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Research Paper

Antibacterial activity of certain folk medicinal plants from Kashmir Himalayas used to treat microbial infections

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ABSTRACT

The aim of our study was to evaluate the in vitro antibacterial activities of five plant extracts against two fish pathogenic bacteria, namely, *Staphylococcus aureus* (Gram positive bacteria) and *Aeromonas hydrophila* (Gram negative bacteria). Ethanolic extracts of the five different herbs namely *Fumaria indica*, *Adiantum capillus*, *Nepata cataria*, *Levandula stoeches* and *Borago officinalis* collected from registered herbal shops were subjected to test antimicrobial properties against *Staphylococcus aureus* and *Aeromonas hydrophila* by disc diffusion method (Inhibition Zone measurement) compared to Novobiocin and Tetracycline as standard antibiotics. The extracts of *F. indica, B. officinalis* and *L. stoeches* showed broad spectrum of inhibitory activity against *S. aureus*with zone of inhibition 7.26±0.25, 5.9±0.1 and 5.2±0.25 respectively at concentration of 100mg/ml. *A. capillus* showed moderate effect against *S. aureus* with zone of inhibition 3.9±0.05. Extracts of *B. officinalis, L. stoeches* and *F. indica* in case of *A. hydrophila* were also seen effective with zone of inhibition 3.2±0.26, 2.83±0.15 and 2.03±0.15 respectively at 100mg/ml. *A. capillus* and *N. cataria* show minimal to no inhibitory activity against *A. hydrophila.* Result revealed that the extracts were found to inhibit the growth of both bacterial strains with the maximum inhibitory effect seen against *S. aureus* and mild inhibitory effect was observed against *A. hydrophilla*. The results of our study suggested that *B. officinalis*, *L. stoeches,* and *F. indica* could be used as active antibacterial ingredients in contemporary drug formulation.

Key words: Antibacterial, drug formulation, herbs, pathogenic bacteria, plant extract

INTRODUCTION

Aquaculture sector is the major contributor towards fish supply as production from marine capture fisheries is almost stagnant over recent years (Jana *et al*., 2018). Due to the rapid advancement of the aquaculture system and rising demand for fish, culture practices are being intensified, which is over stressing the fish and increasing the risk of disease. Fish raised in conditions of high densities are particularly vulnerable to a variety of bacterial infections. Disease outbreaks are responsible for increased mortality rates and a decline in productivity efficiency, which result in significant financial losses for fish farmers. The main method used to treat bacterial fish illness is the use of antibiotics. Due to the emergence of multidrug-resistant bacterial strains and the buildup of residues in the environment and aquaculture products, the use of antibiotics in aquaculture needs to be drastically reduced.(Bulfon *et al*., 2014). Therefore, in recent years, emphasis has been placed on environmentally friendly and sustainable practices for managing aquaculture disease. An innovative and promising replacement for chemical medications for the management of aquaculture disease is the use of plant

products (Reverter *et al*., 2017). Many products from the plant origin have been reported to stimulate appetite, promote growth performance, act as immune stimulants, antibacterial, antiviral and anti-parasitic (protozoans, monogeneans) agent in aquaculture. These activities are observed due to the presence of bioactive compounds such as phenols, sulphur, terpenoids, alkaloids, flavonoids, and saponins (Chakraborty and Hancz, 2011). Different countries use plants as a source of many potent and strong drugs for medicinal purposes (Srivastava *et al.,* 1996).Various medicinal plant parts, such as roots, stems, flowers, fruits,twigs, exudates, and altered plant organs, are used to extract raw drugs. These components havea range of medicinal qualities. The North-Western Himalayan region is home to a wide variety ofmedicinal plants due to its geographic location at the meeting point of the holarctic and paleotropical floral realms (Dar *et al*., 2012). The present study was carried out to evaluate the antibacterial potential of ethanolic extracts of some plants in Kashmir, J&K, India, taking into account the vast potentiality of plants as sources for antimicrobial drugs.

Nepeta cataria: The aromatic perennial herb *Nepeta cataria*, also known as catnip or catmint, isa member of the *Lamiaceae* family and is well known for its therapeutic and medicinal properties**.** Additionally, *N. cataria* is well-known for its essential oil and secondary metabolites, both of which have shown to have enormous potential in the food, agrochemical, and pharmaceutical sectors**.** Like other aromatic plants that have demonstrated flavouring and medicinal properties and act as renewable source for the same, *N. cataria* has been known for these substances. (Ibrahim *et al*., 2022).It includes both annual and perennial herbs. Many cultures use *Nepeta* species as traditional medicines due to their extensive ethnobotanical effects,which include diuretic, diaphoretic, vulnerary, antitussive, antispasmodic, antiasthmatic, tonic, febrifuge, and carminative properties (Ghannadi *et al.,* 2003).

Borago officinalis: *Borago officinalis*, a member of *Boraginaceae* family, is native to some parts of Mediterranean region. The leaves and flowers of Borage have been used in salads and medicine. Borage meal extract's antioxidant and radical-scavenging abilities have been researched. The occurrence of this plant in India occurs from November to January and is sparsely distributed in the Northen-Eastern Himalayas from Kashmir to Kumaon at elevations of 3,500-4,500m above sea level (Gupta and singh, 2010).

Adiantum capillus: *Adiantum capillus-veneris* Linn. (Maidenhair fern) is a tufted fern belongingto Pteridaceae family. The herb is widely grown in warm-temperature to tropical, with high moisture content. It is a hardy, up to 35 cm tall plant with a creeping rhizome and an aromatic scent. The plant fronds typically have two rows, are tender, glabrous, and reach a maximum length of 50 cm. The plant's base is covered in hair and almost has a glossy, black petiole. The species' leaf blades range from ovate to oblong-ovate (Dehdari and Hajimehdipoor, 2018).It is found all over the world, including the Pak-Indian subcontinent, the western Himalaya, Mexico, the drier parts of America, and other tropical and subtropical areas. (Nisar *et al.*, 2012; Reddy, 2010).

Fumaria indica: The annual herb known as "Fumitory" is the member of the *Fumariaceae* family, *Fumaria indica* is a common weed found throughout the plains of Pakistan and India. In the Unani and Ayurvedic systems of medicine, plants are frequently used.Plant is used inisolation as well as in polyherbal formulations (Guna, 2017).Local names for the *Fumaria* genus include "Pitpapra" or "Shahtrah" in India and "Shahterah" in Kashmir. The extracts of Fumariaindica have been shown in the literature to have spasmolytic, analgesic, anti-inflammatory, and antibacterial properties.

Levandula stoeches: Research has been done on the antioxidant and radical-scavenging properties of borage meal extract. The highly potent essential oil produced by lavender, which has overtones of sweetness, is thought to be beneficial for a variety of conditions, including stress, anxiety, exhaustion, irritability, headaches, migraines, insomnia, depression, colds, indigestion, liver, gall bladder, and cancer (Henley *et al.* 2007). Reports of antibacterial effects have also been documented.

MATERIALS AND METHODS

Collection of plant samples: Five plants were collected based on their traditional usage as folk medicine. The leaves ofplants were purchased, in dried form, from the local market in Srinagar, Kashmir. (Table 1) shows the plant species and the parts evaluated in this study.

Fig 1: Five medicinal plants of Kashmir Himalayas

Extraction of plant materials: The leaves of fresh plant material were cleaned with distillated water before being dried at 40 degrees for 48 hours, ground into a fine powder, and stored in a dark, airtight container until use. The powdered samples were subjected to soxhlet extraction using distilled water and ethanol as solvent. Approximately 10g of plant powder was extracted with 100 ml of ethanol (Kais Kassim Ghaima *et al.,* 2013 and Durdi Qujeq *et al.,* 2013).

Preparation of plant extracts and isolation of Bacterial Strains 100 mg/ml concentration of the extracts was prepared. The test microorganisms were cultured into respective media by spread plate method with the 24 hour cultures of bacterial growth in Muller Hilton Agar. The filter paper discs (5 mm in diameter) were placed on test organism- cultured plates after being solidified and impregnated with extracts at concentrations of 100 mg/ml.

Gram positive bacteria S. Aureus and gram negative bacteria A. hydrophila and used for antibacterial test (R Seenivasan., 2012). The antibacterial assay plates were incubated at 37°C for 24h and the diameters of the inhibition zones was measured in mm. Novobiocin and Tetracycline were used as positive control.

Statistical analysis: The data represent mean of three replicates _ standard deviation (SD). Differences between means were considered significant at p-value < 0.05. Percentage yield of extract and Zone of inhibition were determined.

Table 1: Botanical names and the plant parts used for the antibacterial screening (Fig 1)

S. No.	Name of the herb	Local name	Part used
1	*Nepata cataria*	*Gandhsoi*	Leaves
2	*Levandula stoeches*	*Kalwuth*	Leaves
3	*Fumaria indica*	*Shahter*	Leaves
4	*Adianthum capillus*	*Gavtheer*	Leaves
5	*Borago officinalis*	*Kahzaban*	Leaves

RESULTS AND DISCUSSION

Extraction Yields of the Plant Extracts: By using soxhlet extraction method the highest yield was obtained from *Borago officinalis* extract of 15% while least yield was obtained from *Nepata cataria* (Table 2).

Table 2: Yield percentage of ethanolic extract of plants used in the study

Plants	Solvent used	Extract yield (%)
Nepata cataria	Ethanol	2%
Levandula stoeches	Ethanol	7%
Fumaria indica	Ethanol	12%
Adianthum capillus	Ethanol	4%
Borago officinalis	Ethanol	15%

Antibacterial Activities of Plant Extracts: Evaluation of antibacterial activities of five different extracts from *Fumaria indica, Adiantum capillus*, *Nepata cataria, Levandula stoeches* and *Borago officinalis* was determined initially by the disc diffusion method against Gram- positive bacteria i.e. *S. aureus* and Gram-negitive bacteria i.e. *A. hydrophila*. The study showed that all plant extracts used in the study exhibited a varying degree of antimicrobial activity against tested microorganisms (Fig 2). Zones of growth inhibition were measured in mm. Novobiocin and Tetracycline were used as positive controls (Fig 3). The inhibitory effect of these extracts was tested at 100 mg/ml. The antibacterial activity of these extracts at a100mg/ml. Results indicate that the susceptibility of all bacterial strains show significant differences ($p<0.01$).The highest potential was observed in extract of *F.indica*, *B.officinalis* and *L.stoeches* against *S. aureus* with zone of inhibition 7.26 ± 0.25, 5.9 ± 0.1 and 5.2± 0.25, respectively, at 100 mg/ml. *A.capillus* showed moderate effect against *S. aureus* with zone of inhibition 3.9±0.05. While in case of *A. hydrophila* extracts of *B.officinalis, L.stoeches* and *F.indica* were seen effective with zone of inhibition 3.2±0.26, 2.83±0.15 and 2.03±0.15, respectively, at 100 mg/ml. *A.capillus* and *N.cataria* show minimal to no inhibitory activity against *A. hydrophila* (Table 3).

The results of antimicrobial disc susceptibility testing of *S. aureus* and *A. hydrophila* are presented in (Table 4).

Antibiotic resistance is a growing concern worldwide, highlighting the need for alternative sources of antimicrobial substances. The current antibacterial therapy is significantly threatened by the emergence and spread of multidrug resistant pathogens. Due to the fact that plants produce a wide range of bioactive compounds with known therapeutic properties, the search for new sources of antimicrobial compounds has become necessary. The therapeutic properties of the bioactive compounds found in medicinal plants have been acknowledged. The development of new and useful chemotherapeutic agents requires the raw materials in the form of potentially useful compounds provided by plants. Therefore, in recent years, emphasis has been placed on environmentally friendly and sustainable practices for managing aquaculture disease. The

use of plant products is a novel and promising alternative to chemical medications for the management of aquaculture disease (Reverter *et al*., 2017).

Table 3: Zone of inhibition of plant extracts against microorganisms at 100mg/ml concentration

Plant specie	Family	Plant partused	Zone of Inhibition(mm)		P- Value
			A. hydrophila	*S. aureus*	
Fumaria indica	Fumariaceae	Leaves	2.03±0.15	7.26±0.25	<0.01
Borago officinalis	Boraginaceae	Leaves	3.2±0.2	65.9±0.1	<0.01
Levandula stoeches	Lamiaceae	Leaves	2.83±0.15	5.2±0.25	<0.01
Nepata cataria	Lamiaceae	Leaves	-	1.2±0	-
Adianthum capillus	Pteridaceae.	Leaves	0.76± 0.25	3.9±0.05	<0.01
	P-Value		<0.01	<0.01	

In our research we have studied the in vitro antibacterial activity of commercial ethanolic extracts derived from 5 medicinal plants against the fish pathogens *Staphylococcus aureus* (Gram positive bacteria) and *Aeromonas hydrophila* (Gram negative bacteria) with the aim to investigate their possible use in aquaculture in the control of bacterial infections. We found significant differences in the antimicrobial potential of the investigated plant extracts, which were dependent on herbal species and on bacterial strain.Gram-negative bacteria commonly are less susceptible to the inhibitory effects of plant extracts/essential oils compared with Gram positive bacteria, because they possess an outer membrane surrounding the cell wall, which restricts the diffusion of hydrophobic compounds through its lipopolysaccharide covering (Burt 2004; Turker *et al.* 2009b). In our experiments, *Staphylococcus aureus* (Gram positive bacteria) was more susceptible to the investigated plant extracts compared with *Aeromonas hydrophila* (Gram negative bacteria). The plant extract of *Fumaria indica*, *Borago officinalis*and *Levandula stoeches* showed maximum inhbitory activity against *Staphylococcus aureus* and*Aeromonas hydrophila* with zoneof inhibition 7.26±0.25, 5.9±0.1 5.2±0.25 and 3.2±0.26, 2.83±0.15, 2.03±0.15 respectively at 100 mg/ml. Similar results were obtained by Reshi *et al*. (2017) where the extracts showed significant concentration dependent antibacterial activity against *Staphyloccus aureus.* Sanches *et al*. (2005) found that aqueous extract of *guava* was effective against *S. aureus.* In a study by Shinwari *et al.* (2013) on the antibacterial properties of 11 medicinal plant species, the antibacterial activity of F. indica extracts was also investigated. Methanolic extracts of *F. indica* showed antibacterial activity of 11±0.000 against *S .aureus* at a concentration of 50 mg/ml.

Table 4: Antimicrobial activity of five extracts against bacterial strains at 100mg/l

Bacterial strains	*F. indica*	*B. Officinalis*	*L. stoeches*	*N. cataria*	*A. capillus*
A. hydrophila	+	+	+	–	+
S. aureus	+	+	+	+	+

Disc diffusion Method: (+) susceptibility, (-) absence of susceptibility

The antibacterial potential of hexane, ethanol and chloroform extracts of *F. indica* was studied by Fazal *et al*. (2012). Activities in all three solvent extracts were found against a range of bacteria including *S. aureus* as well as *E. coli*. Ethanolic and acetone extracts of *F. indica* were also found effective against *S. aureus* with ZI= 13.66±6.91 and ZI=8.66±1.36 respectively (Shinwari *et al*., 2013). Also Miceli *et al*. (2014) conducted a study regarding the antibacterial activity of *Borago officinalis* and *Brassica juncea* species. Aqueous extracts of *B.officinalis* showed antibacterial activity with zone of inhibition 15-18 mm against *S .aureus*.Additionally, (Abed, 2015) in the studyof chemical composition of *borago officinalis* extraction and antibacterial activity against gastrointestinal infectionconcluded that the aqueous extract of Borage leaves in hot temperature have the highest inhibitory effect against the two species of bacteria (*Escherichia coli* and *Staphylococcus aureus*) 13 mm and 22 mm a diameter, respectively. Furthermore, Abdelhamed *et al.* (2019) reported that trans-cinnamaldehyde (TC) had better antibacterial activity against *A. hydrophila*. Kot *et al*. (2019) conducted a study regarding the antibacterial activity. Commercial Phytochemicals against *Aeromonas* species isolated from fish. They came to the conclusion that Trans-cinnamaldehyde (TC) had the best antibacterial activity and the lowest MIC values, which were between 0.01 mg/mL and 0.19 mg/mL for the majority of *A. hydrophila* strains. This study also agrees with Biradar *et al.* report on the effectiveness of plant extracts from *C. limon* against *A. hydrophila* of gold fish by both *in vivo* and *in vitro* studies; the results showed that *C. limon* was effective with 16 mm zones similar to oxytetracyclin (Biradar*et al*.,2007) The present findings are also in line with the results of Muniruzzaman *et al*.,2004 who studied the sensitivity of plant extracts against three important fish (*H. longifilis*) bacterial pathogens (*A. hydrophila, P. fluorescens* and *E. tarda*). Among the eight species of high

inhibitory responded plants *A. sativum* showed the highest antibacterial effect against *A. hydrophila* and *P. fluorescens.* Scientific study (Musa *et al*., 2008) have found that garlic extracts were effective against the two pathogenic gram positive bacteria(*Staphylococcus aureus* and *Streptococcus agalactiae*).Some authors (Castro *et al*., 2008) also reported that methanolic extracts of 31 Brazilian plants showed antibacterial activity (agar diffusion method) against the fish pathogenic bacteria *A. hydrophila*, *Streptococcus agalactiae* and *Flavobacterium columnare.*

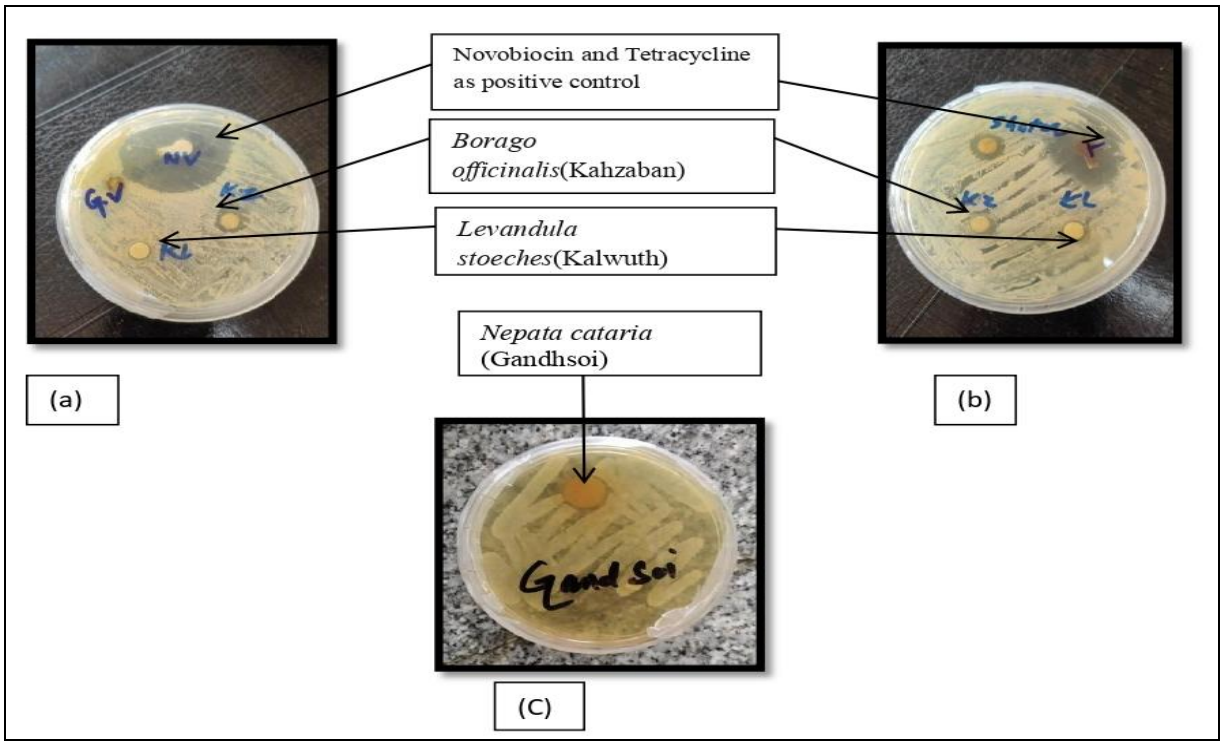

Fig 2. Antimicrobial activity of ethanolic extract of some plants against *A.hydrphila* (a) *and S. aureus*(b), (c)at 100 mg/ml

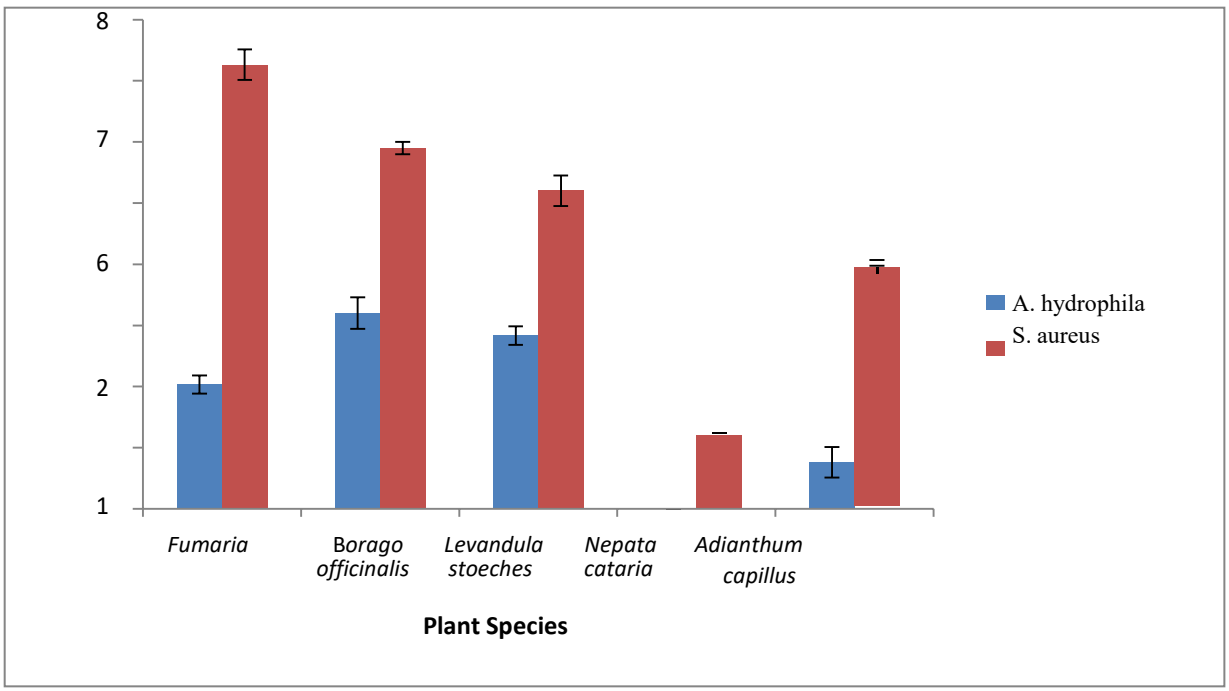

Fig.3: (Mean±SD) Zone of inhibition shown by *F. indica*, *B. officinalis*, *L.stoeches*, *N. cataria*, *A. capilus* against *A. hydrophila* and *S. aureus*.

CONCLUSION

The result of the present study supports the traditional use of these plants practiced by local healers. All the plants investigated in this study exhibited antibacterial activities against the tested bacterial strains. The highest antibacterial response was found in *Fumaria indica* followed by *Borago officinalis* and *Levandula .stoeches. Fumaria indica* and *Borago officinalis* can beused as commercial herbal preparations as an alternative treatment for bacterial infections in fishes. However these findings indicate that further research is necessary to evaluate the antibacterial, antiviral and antiparasitic activity of these plant extracts.

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