Screening of Antibacterial Potentiality of *Thalictrum foliolosum* leaves Extracts

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Abstract: The antibacterial activity of methanol, chloroform, hexane and aqueous extracts of the plant *Thalictrum foliolosum* collected from Nainital, Kumaun Himalaya, has been investigated. The extracts were tested against five pathogenic bacteria (*Agrobacterium tumefaciens, Bacillus subtilis*, *Erwinia chrysanthemi, Escherichia coli* and *Xanthomonas phaseoli*) using disc-diffusion method. Disc treated with standard antibiotic Gentamycin served as positive control in the experiment. Methanol and Hexane extracts showed highest activity against all the pathogens tested followed by Chloroform. However the aqueous extract had almost no inhibitory effect on the tested organisms.

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1. Introduction

The use of natural products with therapeutic properties is as ancient as human civilization and, for a long time, mineral, plant and animal products were the main sources of drugs (De Pasquale, 1984). The plant based, traditional medicine system plays an important role in health care with about 80% of the world's inhabitants relaying mainly on traditional medicines for their primary health care (Owolabi et al., 2007). According to WHO, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated, to better understand their properties, safety and efficacy (Nascimento et al.2000).

To develop alternative antimicrobial drug one approach is to local medicinal plant which represents a rich source of novel antimicrobial agents (Khulbe & Sati, 2009). The Himalaya is comprises of number of medicinal plants which are frequently been reported for their traditional uses in the treatment of various ailments (Sati and Joshi, 2010, 2011). *Thalictrum foliolosum* have been used as a tonic, aperients, and diuretic, stomachic, antiseptic and for the treatment of snake bite, jaundice, rheumatism etc. (Schiff and Doskotch, 1970). *Thalictrum* used to control external parasites (Pande et al., 2007). Dried root powder mixed with *Thymus linearis* in equal proportion is taken regularly to cure stomach pain and gastric trouble (Uniyal et al., 2006). Investigators have identified 290 *Thalictrum* alkaloids in about 80 species of it (Erdemgil, 2001). However, the antibacterial activity of this plant has not been adequately explored. Therefore, in the present investigation antibacterial potential of *Thalictrum foliolosum* following standard methodology (disc diffusion method) is explored.

2. Materials and Methods

2.1 Collection of plant material

Thalictrum foliolosum was collected from Rajbhawan, Ayar Pata and D.S.B. Campus, Nainital during mid Oct-Nov 2010 at an altitude of a 1936 m above sea level. Leaves were dried in normal room temperature for about 10 days.

Thalictrum foliolosum, belongs to family Ranunculaceae, commonly known as "Meadow rue" in Kumaun "Mamira" widely distributed in climatically moderate zones of the Northern Hemisphere. It is a slow growing, dump forming, rhizomatous perennial plant (Fig 1). These like semi-shades, moist, well drained soil and very good for boarder plants and woodland gardens.

Fig 1: *Thalictrum foliolosum*

2.2 Extraction procedure

Leaves of the plant were thoroughly washed and dried at the room temperature (20±2°C). The dried material was powdered in an electric grinder. To prepare stock solution 50g of this powder was added to 200ml of hexane, choloroform, methanol and aqueous solution (w/v, 50g/200ml). Extract was shaken for at least 6h and after that each extract was passed through Whatman filter paper no.1 and the final filtrate as 25% crude extract thus concentrated on a rotary evaporator under vacuum at 20° C and was utilized for the experiments.

2.3 Microorganisms Used

Five (Gram +ve and -ve) bacteria (*Bacillus subtilis* MTCC No. 121, *Escherichia coli* MTCC No.40, *Agrobacterium tumefaciens* MTCC No.609, borrowed from Institute of Microbial Technology, Chandigarh, India and *Xanthomonas phaseoli* and *Erwinia chrysanthemi* were obtained from Plant Pathology Department, G. B. Pant University, Pantnagar, India) were used in this investigation.

2.4 Screening of antibacterial activity

Antibacterial tests of selected microorganisms were carried out using disc-diffusion method (Bauer et al., 1966). Nutrient agar plates (90mm size) were prepared and cooled down at room temperature (20±2°C). A small sterile cotton swab was dipped into the 24h old culture of bacteria and was inoculated by streaking the swab over the entire agar surface. This process was repeated by streaking the swab 2 or more times rotating the plates approximately 60° each time to ensure even distribution of inoculum. After inoculation the plates were allowed to dry at room temperature (20±2°C) for 15min in laminar chamber for settle down of inoculum. The filter paper discs (5 mm) loaded with 40µl of extract were placed on the surface of the bacteria seeded agar plates and it was allowed to diffuse for 5min then these plates were incubated at 37±1°C for 24h. Gentamycin (30 mcg), were placed into agar plates used as positive control and respective solvent were also used as negative control. After 24h of incubation, the diameter was observed for inhibition zone (measured in mm including disc size). All tests were performed in triplicates and observed values of ZOI are expressed as mean value with standard error of means (SEM).

3. Results

The results of screened plant extracts for antibacterial activity are summarized in Table1. Methanol, chloroform and hexane fraction showed a variable activity against all the test strains. Highest zone of inhibition (15±0.6mm) was recorded against *X. phaseoli* followed by *A. tumefaciens, E. coli, B. subtilis* (13±0.0mm) each, however lowest level was observed against *E. chrysanthemi* (12±0.3 mm).

Hexane extract was also found active against all strains. The highest zone of inhibition (15±0.6mm) was observed against *X. phaseoli* followed by *B. subtilis* (14±0.3mm). Significant inhibition was observed against *E. coli* (13±0.6mm) followed by *A. tumefaciens* and *E. chrysanthemi* (12±0.9mm) each (Plate1).

Similarly, chloroform extract was also found significantly active against *B. subtalis, X. phaseoli, E.coli* (13±0.3mm) each followed by *A. tumefaciens* (12±0.3mm) and *E. chrysanthemi* (11±0.0 mm). Aqueous extract did show no inhibitory activity against all the bacterial strains tested (Fig.2).

4. Discussion

Due to stressful climate and geographical conditions, Kumaun Himalayan region plants offer greater possibilities of active compounds. The high altitude grown plant *Thalictrum foliolosum* has not been investigated for its defined antimicrobial potentiality. The available literature indicates that *T. foliolosum* well known for its use in the treatment of various ailments but this indigenous plant has not been studied adequately as antibacterial agents. Therefore, this study highlights for the first time the ability of different solvent fractions of the plant as antibacterial though in vitro assay.

In this study, four extractant i.e. hexane, methanol, chloroform and aqueous were used to obtain active compounds in the extracts. The result obtained in this investigation for the antibacterial activity of *T. foliolosum* using disc diffusion method showed that strains are sensitive to all the tested extracts except aqueous (Table 1). The effectiveness of different extracts of *T. foliolosum* was different. Methanol and Hexane extracts showed highest activity against all the pathogens tested followed by Chloroform. Aqueous extract were inactive this might be due to the various substances that show activity against bacteria are more soluble in organic solvents than aqueous extract (Sati and Joshi, 2010).

In previous studies, the antimicrobial properties of some *Thalictrum* species and their alkaloids were reported. Abraham et al., (1986) studied antibacterial and antifungal activities of some *Thalictrum* species. Wu et al., (1977a) reported that, only thaliglucinone among *T. rugosum* alkaloids exhibited antimicrobial activity against 6 different organisms. Mitscher et al., (1972) reported that obamegine and thalidasine alkaloids that were ethanolic extract of *T. rugosum* the most active antimicrobial alkaloids against *Mycobacterium smegmatis*. It was reported that *T. longistylum* and *T. revolutum* were effective against some bacteria in the literature (Wu et al., 1977b).

Desta, (1993) use five extracts (direct aqueous extract, petroleum ether fraction, dichloromethane

fraction, methanol fraction and residual aqueous fraction) of Ethopian traditional drug *T. rhynchocarpum* (Ranunculaceae) and were tested against *Staphylococcus aureus*, *Salmonella gallinarum*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Candida albicans* at a concentration of 1000 µl/ml. According to the his results, methanolic extract, residual aqueous extract and direct aqueous extract fractions exhibited the highest degree of antimicrobial activity against *S. aureus*, *P. vulgaris*, *C. albicans*, *S. gallinarum* and *E. coli*.

Methanolic extract fraction exhibited antimicrobial activity as less than of the standard antibiotic against *S. aureus*, and *P. vulgaris*. Otherwise, residual aqueous of this plant extract showed antimicrobial activity as less than of the standard antibiotic for *S. aureus*, *S. gallinarum* and *E. coli*. It was recorded that direct aqueous extract exhibited antimicrobial activity as less than of the standard antibiotic for *S. aureus* and *E. coli*. Petroleum ether fraction was no activity against these microorganisms. Rawat *et al.,* (1992) reported that the root and rhizome of *Thalictrum foliolosum* are thought to be of therapeutic value as a purgative, diuretic and febrifuge, and in the treatment of atonic dyspepsia. A root ethanolic extract at 1000, 2000 or 3000 mcg/paper disc was assayed against 5 species of pathogenic bacteria, while 2000 and 3000 mcg inhibited bacterial growth. Maximum effectiveness against *Pseudomonas aeruginosa* and *Staphylococcus aureus* was obtained with 3000 mcg.

From a clinical point of view, *E. coli* can infect the gall bladder, surgical wounds, skin lesions and the lungs (Black, 1966). Similarly, *B. subtilis* have been known to act as primary invaders or secondary infectious agents in a number of diseases and have been implicated in some cases of food poisoning (Thrunbull and Kramer, 1991), thus the use of *T. foliolosum* extracts is an effective measure to fight against such infections. This study also provide a new leads as there is no previous record on the antibacterial activity of *T. foliolosum* extracts against the plant pathogenic strains (*X. phaseoli, A. tumefaciens,* and *E. chrysanthemi*) responsible for various plant diseases like crown gall, leaf blight, leaf spot and rot diseases.

It is interesting to note that this is the first report to demonstrate that methanol, chloroform and hexane extract of *T. foliolosum* contains antibacterial substances for controlling bacterial pathogens. In addition these results also confirmed the evidence in previous studies which reported that methanol is a better solvent for more consistent extraction of antimicrobial substances from medicinal plants compared to other solvents such as water, ethanol, chloroform and hexane (Karaman et al., 2003; Ahmad et al., 1998; Eloff, 1998).

The present work on the antibacterial potentiality also strongly reports the frequent use of this plant in folk medicine for the treatment of various ailments. It also underlines the importance of the ethnobotanical approach for the selection of plant in the discovery of new bioactive compounds.

Conclusion

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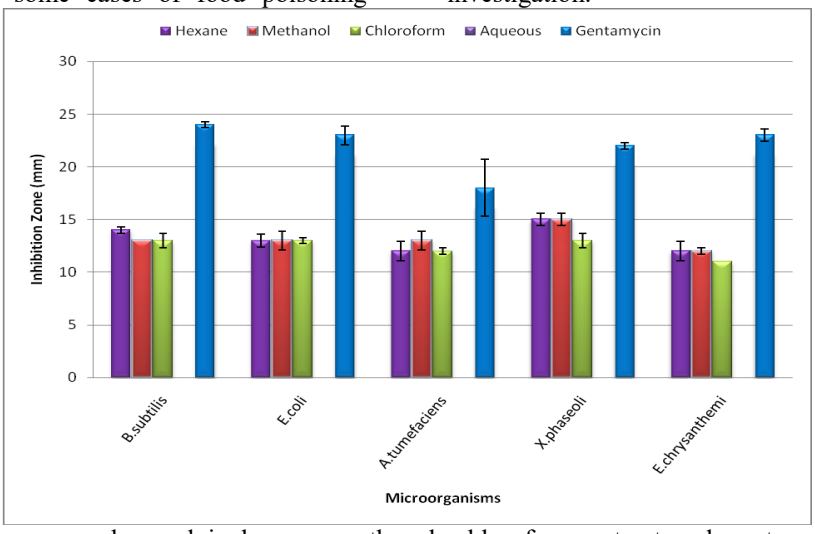

Figure 2. Inhibition zones observed in hexane, methanol, chloroform extract and gentamycin. Bar represents standard errors and mean values of inhibition zone.

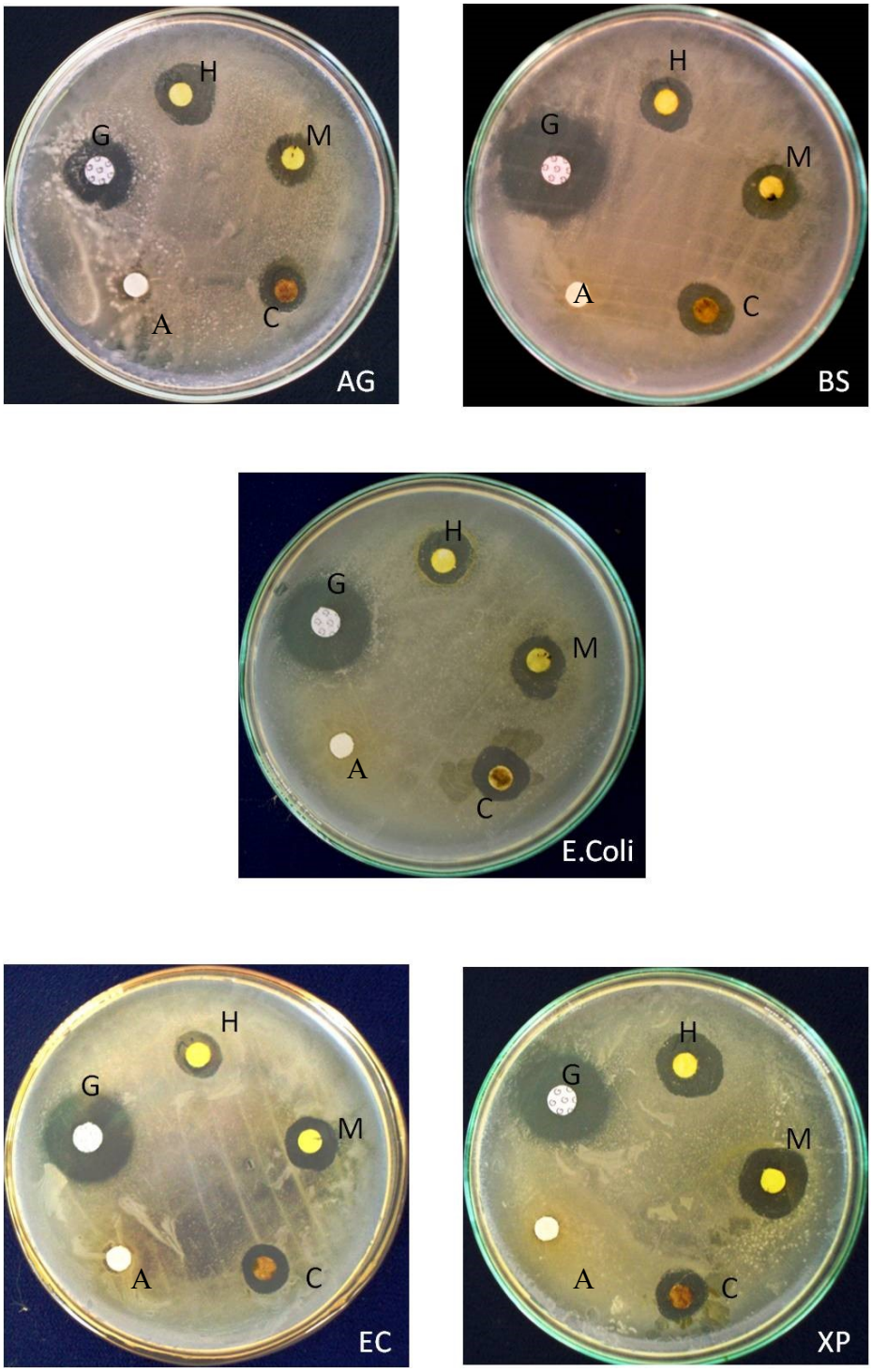

Plate 1: Antibacterial activity of *Thalictrum foliolosum* against some pathogenic bacteria. **H, M, C, A, G:** Hexane extract, Methanol extract; Chloroform extract, Aqueous extract and Gentamycin (positive control); **AG-** *Agrobacterium tumefaciens;* **E.coli -** *Escherichia coli;* **EC-** *Erwinia chrysanthemi;* **XP-***Xanthomonas phaseoli;* **BS-** *Bacillus subtilis*

Table 1: Zone of inhibition of different extracts of *Thalictrum foliolosum*

Microorganisms	Diameter of Inhibition Zone (mm)*						
	H	**M**		**C**	**A**		**G**
B. subtilis	14±0.3	13±0.0		13±0.7	Na		24±0.3
E. coli	13±0.6	13±0.9		13±0.3	Na		23±0.9
A. tumefaciens	12±0.9	13±0.9		12±0.3	Na		18±2.7
X. phaseoli	15±0.6	15±0.6		13±0.7	Na		22±0.3
E. chrysanthemi	12±0.9	12±0.3		11±0.0	Na		23±0.6

*All the values are mean ± SEM of three determinations. **H, M, C, A:** Hexane, Methanol Chloroform, Aqueous extracts; **G:** Gentamycin (positive control); **N/A:** not active

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