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IN VITRO ANTIBACTERIAL ACTIVITY OF RIBES GROSSULARIA AGAINST VARIOUS PATHOGENIC MICRO-ORGANISMS

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ABSTRACT:

The present study is to evaluate the antimicrobial efficacy of *Ribes grossularia* (Grossulariaceae) against bacterial strains *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Proteus vulgaris*. Aqueous, methanol and chloroform extracts of stem and leaves of *R. grossularia* were tested for antibacterial activity in vitro by agar well diffusion method. Highest zone of inhibition was observed in chloroform and methanol extract of stem against *P. vulgaris* (19.9 and 17.93mm respectively) followed by methanolic extract of stem against *S. aureus* (14.9 mm). Overall the methanol and chloroform extract of stem and methanol extract of leaves was found to be more effective for *P. vulgaris* and *S. aureus*. The results of this extracts were compared with standard antibiotics ampicillin, streptomycin and tetracycline.

KEY WORDS: *Ribes grossularia*, Antibacterial activity, Agar well diffusion, Antibiotics

INTRODUCTION:

The Himalaya region covered 18% of total geographical area of India. It is mainly divided into Northern, Western, Central and North Eastern Himalaya. Uttarakhand is centrally located in the Indian Himalayan mountain chain (Nautiyal, *et al.*, 2005). It is extremely rich in plant life and diversity of medicinal plants. It is well evident that about 8000 species of medicinal plants is growing in the Himalaya and it has been more than 3500 species in Garhwal region (Khoshoo, 1991; Rao 1994; Sundariyal, *et al.*, 2001). In recent years interest in plants having antimicrobial activity is raised so that new natural antibiotics could be developed because most of bacterial strains have developed resistance against most of commercial antibiotics (Rao, *et al.*, 1994; Ahmad , *et al.*, 2001; Bisht, *et al.*, 2009; Pandey, *et al.*, 2011; Raman, *et al.*, 2012; Negi, *et al.*, 2012). Medicinal plants are a very rich source of antimicrobial compounds and potentially useful agents for the development of new chemotherapeutic products. In different countries, plants are used medicinally and these

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are source of many potent and powerful drugs (Srivastava, *et al.,* 1996). There are many reports which show the antiviral, antibacterial, antifungal and anti-inflammatory properties of plant (Joshi, *et al.,* 1990; Samy, *et al.,* 2000; Palombo, *et al.,* 2001; Behera, *et al.,* 2005; Joshi, *et al.,* 2009; Shaba, *et al.,* 2012). *Ribes grossularia* is also known as Himalayan currant. It is an erect, deciduous shrub with hairless branches to 4 m high, which is found in open rocky slopes above 2700 m. It is also called as asian gooseberry and the rough or hairy gooseberry. The other hindi names are amala, amlanch, kansi, pilsa, teila, caktu and kontilo. Flowers are small and followed by red, green, yellow, purple or black berries. Berries are globose, sparsely hairy and about 8 mm across in hanging clusters. In rural areas, one palmful powder of whole plant is given daily for preventing abortion. Leaves are used as an anti diuretic. In other countries, fruit of Ribes is used as a fertility enhancer to assist women in becoming pregnant. Roots and seeds are high in gamma-Linolenic acid, which has been clinically verified as an effective treatment for pre-menstrual syndrome. A root tea is effective for women in uterine difficulties (Gaur, 1999; Sood, *et al.,* 2005; Kumari Priti, *et al.,* 2009). The present study was carried out to evaluate the antibacterial activity of leaf and stem extracts of *Ribes grossularia* with the hope that the result will further confirm the folk therapy of infection.

MATERIALS AND METHODS:

Collection of Sample:

Plant parts such as stem and leaves were collected from Mana, District- Chamoli, Uttarakhand, India. The plant material was washed thoroughly 2-3 times with running water and once with distilled water and shaded dried, then grinded into powdered form. Powdered material stored in air tight container at room temperature (30-32^{0}C) until use. The taxonomic identity of this plant was confirmed at Botanical Survey of India, Northern region of India, Dehradun, at flowering stage.

Chemicals:

All chemicals used of Hi-media (analytical grade) and purchased from chemical companies.

Extract preparations:

To obtain the aqueous extracts, dried and fine powdered bark and leaves of *Ribes grossularia* were weighed about 2 gm each and soaked in 25 ml of distilled water, methanol and chloroform in different conical flask for 24 hours and filtered using standard filter paper. The material was again mixed with 25 ml of each of three solvents and filtered after 24 hours. Same process was repeated once again. The extract after treating with 75 ml (25 ml×3 times) distilled water, methanol and chloroform was then filtered, and filtrate was transferred into vials and allowed to evaporate until completely dry. Once dry, the extract was re-suspended in 2 ml of distilled water, methanol and chloroform. The concentration of the

final extract was 1 g material/1 ml (Panthi, *et al.*, 2006 and joshi, *et al.*, 2009).

Culture condition of Micro-organisms: The test organisms included four bacterial strains *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Proteus vulgaris* which were obtained from Department of Microbiology, H. N. B. Garhwal University, Srinagar,Uttarakhand.

Preparation of test organisms:

For bacterial strains, three to four isolated colonies were inoculated in 2 ml nutrient broth and incubated till the growth in the broth was equivalent with Mac-Farland standard (0.5%) as recommended by WHO at which the number of cells was assumed to be 1.5 x 108 cfu/ml.

Evaluation of anti bacterial activity:

Anti-bacterial activity was evaluated by agar well diffusion method (Perez, *et al.*, 1990). Zone of inhibition diameter was measured for the estimation of potency of the antimicrobial substance. Muller Hinton agar medium was prepared by using 15g agar dissolved in 1L distilled water. Muller Hinton agar medium was poured into each Petri plate of 20 x 90mm and allowed to cool to 45°C to solidify. The freshly prepared inoculums were swabbed all over the surface of the MHA plate using sterile cotton swab. Wells of 8 mm diameter were made in the agar with a sterile cork borer. Hundred micro-liters of the working suspension/solution of different plant extracts were loaded in each well and same volume of extraction solvent for control was filled in the wells with the help of micropipette. Plates were left for some time till the extracts diffused in the medium with the lid closed and incubated at 37°C for 24 hour. The tests were performed three times and the zones of inhibition were measured for each extract using a ruler and the results were recorded. During determination of zone of inhibition, the diameter of well was 6 mm and the height of the well was 4 mm and the concentration of loaded extract in each well was 100 µl.

RESULTS AND DISCUSSION:

The aqueous, methanol and chloroform extract of leaves and stem of *R. grossularia* were tested against the *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli* and *Proteus vulgaris*. The result of experiment is shown in Table no. I. In a control experiment the antimicrobial activity of three standard antibiotics against test organisms were also observed and are summarized in Table no. II. All antibiotics shown antibacterial activity but *P. vulgaris* could not be inhibited and showed negative results against Tetracycline. The results of both tables when compared confirm the potential antibacterial activity of the R. grossularia. All extracts have exhibited greater inhibitory effect against all pathogen than the antibiotics. The antimicrobial potential of extracts was found higher than the antibiotics.

In case of *P. aeruginosa*, activity of methanolic extract of leaves was found 12.2 mm followed by aqueous (9.93 mm) and chloroform (8 mm). In the stem extract of methanol 12.2 mm zone of inhibition was found which is followed by chloroform 9.9 mm but there is no activity found in aqueous extract. It was seen that among three antibiotics only streptomycin was functional while ampicillin and tetracycline were not effective at all.

In case of *S. aureus*, only methanolic extract was effective with 12.1 mm zone of inhibition and no activity was found in aqueous and chloroform extracts. In the stem extract of methanol the zone of inhibition was found 14.9 mm followed by chloroform 11.1 mm and there is no significant zone of inhibition in aqueous extract. In comparison with the standard antibiotics, the zone of inhibition in streptomycin, ampicillin and tetracycline was found 9 mm, 7.96 mm and 13.93 mm respectively. So the leave extract of methanol and stem extract of methanol and chloroform was found more effective than the standard antibiotics.

In case of *E. coli*, only methanol extracts of leave and stem shows the zone of inhibition i.e. 12.06 mm and 9.9 mm respectively.

In case of *P. vulgaris*, leave extract of methanol shows the maximum inhibition 15.03 mm which is followed by aqueous extract and chloroform extract i.e. 11.1 mm and 8 mm respectively. In the stem extract of chloroform maximum zone of inhibition

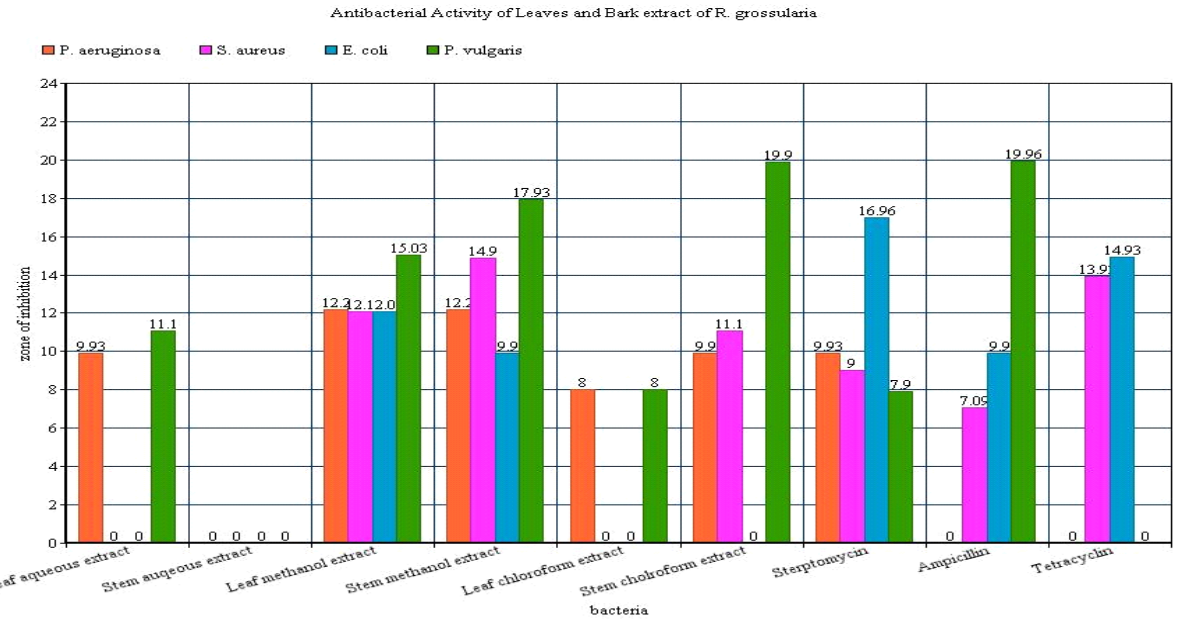

Fig: 1: Anti-bacterial activity of leaves and stem extracts of *R. grossularia* against different pathogenic microorganisms in comparison with standard antibiotics.

19.9 mm was found which is followed by methanol extract i.e. 17.93 mm. there is no zone of inhibition found in aqueous extract. In comparison with standard antibiotics streptomycin and ampicillin shows the zone of inhibition i.e. 7.9 mm and 19.96 mm respectively. Tetracycline shows no activity against the bacteria.

On comparing the aqueous, methanol and chloroform extract of leave and stem of *R. grossularia,* it is confirmed that methanol extract is more active than the chloroform and aqueous. This limelight's that the methanolic extract of this plant may contain active components and has the ability to extract the active phytochemicals present in the plant parts, which produced zones of inhibition against the bacterial strains. The value of this plant lies in the chemical substances that produce a definite physiological action on the bacteria The results support the use of this plant in traditional medicine for the treatment of infections and can be further subjected to isolation of the therapeutic antimicrobial compounds. Other activities of this plant can be studied in Uttarakhand which will add the existing knowledge about potential of this species in the region.

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