleshy pericarp and pear shaped with 8–12 conspicuous longitudinal ridges, 3–5 cm in diameter and with achenes embedded in them, riped or mature fruit is dark red in colou

1.3 Traditional uses: Leaves of *F. auriculata* are crushed and the paste is applied to the wounds. They are also used in diarrhoea and dysentery. It's stem bark juice is effective for cuts and wounds and diarrhoea. Roasted figs are taken for diarrhoea and dysentery. Latex of roots is used in diarrhea, cholera mumps, and vomiting. Mixture of root powder of *F. auriculata* and bark of *Oroxylum indicum* is taken in jaundice (Manandhar S *et al*., 2019) [11]. Ethnic people in kharagchari hill district use *F. auriculata* as food and medicinal plant (Singh R *et al*., 2005) [19].
Ficus species are rich in flavonoids, polyphenolic compounds, which have strong antioxidant properties that help in prevention and treatment of various oxidative stress related diseases such as and hepatic and neurodegenerative diseases (Gaire BP *et al*., 2011) [4].
Currently the worlds challenges the problems and potential of antimicrobial drugs against microorganism. On the other hand, plant-based antimicrobials agents due it is devoid of the many side effects and overcome the side effects associated with synthetic antimicrobials agents(Parekh *et al*., 2005) [14].
There is important hereditary diversity among distinct varieties of fig, which contain wonderful pharmacological activities and are of commercial significance. The medicinal plants of the genus *Ficus auriculata* consist of triterpenes, flavonoids, polyphenols, alkaloids, sterols, coumarins and other secondary metabolites that are responsible for various pharmacological activities (Shilpakar A *et al*.,2009) [18]. Our work is directed to investigate antimicrobial activity of *Ficus auriculata* fruit, leaf and bark extracted in three different solvents i.e., hexane, chloroform and methanol. Since there is no scientific study to substantiate the traditional claim on antimicrobial activity of the plant, the present study is being taken up (Murti. K *et al*., 2011) [12].

2. Materials and Methods
2.1. Collection of plant materials
We took three parts of plant fruits, leaves and bark of *Ficus auriculata* from two different altitudes of Uttarakhand. Places near to the Almora and Haldwani were selected for the collection of fruits, leaves and barks of *Ficus auriculata*. Washed off the sample to remove dust.After washed off cut the fruits, leaves and bark in to small pieces. They were kept in shade drying for the two weeks till then moisture content has been removed and then start the extraction process for the further progress.

2.2. Extraction
Extraction was done by successive soxhlet extraction technique.

2.2.1. Soxhlet extraction
This technique was carried out to obtain extracts for the phytochemical screening, biological and pharmacological activity. Fruit, leaves and Bark were meshed in grinder to get the powder of uniform size. Powder was packed in a thimble of filter paper. The thimble was then inserted into the Soxhlet apparatus and extraction was done by using hexane, chloroform and methanol as a solvent in a successive manner from non polar to polar solvent and extraction was continued for 9-10 hrs (Murugan. R *et al*., 2014) [13]. Then hexane extract was collected and powder from the thimble was used for the next successive extraction of choloroform after drying it again yield was calculated. The same procedure has been followed by drying and powder in thimble was dried used for methanol extraction. Finally methanol, hexane and chloroform soluble fractions were obtained. All extracts obtained from two regions of uttarakhand were then evaporate in water bath and dried in a vacuum oven at 40-45 ℃, and yield value for each extract was calculated. At last the extract were put on rotary evaporator or distillate to evaporate or collect solvent fo further use at 50^0 C to get the crude extract (Manandhar *et al*., 2019) [11].

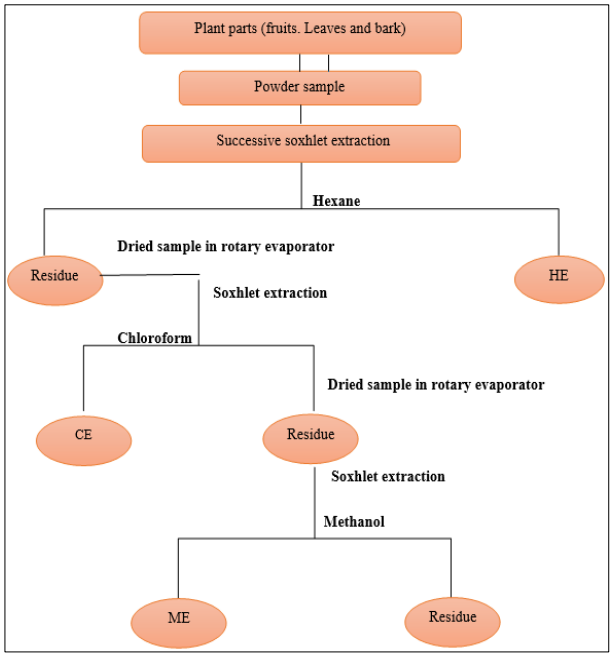

Fig 1: Flow chart of successive soxhlet extraction of fruits, leaves and bark of *Ficus auriculata* (HE-Hexane extract, CE-Chloroform extract, ME-methanol extract)

2.3. Determination of antibacterial activity FLB extracts of *F. auriculata*

2.3.1. Test organism collection: For antibacterial screening we collected human pathogenic bacterial strains i.e *Escherischia coli, Salmonella typhi, Bacillus subtilis* and *Staphylococcus aureus* For the antibacterial study. We isolate these bacterial strains from Department of Public Health and Epidemiology, College of Veterinery and Animal Sciences, GBPUA&T pantnagar (INDIA). FLB extract of hexane, chloroform and from two different regions i.e., Almora and Haldwani. These sample gives the antibacterial screening against the bacterial strain and shows the effect against these bacterias. Two of them are gram positive strain (*Bacillus subtilis, Staphylococcus aureus*) and two of them are gram negative strain (*Escherichia coli, Salmonella typhi*). Antibacterial screening activity was done by disc-diffusion method (Singh R *et al*., 2005) [19].

2.3.2. Preparation of bacterial inoculums: Bacterial inoculation prepared by the Luria bertani broth (HIMEDIA) for *Escherichia coli, Salmonella typhi, Bacillus subtilis* and *Staphylococcus aureus*. Nutrient agar was well dissolved in distilled water and then autoclaved for 30 minute about 120℃ and 15 to 20 lbs pressure for bacterial colonies inoculation.

2.3.3. Preparation of agar plates: We prepared nutrient agar medium for bacterial culture and then poured in to sterile plates. Which should be done under laminar flow in undisturbed manner that kept the medium contamination free. Agar media uniformly spread over the sterile plates. Now agar nutrient plates was put in the incubator at 37° C for overnight until solidification of agar nutrient in sterile plates.

2.3.4. Placements of plates: After uniformly spread of agar in sterile plates, sterilized paper disc of 5 mm was dipped in FLB extract of hexane, chloroform and methanol of different concentration (200-1000 µL). Now these plates again put in to the incubator for overnight at 37° C for growing bacterial cultures. Next day measure the inhibition zone of different concentration and compared the zone of inhibition with standard Gentamicin (10µg/disc), amiacin (10µg/disc) and ofloxacin. Diameter of inhibition zone measured by scale (mm).

3. Statistical analysis

Each experiment was performed in triplicates and the mean values with standard deviation for the inhibition zones. The triplicate results were applied to represent the antibacterial activity of the leaves, bark and fruits in three different solvents.

4. Results and Discussion

Fruits, leaves and bark extract of *F. auriculata* which is collected from two different regions Almora and Haldwani (Nainital) were extracted in three different solvents i.e, hexane, chloroform and methanol. Different concentration of these extracts were used to antimicrobial assays. The *in vitro* antimicrobial activity were evaluated against two gram positive (*B. subtilis* and *S. aureus*) and two gram negative (*E. coli* and *S.typhi*) human pathogenic bacteria. Hexane extract of AL and HF shows the maximum zone of inhibition in increasing dose dependent manner from 200-800 µg/mL (9.2±0 mm to 15.75±0.25 mm) and (8±0.40 mm to 15.5±0.25 mm) against *E. coli*, AF and HL shows the maximum zone of inhibition (7±0 mm to 13.2±0.25 mm) and (7.45±042 mm to 11.1±0.28 mm) against *B. subtilis*, AB and HF shows the maximum zone of inhibition (7.1±0.1 mm to 9.5±0 mm) and (5.5±0.5 mm to 7.1±0.28 mm) against *S. typhi*, AF and HF shows the maximum zone of inhibition (8.1±0.15 mm to 11±0.05 mm) and (7±0 mm to 11.4±0.38 mm) against *S. aureus* in (Table 1 for Haldwani species, Table 2 for Almora species and Figure 2). Chloroform extract of AL and HL shows the maximum zone of inhibition from 200-800 µg/mL (7±0 mm to 11.9±0.17 mm) and (7.16±0.28 mm to 10.7±0.25 mm) against *S. typhi*, AB and HF shows the maximum zone of inhibition (10.2±0.20mm to 14±0.11 mm) and (5±0 mm to 9.8±0.28 mm) against *E. coli*, AF and HL shows the maximum zone of inhibition (5±0 mm to 12.3±0.32 mm) and (7.10±0 0.28 mm to 10.8±0.104 mm) against *B. subtilis*, AB and HL shows the maximum inhibition zone (9±0.11 mm to 12.4±0.15 mm) and (7.36±0.32 mm to 12.1±0.23 mm) against *S. aureus* in (Table 3 for Haldwani species, Table 4 for Almora species and Figure 3). Methanol extract of AB and HB shows the maximum inhibition zone from 200-800 µg/mL (7.25±0 0.25 mm to 12.1±0.28 mm) and (8.20±0.34 mm to 12.9±0.057 mm) against *S. typhi*, AF and HL shows the maximum inhibition zone (6±0 mm to 8.2±0.2 mm) and (6±0.28 mm to 13±0.57 mm) against *E. coli*, AF and HL shows the maximum inhibition zone (7.13±0.15 mm to 11.16±0.15 mm) and 7.45±0.28 mm to 11.1±0.5 mm) against *B. subtilis*, AB and HF shows the maximum inhibition zone (7.1±0.1 mm to 12±0 mm) and (9.6±0.36 mm to 12.5±0.28 mm) against *S. aureus* in (Table 5 for Haldwani species, Table 6 for Almora species and Figure 4). Methanolic extract of stem bark of *F. auriculata* shown the maximum zone of inhibition against *E. coli* and hexane leaf extract shown the maximum inhibition zone against *S. aureus* (Gaire *et al*., 2011) [4]. Leaf extract of methanol and chloroform showed the maximum inhibition zone against *E. coli* and *S.typhi* (Kumari A *et al*., 2018) [7]. Ethanolic fruit extract showed the higher antibacterial potential against food poisoning bacteria (*Escherichia coli, Shigella flexneri* and *Staphylococcus epidermis*) and fruits showed the maximum potential against *Shigella flexneri* (Saklani *et al*., 2012) [16]. Alcoholic extract of leaves and fruits of *F. auriculata* showed the effective antibacterial potential against *S. aureus, B. aureus* and *B. subtilis.* Leaves shown the effective antibacterial potential than fruits, while fruits shown the effective antibacterial potential against *B. subtilis* (Fishawy *et al*., 2011) [3].

Table 1: Zone of inhibition (mm) of Haldwani leaves, bark and fruits hexane extract

Haldwani	Concen.(µg/ml)	*S. typhi*	*E. coli*	*B. subtilis*	*S. aureus*
HFHE	200	5.5±0.5	8±0.40	0±0.14	7±0
	400	5.85±0.57	10±0.28	7±0	9.16±0.28
	600	6.16±0.28	15±0.57	8.25±0.43	10±0
	800	7.1±0.28	15.5±0.25	9.16±0.28	11.4±0.38
HLHE	200	6±0.11	6.8±0.0	7.45±0.42	6±0.0
	400	7.2±0.25	7±0.11	8.23±0.40	7.16±0.28
	600	7.8±0.28	10.9±0.11	9.9±0.14	7.4±0.40

	800	8.6±0.23	13±0.0	11.1±0.28	10.2±0.34
HBHE	200	6±0.11	6.5±0.10	6±0.0	7±0.0
	400	7±0.11	7±0.11	6.5±0.11	9±0.0
	600	7.5±0.0	8±0.0	7±0.0	9.56±0.11
	800	8.5±0.05	9.4±0.11	8.1±0.17	10.1±0.28
Amikacin	(10 µg/ml)	23	20	20	22
Control		0	0	0	0

*[HB- Haldwani bark, HL- Haldwani leaves, HF-Haldwani fruits, AB-Almora bark, AL-Almora leaves, AF-Almora fruits] *Mean of three replications*Mean of three replications with ± standard deviation

Table 2: Zone of inhibition (mm) of Almora leaves, bark and fruits hexane extract

Almora	Concen.(µg/ml)	*S. typhi*	*E. coli*	*B. subtilis*	*S. aureus*
AFHE	200	5.2±0.25	0±0.0	8.23±0.25	8.1±0.15
	400	7.3±0.30	6.3±0.15	9.3±0.35	9.1±0.28
	600	8±0.05	7±0.0	10±0.0	10.1±0.0
	800	9.16±0.20	8±0.05	13.2±0.25	11±0.05
ABHE	200	7.1±0.10	5±0.0	7±0.05	7.2±0.3
	400	8±0.05	6.3±0.15	8.18±0.16	8±0.0
	600	9.1±0.23	7.1±0.20	9.3±0.20	9.1±0.10
	800	9.5±0.0	7.4±0.1	10±0.0	10.8±0.28
ALHE	200	11.1±0.0	9.2±0.0	8.2±0.34	6.16±0.15
	400	6±0.11	10±0.2	9.2±0.20	8.18±0.31
	600	6.2±0.25	12±0.0	11.1±0.23	8.9±0.11
	800	7±0.0	15.7±0.25	12.2±0.25	10±0.05
Amikacin	(10 µg/ml)	23	20	20	22
Control		0	0	0	0

*Mean of three replications with ± standard deviation

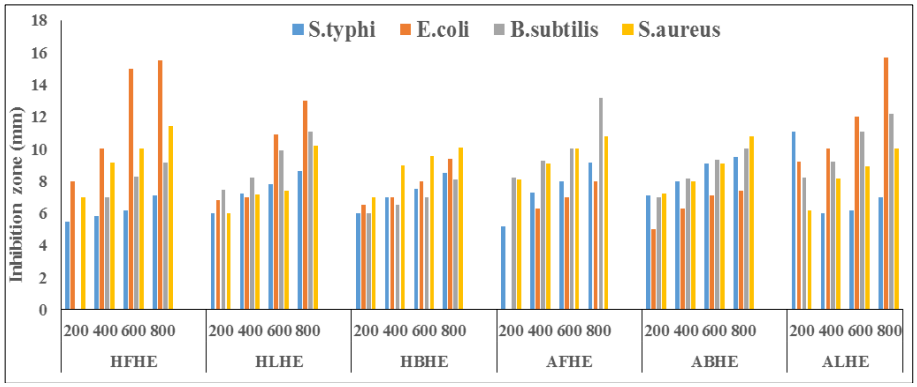

Fig 2: Zone of inhibition of Haldwani and Almora leaves, bark and fruits in hexane extract

Table 3: Zone of inhibition (mm) of Haldwani leaves, bark and fruits chloroform extract

Haldwani	Concen. (µg/ml)	*S.typhi*	*E.coli*	*B.subtilis*	*S.aureus*
HFCE	200	5.1±0.28	5±0.0	5±0.0	7±0.11
	400	5.8±0.28	6.16±0.28	7.6±0.57	9.8±0.28
	600	7.5±0.5	7.16±0	8.6±0.0	10.6±0.57
	800	9.5±0.5	9.8±0.28	10.16±0.28	11.58±0.14
HLCE	200	7.16±0.28	5.25±0.43	7.1±0.28	7.36±0.32
	400	8.25±0.43	6.25±0.25	8.25±0.25	10.26±0.25
	600	8.75±0.43	7.08±0.144	9.1±0.17	11.4±0.41
	800	10.78±0.25	8.25±0.43	10.8±0.104	12.1±0.23
HBCE	200	0±0.0	5±0.0	7±0.0	6.9±0.05
	400	0±0.0	5.3±0.28	8.1±0.15	7.5±0.05
	600	8.1±0.17	6.1±0.17	8.5±0.05	8.36±0.32
	800	9.2±0.2	7.16±0.15	9.1±0.17	9.2±0.20
Amikacin	(10 µg/ml)	23	20	20	22
Control		0	0	0	0

*Mean of three replications with ± standard deviation

Table 4: Zone of inhibition (mm) of Almora leaves, bark and fruits chloroform extract

Almora	Concen.(µg/ml)	*S.typhi*	*E.coli*	*B.subtilis*	*S.aureus*
AFCE	200	7.1±0.15	6±0	5±0.0	5±0.0
	400	8±0.11	7±0.11	9.16±0.15	8±0.20
	600	9±0.0	7.6±0.1	11±0.0	8.4±0.11
	800	10.2±0.2	8.16±0.15	12.3±0.32	9.1±0.17

ABCE	200	7±0.0	10.2±0.20	5±0.0	9±0.11
	400	7.4±0.11	11.1±0.23	7±0.15	10.1±0.15
	600	8±0.11	13.1±0.18	8±0.0	12±0.02
	800	11.9±0.17	14±0.11	9.08±0.18	12.4±0.15
ALCE	200	6±0.05	7.1±0.23	7.16±0.15	7±0.0
	400	6.9±0.15	8.26±0.25	9.16±0.20	8.1±0.15
	600	8.1±0.17	10.3±0.26	10.16±0.28	10.1±0.17
	800	9±0.0	11.16±0.28	11.1±0.18	11.3±0.28
Amikacin	(10 µg/ml)	23	20	20	22
Control		0	0	0	0

*Mean of three replications with ± standard deviation

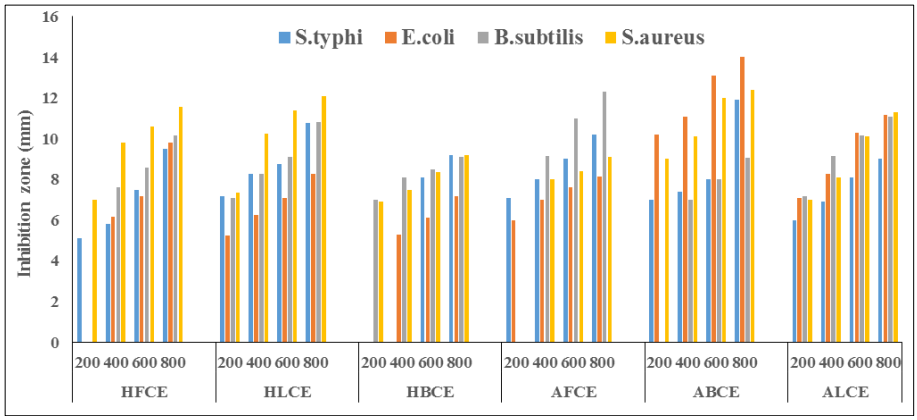

Fig 3: Zone of inhibition of Haldwani and Almora leaves, bark and fruits in chloroform extract

Table 5: Zone of inhibition (mm) of Haldwani leaves, bark and fruits methanol extract

Haldwani	**Concen.(µg/ml)**	***S.typhi***	***E.coli***	***B.subtilis***	***S.aureus***
HFME	200	±0.57	7.1±0.28	6.1±0.28	9.3±0.76
	400	7.1±0.28	7.5±0.5	7.3±0.57	10.8±0.28
	600	7.5±0.5	9.5±0.5	8.3±0.28	11.5±0.5
	800	8.5±0.5	10.3±0.57	9.3±0.57	12.5±0.28
HLME	200	6.0±0.57	6±0.28	7.45±0.28	6±0.76
	400	7.2±0.28	7.06±0.5	8.23±0.57	7.16±0.28
	600	7.8±0.5	10.9±0.5	9.9±0.28	7.4±0.5
	800	8.6±0.5	13±0.57	11.1±0.57	10.2±0.86
HBME	200	8.2±0.34	5.1±0.28	6.8±0.15	9.6±0.36
	400	9.06±0.11	6.1±0.10	7.3±0.28	10.1±0.36
	600	10±0.0	7 ±0.11	8±0.50	11±0
	800	12.9±0.05	8±0.00	9.1±0.28	12.1±0.23
Amikacin	(10 µg/ml)	23	20	20	22
Control		0	0	0	0

*Mean of three replications with ± standard deviation

Table 6: Zone of inhibition (mm) of Almora leaves, bark and fruits methanol extract

Almora	**Concen.(µg/ml)**	***S.typhi***	***E.coli***	***B.subtilis***	***S.aureus***
AFME	200	5.26±0.25	6±0	7.13±0.15	9±0.10
	400	6±0.0	6.3±0.2	8.1±0.17	9.8±0.28
	600	8±0.11	7±0.05	9±0.0	11±0.0
	800	10.1±0.17	8.2±0.2	11.2±0.15	11.4±0.11
ABME	200	7.2±0.25	6±0.0	8±0.0	7.1±0.1
	400	8±0.0	7±0.05	9.1±0.15	8±0.0
	600	9.26±0.25	7.3±0.15	9.2±0.30	9±0.10
	800	12.1±0.28	8.1±0.15	9.8±0.62	12±0.0
ALME	200	0±0.0	0±0.0	7±0.11	0±0.0
	400	5.1±0.28	0±.00	8.1±0.23	6.1±0.15
	600	6.2±0.25	6.3±0.26	9.1±0.1	7.2±0.2
	800	7.3±0.26	7.2±0.20	10±0.11	8.1±0.17
Amikacin	(10 µg/ml)	23	20	20	22
Control		0	0	0	0

*Mean of three replications with ± standard deviation

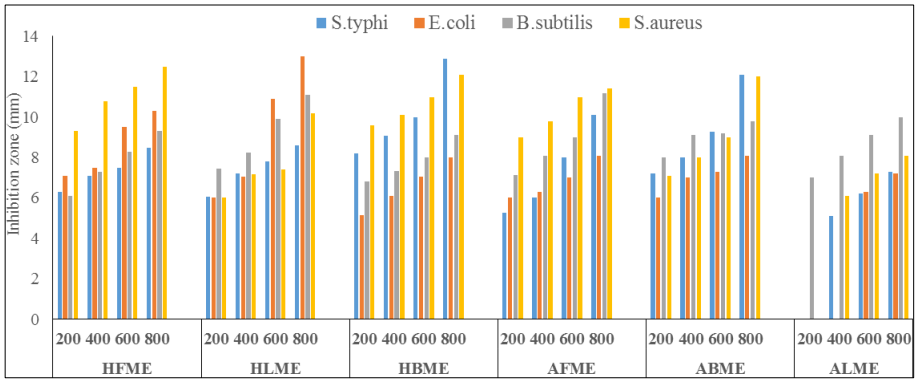

Fig 4: Zone of inhibition of Haldwani and Almora leaves, bark and fruits in methanol extract

5. Conclusion

In present study hexane extract and chloroform extract of Almora species shows the maximum zone of inhibition against human pathogenic bacteria while methanol extract of Haldwani (Nainital) species shows the maximum zone of inhibition against human pathogenic bacteria. A number of antibiotics are becoming less effective due to development of resistance and this has caused serious clinical problems in the treatment of infectious diseases. So, It also confirming the more possiblility of bioactive compounds that we will isolate for future aspects and are useful for rationalizing the use of this plant in primary health care.

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