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Antibacterial Activity of Some Medicinal Plants of Kashmir, J&K, India

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The study was conducted to find out the activity of some medicinal plants against bacterial isolates. The bacterial isolates from milk samples of infected quarters of cows were investigated for *in-vitro* drug sensitivity by standard disc diffusion technique (Bauer *et al.,* 1966). Cultural examination of milk samples was done by method described by Quin *et al.* (2004) in which predominant isolates were *Staph.* (55.55%), *E. coli* (22.22%), *Strepto. agalactiae* (16.66%) and other isolates as 5.5% and were subjected to *in-vitro* antibacterial sensitivity test to selected herbal extracts and standard antibiotic (cefuroxime) (Table-1). The four different aqueous concentrations of the herbs namely *Fumaria indica, Adiantum capillus*, *Nepata cataria, Levandula stoeches and Borago officinalis* collected from registered herbal shops prepared by standard procedure as 25mg/ml, 50mg/ml, 75mg/ml and 100mg/ml were used. The results indicated that aqueous extract of *Fumaria indica, Adiantum capillus and Napeta cataria* against *Staphylococcus aureus, E.coli, Streptococcus agalactia* and *K. pneumonia* exhibited maximum zone of inhibition 20.0±1.21, 21.0±0.19, 13.0±0.37 17.0±0.21; 18±0.41, 12±0.21, 13±0.31, 15±0.31 and 15±0.33, 16.01±0.19 14.09±0.37, 13.31±0.41 at 100 mg/ml respectively which was significantly low as compared to standard drug (cefuroxime) at 30 microgram concentration. Aqueous extract of *Levandula stoeches* against *Staphylococcus aureus* and *E.coli* exhibited maximum zone of inhibition 18.0±0.33 and 13.0±0.141 at 100 mg/ml respectively. *Borago officinalis* has shown nil to non-significant bacterial growth inhibition activity.

Keywords: Antibacterial, herbs, Aqueous extract, Zone of inhibition.

Plants being a source of many potent and powerful drugs are used medicinally in different countries (Srivastava *et al.*, 1996). A wide range of medicinal plant parts like roots, stems, flowers, fruits, twigs, exudates and modified plant organs are used for extract of raw drugs and they possess varied medicinal properties. Considering the vast potentiality of plants as source for antimicrobial drugs the present study was undertaken to screen

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the antibacterial potential of aqueous extracts of some plants of Kashmir, J&K, India.

1) *Nepeta cataria*: *Nepeta*, a genus of annual or perennial herbs, belonging to the Lamiaceae family, includes approximately 250 species and are localized in central and southern Europe, Asia, the Middle East, northern Africa and tropical mountains in Africa (Ghannadi *et al*, 2003). *Nepeta* species are used as the traditional medicine in many countries and have a large ethno-botanical effects like diuretic, diaphoretic, vulnerary, antitussive, antispasmodic, antiasthmatic, tonic, febrifuge and carminative (Ghannadi *et al.*, 2003). The present study was aimed to evaluate the antimicrobial potential of the *Nepeta* species found in Kashmir.

2) *Fumaria indica/officinalis:* The genus *Fumaria* (Fumariaceae) consists of 46 species in the world and are known as "fumitory, earth smoke, beggary, fumus, vapor, fumittery or wax dolls" in English. *Fumaria officinalis* is a small, branched, annual herb growing wild in plains and lower hills and is locally known as "Pitpapra" or "Shahtrah" in India "Shahterah" in Kashmir. Literature reveals that the extracts of *Fumaria indica* posses spasmolytic, analgesic, anti-inflamatory, antibacterial properties.

3) *Borago officinalis:* This plant in India is sparsly distributed in Northen-Eastern Himalayas from Kashmir to Kumaon at altitudes of 3,500-4,500m ASL and occurs during November to January.

4) *Adiantum capillus:* It has a worldwide distribution, found in Pak-Indian subcontinent, western Himalaya, Mexico, warmer parts of America and other tropical and subtropical regions of the world (Nisar *et al.*, 2012; Reddy, 2010). *Adiantum* is used as expectorant, astringent, demulcent, antitussive, emmenagogue, febrifuge, diuretic and in catarrhal affections.

5) *Levandula stoeches:* The lavenders are a genus of about 25-30 species of flowering plants in the mint family, Lamiaceae, native of Mediterranean region south to tropical Africa and many regions of Asia and has been used for centuries as a herbal remedy for many ailments. Lavender yields highly effective essential oil with very sweet overtones which is believed to be of benefit for a multitude of problems including stress, anxiety, exhaustion, irritability, head ache, migraine, insomnia, depression, cold, indigestion, liver, gall bladder problems and cancer (Hudson 1996; Henley *et al.* 2007). Reports of antibacterial effects have also been documented.

MATERIALS AND METHODS

Following herbs (aqueous extract) were evaluated for their *in vitro* toxicity trial on cell line (HeLA cell lines):

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

Plant material

The selected herbs (leaves) were purchased from registered herbal shops from local market Srinagar, J&K.

Extraction of plant material

Plant material was washed with distilled water, dried in shade, grinded to fine powder and stored in airtight container at room temperature in the dark until used. The powdered samples were subjected to extraction using distilled water following the method of Nair *et al.* (2005)

Preparation of extracts

Different concentrations of the extracts of herbals were prepared. The test microorganisms were seeded into respective media by spread plate method with the 24 h cultures of bacterial growth in nutrient broth. After solidification the filter paper discs (5 mm in diameter) impregnated with the

extracts of different concentrations were placed on test organism-seeded plates. *S. , E. coli* and other isolates were used for antibacterial test. The antibacterial assay plates were incubated at 37°C for 24h and the diameters of the inhibition zones was measured in mm, Cefuroxime was used as positive control.

***In vitro* antibacterial sensitivity test of herbs in comparison with standard antibiotic**

The bacterial isolates from milk samples of infected quarter were further investigated for *in vitro* drug sensitivity by standard disc diffusion technique (Bauer *et al.,* 1966) using commercially available selective antibiotic discs (M/S Hi-Media Laboratories).

Antibiogram

The different concentrations of herbs were studied for *in vitro* AST in comparison to standard antibiotic. The diameter of the zone of inhibition was measured by paper scale after 18 hr incubation for identifying the sensitivity of microbial culture against above mentioned extracts according to laboratory manual.

Cultural examination of milk

Cultural examination of milk samples was done by method described by Quin *et al.* (2004). Milk samples collected in sterile glass vials were streaked primarily on ovine blood agar plates with a sterile platinum loop under strict sterile environment. The inoculated plates were incubated at 37°C for 24 hours. The causative organisms were identified initially by colony characteristics on blood agar, Gram staining and biochemical characteristics for presence of catalase and cytochrome C oxidase. Further, the organisms grown on blood agar plates were streaked on selective media e.g. Mannitol Salt Agar (for *Staphylococcus* spp.), Edward's media (for Streptococci), MacConkey Agar (for Coliforms and Enterococci). Hotis test was done to identify *Streptococcus agalactiae*.

RESULT AND DISCUSSION

Bacterial isolation

Bacterial Isolation

The predominant isolates were *Staph.* (55.55%), *E. coli* (22.22%), *Strepto. agalactiae* (16.66%) and other isolates as 5.5%. Thus *Staph. , E. coli, K. pneumonia* and *Strepto. agalactiae* were selected for *in vitro* antibacterial sensitivity test to selected herbal extracts. Among all the pathogens of bovine mastitis, *Staphylococcus* is the predominant organism (Allore, 1993; Kapur *et al.,* 1992). In India, previous and several studies about bovine mastitis (1994; Costa *et al.,* 1997; Naiknaware *et al.,* 1998) have assessed that the coagulase negative *Staphylococci* were the most frequent isolated bacteria

***In vitro* antibacterial sensitivity test of herbs in comparison to standard antibiotic**

Four different concentrations of herbs were studied for *in vitro* AST in comparison with standard antibiotic.

Fumaria indica

Aqueous extract of *Fumaria indica* against *Staphylococcus aureus* and *E. coli* exhibited maximum zone of inhibition 20.0±1.21 and 21.0±0.19 at 100 mg/ml and minimum zone of inhibition 9.0±0.51 and 11.0±0.22 was recorded at 25 mg/ml respectively which significantly increased at 50 and 75 mg/ml where zone of inhibition was 11.0±0.33 and 17.0±0.19, 13.0±0.41 and 19.0±0.16 respectively. Against *Streptococcus agalactia* exhibited maximum zone of inhibition 13.0±0.37 was recorded at 100 mg/ml and minimum zone of inhibition (1.0±0.12) was recorded at 25 mg/ml which significantly increased at 50 and 75 mg/ml where zone of inhibition were 6.00±0.41 and 7.0±0.04, respectively while as, against *K. pneumonia*, maximum zone of inhibition 17.0±0.21 was reported at 100 mg/ml and minimum zone of inhibition (4.0±0.90) was recorded at 25 mg/ml which significantly increased at 50 and 75 mg/ml where zone of inhibition were 9.0±0.19 and 12.0±0.57, respectively. Aqueous extract of *Fumaria indica* exhibited high antibacterial activity against *Staphylococcus aureus, E. coli, K. pneumonia* and and the findings match with Khan *et al.* (2014), Gupta *et al.* (2012), Fatima *et al.* (2014). Parekh and Chanda (2007) and Fazal *et al.* (2012) and the findings are in contrast to Shinwari *et al.* (2015). The antimicrobial activity of aqueous extract of *Fumaria indica* against the different clinical strains of bacteria supported the scientific validity of the plant being used traditionally as a medicine. The inhibition of bacterial strains by aqueous extract may be attributed to the presence of soluble phenolic and polyphenolic compounds in the extract. The significant antimicrobial effects of this

extract could be explained by disturbance of permeability barrier of bacterial membrane structures (Cowan *et al.*, 1999 and Cowan *et al.*, 2008). Recent findings revealed that tea tree oil damages the cell membrane structure of *E. coli*, *S.* and *C. albicans.*

Adiantum capillus

Aqueous extract of *Adiantum capillus* against *Staphylococcus aureus* exhibited maximum zone of inhibition 18±0.41 at 100 mg/ml and minimum zone of inhibition (6±0.03) was recorded at 25 mg/ml which significantly increased at 50 and 75 mg/ml where zone of inhibition was 9±0.31 and 14.0±0.14, respectively. Furthermore, against *E. coli* maximum zone of inhibition 12±0.21 was shown 100 mg/ml and minimum zone of inhibition (4±0.39) was recorded at 25 mg/ml which significantly increased at 50 and 75 mg/ml where zone of inhibition was 6±0.07 and 8±0.09 respectively. Against *streptococcus agalactia* exhibited maximum zone of inhibition 13±0.31 at 100 mg/ml and minimum zone of inhibition (5±0.32) was recorded at 25 mg/ml which significantly increased at 50 and 75 mg/ml where zone of inhibition was 7.0±0.43 and 11±0.34 respectively, while as, against *K. pneumoniae* maximum zone of inhibition 15±0.31was recorded at 100 mg/ml while as minimum zone of inhibition (5±0.12) was recorded at 25 mg/ml which significantly increased at 50 and 75 mg/ml where zone of inhibition was 7.0±0.21 and 9.0±0.14 respectively. Aqueous extract of *Adiantum capillus* exhibited high antibacterial activity against *Staphylococcus aureus*, *E. coli* followed by *K. pneumonia* and. and the findings corraborates with Hussain *et al.* (2014), Meenakshi *et al.* (2008), Ishaq *et al.* (2014) and Ansari and Ekhlasi-Kazaj (2012).

Nepata cataria

Aqueous extract of *Napeta cataria* against *Staphylococcus aureus* exhibited maximum zone of inhibition 15±0.33 at 100 mg/ml and minimum zone of inhibition (8.03±0.02) was recorded at 25 mg/ml which significantly increased at 50 and 75 mg/ml where zone of inhibition was 12.03±0.03 and 14±0.01 respectively, while as against *E. coli* exhibited maximum zone of inhibition 16.01±0.19 at 100 mg/ml and -(nil) zone of inhibition was recorded at 25 mg/ml which significantly increased at 50 and

Table 1. *In vitro* antibacterial AST of aqueous herbal leaf extracts

Test extract	Test organism	Test concentration (zone of inhibition) (mg/ml) 25	50	75	100	Standard drug (30 mg)
Fumaria indica	A	9.0±0.51^{a}	11.0±0.33^{a}	17.0±0.19^{b}	20.0±1.21^{c}	23.11±0.42
	B	11.0±0.22^{a}	13.0±0.41^{a}	19.0±0.16^{b}	21.0±0.19^{b}	28.00±0.59
	C	5±0.32^{a}	6.00±0.41^{b}	12.0±0.04^{c}	13.0±0.37^{c}	19.00±0.21
	D	4.0±0.90^{a}	9.0±0.19^{b}	12.0±0.57^{b}	17.0±0.49^{c}	18.00±0.21
Adiantum capillus	A	6±0.03^{a}	9±0.31^{a}	14.0±0.14^{b}	18±0.41^{c}	23.11±0.42
	B	4±0.39^{a}	6±0.07^{a}	8±0.09^{b}	12±0.21^{b}	28.00±0.59
	C	5±0.32^{a}	7±0.43^{a}	11±0.34^{b}	13±0.31^{b}	19.00±0.21
	D	5±0.12^{a}	7±0.21^{a}	9±0.14^{b}	15±0.31^{c}	19.00±0.29
Nepata cataria	A	8.03±0.02^{a}	12.03±0.03^{a}	14±0.01^{b}	15±0.33^{b}	23.11±0.42
	B	-	5.02±0.07^{a}	8.09±0.02^{b}	16.01±0.19^{b}	28.00±0.59
	C	4.90±0.02^{a}	7.03±0.04^{b}	10.01±0.04^{c}	16.09±0.37^{d}	19.00±0.21
	D	3.01±0.03^{a}	5.03±0.12^{a}	9.03±0.1^{b}	13.31±0.41^{c}	19.00±0.29
Levandula stoeches	A	10.0±0.71^{a}	12.0±0.31^{a}	14±0.31^{b}	18.0±0.33^{c}	22.0±0.12
	B	6.0±0.14^{a}	8.0±0.41^{a}	8.0±0.414^{a}	13.0±0.14^{b}	17.0±0.43
	C	8.0±0.32^{a}	8.0±0.31^{a}	10.0±0.33^{a}	14.0±0.19^{b}	13.0±0.77
	D	4.0±0.12^{a}	8.0±0.41^{a}	9.0±0.00^{a}	11.0±0.18^{a}	20.0±0.37
Borago officinalis	A	-	-	3.0±0.14^{a}	5.0±0.31^{a}	25.0±0.18
	B	-	4.0±0.31^{a}	6.0±0.11^{a}	9.0±0.29^{b}	16.9±0.33
	C	-	-	-	1.0±0.13^{a}	12.5±0.67
	D	-	-	-	-	22.0±0.47

Values with different superscript in rows differ significantly (P< 0.05).
A= *Staph.* , B= *E. coli*, C= *streptococci agalactia*, *D= K.pneumoniae*

75 mg/ml where zone of inhibition was 5.02±0.07 and 8.09±0.02 respectively. Against *streptococcus agalactia* exhibited maximum zone of inhibition 14.09±0.37 at 100 mg/ml and minimum zone of inhibition (4.90±0.02) was recorded at 25 mg/ml which significantly increased at 50 and 75 mg/ml where zone of inhibition was 7.03±0.04 and 10.01±0.04 respectively. Against *K. pneumoniae* maximum zone of inhibition 13.31±0.41 was recordedat 100 mg/ml and 3.01±0.03 zone of inhibition was recorded at 25 mg/ml which increased at 50 and 75 mg/ml where zone of inhibition was 5.03±0.12 and 9.03±0.1 respectively. Aqueous extract of *Nepata cataria* exhibited high antibacterial activity against *Staphylococcus aureus*, *E. coli* and the findings are in corroboration with the findings of Bandh *et al.* (2011). The antimicrobial activity of AENC may be related to the monoterpenoid component *i.e.* nepetalactone present in the extracts as confirmed by a study.

Levandula stoeches

Aqueous extract of *Levandula stoeches* against *Staphylococcus aureus* exhibited maximum zone of inhibition 18.0±0.33 at 100 mg/ml and minimum zone of inhibition (10.0±0.71) was recorded at 25 mg/ml which significantly increased at 50 and 75 mg/ml where zone of inhibition was 12.0±0.314 and 14±0.31 respectively, While as, against *E. coli* maximum zone of inhibition was recorded at 13.0±0.141 at 100 mg/ml and minimum zone of inhibition (6.0±0.14) was recorded at 25 mg/ml which significantly increased at 50 and 75 mg/ml where zone of inhibition was 8.0±0.414 and 8.0±0.414 respectively. Against *streptococcus agalactia* maximum zone of inhibition 14.0±0.191 was shown at 100 mg/ml and minimum zone of inhibition (8.0±0.32) was recorded at 25 mg/ml which increased at 50 and 75 mg/ml where zone of inhibition was 8.0±0.313 and 10.0±0.333 respectively. Against *K. pneumoniae* maximum zone of inhibition 11.0±0.181 at was recorded 100 mg/ml and 4.0±0.12 zone of inhibition was recorded at 25 mg/ml which significantly increased at 50 and 75 mg/ml where zone of inhibition was 8.0±0.419 and 9.0±0.00 respectively.

Borago officinalis

Based on the *in vitro* antibacterial sensitivity test of herbal aqueous extracts, three extracts, *Fumaria indica*, *Adiantum capillus*, *Nepata cataria* significantly inhibited bacterial growth under study and maximum inhibition was recorded at concentration 100 mg/ml, while the extracts, *Levandula stoeches* significantly inhibited bacterial growth under study however, due to increased cytotoxicity, it was ignored for further study. Moreover *Borago officinalis* has shown nil to non-significant bacterial growth inhibition activity in addition with cytotoxicity effect. Aqueous extract of *Borago officinalis* against *Staphylococcus aureus* exhibited maximum zone of inhibition 5.0±0.31 at 100 mg/ml and nil/no zone of inhibition was recorded at 25 and 50 mg/ml which significantly increased to 3.0±0.14 at 75 mg/ml while as, against *E. coli* maximum zone of inhibition 9.0±0.29 was exhibited at 100 mg/ml and nil/no zone of inhibition was recorded at 25 mg/ml which significanttly increased at 50 and 75 mg/ml where zone of inhibition was 4.0±0.31 and 6.0±0.11 respectively. Against *streptococcus agalactia* maximum zone of inhibition 1.0±0.13 at 100 mg/ml and nil/no zone of inhibition was recorded at 25, 50 and 75 mg/ml while as, against *K. pneumoniae* exhibited no/nil zone of inhibition at 25, 50, 75 and 100 mg/ml.

CONCLUSION:

The highest AST response was found in *Fumaria indica* followed by *Adiantum capillus* and *Nepata cataria*. *Fumaria indica and Adiantum capillus* can be used as a commercial herbal preperations as an alternative treatment for bacterial infections.

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