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IN VITRO ANTIBACTERIAL ACTIVITY OF *PIPER LONGUM* L. AND *FICUS RELIGIOSA* AGAINST *ESCHERICHIA COLI*

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ABSTRACT

In this study an attempt was made to evaluate the antimicrobial activity of water and ethyl alcohal solvent extracts of leaves of *Piper longum* L and *F. religiosa* against *E. coli* isolated from tap water by using disk diffusion method. Aqueous and ethyl alcohol extract of *F. religiosa* showed antibacterial activity. It was observed that aqueous extract of *Ficus religiosa* was highly active against *E. coli* which showed 27mm diameter of zone of inhibition whereas aqueous extract of *Piper longum* was also active against *E. coli* with 17mm zone of inhibition while no activity was shown by ethyl alcohol extract. Test organism was resistant against most of the antibiotics whereas co-trimoxazole was highly active against *E. coli* with 27mm zone of inhibition.

INTRODUCTION

The medicinal plant products and their active constituents played an important role to treat various diseases. Natural products, such as those found in plants, or their semi-synthetic derivatives, may provide novel compounds that can be developed into antimicrobial drugs (Bisht et al., 2006; McRae et al., 2007). Today, there is a renewed interest in traditional medicine and an increasing demand for more drugs from plant sources. This revival of interest in plant derived drugs is mainly due to the current widespread belief that "green medicine" is safe and more dependable than the costly synthetic drugs, many of which have adverse side effects (Nair and Chanda, 2007). In order to promote the use of medicinal plants as potential sources of antimicrobial compounds, it is pertinent to thoroughly investigate their composition and activity and thus validate their use (Nair and Chanda, 2006). Plants have been the source of medicines for thousands of years; species of the genus *Piper* and *Ficus* are among the important medicinal plants used in various systems of medicine. *Piper longum* Linn (Piperaceae), commonly known as Indian Long pepper, used as a spice and seasoning, is known to possess multitude of pharmacological activities. The fruits and roots are attributed with numerous medicinal uses, and may be used for diseases of respiratory tract, viz. cough, bronchitis, asthma also as anti- irritant and analgesic. The fruits have been used as liver tonic, stomachic, emmenagogue, abortifacient, aphrodisiac and digestive (Kirtikar and Basu, 1987). *Ficus religiosa* belongs to Moraceae family. It is native from India to south East Asia. Bark powder is used to treat hypoglycemic, stomatitis and aphrodisiac. Stem bark is used for the treatment of ulcers, asthma, parasympatholytic, viral infection, and bacterial infection, protozoan infections, relaxant possess spasmolytic effects on smooth muscles and skin diseases. Latex has curing activity for toothache. The fruit extract have antitumor activity and is used to cure purgative and aphrodisiac (Asolkar et al., 1992).

The present work was aimed to evaluate the antibacterial activity of extracts of *P. longum* and *F. religiosa* against *E. coli* isolated from water.

MATERIALS AND METHODS

Isolation and identification of test organism

Test microorganism i.e. *E. coli* was isolated from tap water of Kanya Gurukul Laboratory. Test microorganism was identified by Gram staining and further identification was carried out by biochemical testing.

Drug sensitivity (Kirby and Bauer, 1966)

Screening of sensitivity of *E. coli* towards antibiotics was determined by disc diffusion method. Multidisc of antibiotic was used to check sensitivity against *E. coli.*

Screening of antibacterial activity of plant extracts

Plant materials

The fresh leaves of *P. longum* L. And *F. religiosa* were collected locally. Plant's leaves were identified by the taxonomic literature present in Gurukul Kangri University library and from herbarium.

Preparation of plant extracts (Singh and Rai, 2013)

The collected leaves were washed thrice with distilled water and dried in laboratory at 37±1 ˚C for 24 hours. After drying, the leaves were ground in grinding machine. Dry powdered leaves were extracted in soxhelet apparatus separately in water, ethyl alcohol to obtain their extracts. Extracts were filtered by filter paper (Whatmann No.1), concentrated and dried under reduced pressure in a rotary evaporator. All the dried extracts were stored in airtight vials at 4˚C for subsequent use. Different dilutions of extract were used i.e. 100%, 80%, 60%, 40%, 20%.

Preparation of Disc

Whatmann No.1 filter paper was punched into discs using a paper punch. The discs were sterilized. 20 µL of each dilution was impregnated into sterile, blank discs 6 mm in diameter.

These prepared discs were used in the study.

Antibacterial susceptibility of plant extracts (Kirby and Bauer, 1966)

Antibacterial activity of plant extract was assayed by disc diffusion method The bacterial strain (4-5 colonies) to be tested was suspended in 4ml of normal saline (0.85 %) and the density of suspension adjusted to approximately 10^8 CFU ml-1 using a 0.5 M barium sulphate suspension as the standard turbidity (McFarland, 1907). The surface of the sterile 3.8% Mueller Hinton Agar (Hi-Media) in Petri dishes was dried and the test strain was inoculated with a sterile swab to obtain a bacterial lawn. Sterilized discs were placed on the agar. The plates were incubated at 37ºC for 24 to 48 hrs.

The antibacterial activity was evaluated by measuring the zone of inhibition around the well.

RESULTS AND DISCUSSION

During investigation the microorganisms were isolated from water sample. After Gram staining it was observed that isolated microorganism was Gram negative rods. On the basis of biochemical testing it was found that microorganism was *E. coli* and the results of biochemical tests are tabulated in Table 1. The results of these biochemical tests were similar to those observed by other authors who studied *E. coli* isolated from wastewater effluents of food and beverage industry (Silva et al., 1980; Farasat et al., 2012). Results of drug sensitivity showed that maximum zone of inhibition were exhibited by ciprofloxacin followed by co-trimoxazole which was found to be 37 mm, 27mm diameter respectively which cleared that *Escherichia coli* was sensitive towards these antibiotics. No antibacterial activity was seen by ampicillin and amikacin which showed that *E. coli* was resistant towards these antibiotics. Results have been shown in Table2. During the study on test plants for their activity against *E. coli*, which was compared with standard antibiotics to calculate its efficacy, it was found that aqueous extract of *F. religiosa* exhibited maximum zone of inhibition in comparison to ethyl alcohol extract which was found to be 27 mm and 20 mm respectively at 100% concentration and it was comparable to zone of inhibition of standard antibiotic (co-trimoxazole).Similar study was done by Rajiv and Sivaraj (2012). In their research it was observed that aqueous extract of *F. religiosa* having best antimicrobial activity. In case of *P. longum* Aqueous extract showed maximum zone of inhibition (22mm) and no antibacterial activity was shown by ethyl alcohol extract. Results are tabulated in table 3. Anu et al., 2013 also studied on *P. longum*. In their study maximum activity was shown by acetone extract followed by ethyl alcohol then aqueous extract. Difference in the results may be due to the strain and method they used for extraction in their study. Antibacterial activity of extract may be due to the presence of phytochemical constituents present in the plant. Previous studies which have been done cleared that these plant contained bioactive compounds like alkaloids, glycosides, terpenoids, flavonoids, and tannins (Trivedi et al., 1969; Rami et al., 2013).

Table 1. Microbiological characteristics of *E. coli* isolated from water sample.

S. NO.	Characteristics		Observations
1	**Colony Characteristics**	NAM	Smooth White colonies
		Mac conkey	Pink colonies
		EMB	Green metallic sheen
2	**Microscopic Characteristics**	Gram staining	Negative
		Motility	Motile
		Shape	Short rods
3	**Biochemical Characteristics**	Indole	+
		MR Test	+
		VP Test	_
		Citrate utilization	_
		Gelatine liquification	–
		Catalase test	+
		H_2S Production	-
		Fermentation	
		Glucose	+
		Lactose	+
		Sucrose	+
		Carbohydrate	+

Table 2: Antibiotic sensitivity tests for *E. coli* (Average of triplicate)

Antibiotics	Zone of inhibition(mm)	Interpretation*
Ampicillin(AS)	--*	R
Co-trimoxazole(CO)	**27**	S
Chloramphenicol(CH)	17	S
Ciprofloxacin(CP)	**37**	S
Tetracycline(TE)	15	S
Ofloxacin(OF)	20	S
Gentamycin(GM)	10	I
Bacitracin(BC)	5	I
Amikacin(AK)	--	R
Erythromycin(E)	5	I
Cefotaxime(CF)	11	I
Perfloxacin(PF)	20	S

* R “Resistant”, S “Sensitive”, I “Intermediate”, --“Nil”

Table 3: Antibacterial susceptibility of *Ficus religiosa and Piper longum* extract against *E. coli* (Average of triplicate)

Concentration (%)	Zone of inhibition(mm)			
	Ficus religiosa		*Piper longum*	
	Aqueous extract	**Ethyl alcohol extract**	**Aqueous extract**	**Ethyl alcohol extract**
100	**27**	**20**	**22**	-
80	24	15	20	-
60	20	2	18	-
40	19	2	14	-
20	15	2	11	-

CONCLUSION

Due to indiscriminate use of antimicrobial drugs microorganisms develop resistance to many antibiotics. In addition to this many of them are known to have side effects. Therefore there is a need to screen local medicinal plants for possible antibacterial properties. In present study aqueous extract of *P. longum* and *F. religiosa* has antimicrobial activity against *E. coli* and it is very similar to commercial antibiotics indicating that plant can be used for production of new antibiotics. These plants have great medicinal values as it has been reported to have enormous phytochemical constituents including tannins, flavonols and flavonoids, terpenoids, coumarins, glycosides, esters, carbohydrates, serine protease, etc. Thus, *P. longum* and *F. religiosa* have great medicinal potential for the therapy of infection.

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