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In vitro Approaches of *Primula vulgaris* Leaves and Roots Extraction against Human Pathogenic Bacterial Strains

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Abstract: Primula is a medicinal plant bearing flowers which belongs to family *Primulaceae.* These are low growing herbs. Methanol and cold water extraction methods were used for leaves and roots extractions of *Primulavulgaris.* Antimicrobial activity ofleaves and roots extracts was evaluated by well diffusion method. Results revealed that *P. vulgaris* leaves extractexhibit excellent inhibitory effects against *Escherichia coli* that showed zone of inhibition 18 mm with methanol extract and 14 mm with cold water extract and *Pseudomonas aeruginosa* showed zone of inhibition 14 mm with methanol extract and 13 mm with cold water extract. Roots extract of *P. vulgaris* also exhibited inhibitory effects against *E. coli* showed zone of inhibition 13 mm with methanol extract and 16 mm with cold water extract and *P. aeruginosa* showed zone of inhibition 13 mm with methanol extract and 14mm with cold water extract while *Staphylococcus aureus* did not show any inhibitory zone with leaves or roots extracts. This research summarized that leaves and roots extracts of *P. vulgaris* contains a brought range of antimicrobial activity against the microorganism. Advance studies should carry out to find out the precise method of action by which extracts exert their antimicrobial effect to recognize which can be used in drug development.

Key words: Bacteria · Antibacterial · Well Diffusion Method · Root and Leaf Extract · *P. Vulgaris.*

INTRODUCTION

The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization [1]. *Primula* is a medicinal plant bearing flowers which belongs to family *Primulaceae.* Primula genus consists of 400 to 500 species. These are low growing herbs. These are low growing herbs. This species is found throughout the temperate Europe and Asia [2].

*Primula*is low emerging plant with a badge of leaves 5-15 cm long and 2-6 cm broad. The bright colour flowers are formed in the springs between April and May. They are in bunches of 10-30 collectively on a lonely stem 5-20cm tall, every flower is 9-15 mm wide. Reproduction occurs by seeds, which are dispersed by ants. A single primrose plant may live for 15-25 years [3].

The *Primula*has been cultivated for ornamental purposes and for many years it is used in traditional herbal medicine [4]. The edible parts are flower and leaves. Young leaves of *Primula* that is raw or cooked in soups. The dried or fresh leaves are used as a tea substitute [5]. They create a decorative addition to the salad bowl. This species has become much decreased due to the destruction of their habitats, farming practices and over collection in the past 100 years. When it was available in increased amount, the flowers were harvested in high quantity in the spring and used for preparation of a tasty wine with sedative and nerving properties [6].

Primula plants are an underused but are very valuable medicinal herb. They have a very extensive history of medicinal use and have been chiefly use in treating conditions involving cramps, spasms, paralysis

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and rheumatic pains. *Primula* plant contains saponins, which have an expectorant effect and salicylates which are the main component of aspirin and have anodyne, anti-inflammatory and febrifuge effects. Flowers are anodyne, diaphoretic, diuretic and expectorant [6]. *Primula* is also recommended for treating over-activity and insomnia, especially in children. They are potentially precious in the treatment of asthma and other allergic conditions. *Primula* species can also contains allergens and some species are used traditionally to treat epilepsy and convulsions [2, 7]. Another *Primula* species has ?avonoids that possessed strong cytostatic properties against HL 60 cells even at low concentrations [8].

Extract from primula contains saponins which have antibacterial activity. When saponins were tested for its antibacterial activity, saponins showed inhibitory zones against both Gram positive and Gram negative bacteria. Pure saponin exhibits remarkable antibacterial activity as compared to crude extract. The aim of this study is to determine antibacterial effects of leaf and root extraction from *Primula vulgaris* against selected bacterial strains of human origin.

MATERIALS AND METHODS

Primula plants free from disease were collected from Awan Nursery Haripur. These plants were transported to the Microbiology Research laboratory, Department of Microbiology, Hazara University, Mansehra. Fresh leaves and roots were separated from primula plants and then washed and were kept separated in shade and allowed it to air dry. After drying these materials was mashed into powder form with the help of piston motor and powder form of root and leaf were stored separately.

Three bacterial strains were used in the research (antibacterial activity of primula plant). These microbial strains included:*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa*ATCC 27853 and *Staphylococcus aureus*ATCC 6538.

The Adebayo and Ishola (2009) method of extraction was used. Five grams powdered samples of leaf and root was soaked in 50 ml methanol and cold water in 250ml sterile flask and rotated on shaker at 150 rpm for 24 hours at room temperature. The extract was filtered through a muslin cloth and then centrifuged at 4400 rpm for 7 minutes. The supernatant were collected and the pellet was discarded. These steps were repeated three times.

Nutrient broth and Muller Hinton Agar was an enrichment fluid medium (NB) for the growth of microorganisms.Medium was prepared by adding 13g of dehydrated powder using electrical balance (SHIMADZU) into 1 liter of distilled water. pH was adjusted by electrical pH meter (JENWAY, 3305) at 7.4 and was boiled to dissolve completely. All Media were sterilized by using automatic autoclave (REXALL, CO) at 121°C for 15 minutes.

Media was poured in pre-sterilized glass petri plates of 90mm in Laminar Flow Hood (ESCO) which was sterilized by overnight exposure of UV light and disinfected with 70% ethanol solution. Media plates were kept open for half an hour in the Laminar Flow Hood for drying and solidifying media.

The *in-vitro* activity of the extracts was assayed against the selected bacterial strains. All the ATCC (MicroBioLogics) strains were maintained in Nutrient Broth tubes at 4°C. The antimicrobial efficacy of the plant extracts was evaluated against given strains.

Antimicrobial activity of *Primula*leave and roots extracts was tested using Agar well diffusion techniques as described by Adeniyi *et al.*, (1996) Wells of 6mm diameter with sterile cock borer were aseptically punched in the 90mm MHA agar plates. With the help of sterile micropipette tips Primula leaves and roots extracts (methanol and cold water) 100µl were poured into the wells. The plates were incubated at 37°C for 24h. After incubation, the diameter of the resulting zone of inhibition was measured with the help of Digital Vernier Caliper (Mitutoyo) and the average values were recorded. Each antimicrobial assay was performed three times. The mean of values were recorded.

RESULTS

In the present research leaves and roots extracts from primula was tested for antimicrobial activity.

We used Gram negative bacteria *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and Gram positive *S. aureus* ATCC 6538 to test the *Primula* antibacterial activity. The Figures (1, 2, 3, 4, 5, 6, 7 and 8) and Table 1and 2 showed that the leaves and roots extract of primula in methanol and cold water inhibited *E. coli* and *P. aeruginosa*, while they did not show any inhibitory activity against *S. aureus* (Figures 9, 10, 11, 12, 13, 14 and 15).

Table 1: Zones of inhibition (mm) produced by leaves and roots methanolic extracts of *P. vulgaris* against selected Gram negative and Gram positive bacterial strains

	Zone of Inhibition (mm) of Methanol Extraction	
Bacterial Strains	Leaf extract	Root extract
E. coli	18 mm	13 mm
P. aeruginosa	14 mm	13 mm
S. aureus	01 mm	01 mm

Table 2: Zones of inhibitions (mm) produced by leaves and roots Cold water extracts of *P. vulgaris* against selected Gram negative and Gram positive bacterial strains

	Zone of Inhibition (mm) of Cold water Extraction	
Bacterial Strains	Leaf extract	Root extract
E. coli	14 mm	16 mm
P. aeruginosa	13 mm	14 mm
S. aureus	02 mm	02 mm

Zones of inhibition of leaves and roots (methanol and cold water) extracts of *P. vulgaris* plant against *E. coli*

DISCUSSIONS

During the last two decades, several groups have sought application of primula extracts used for antimicrobial activity against different bacterial spp. [5,6,11]. Subsequently, the aim of this research was to find out the antibacterial effects of *P. vulgaris* against selected Gram positive and Gram negative bacteria.

The antimicrobial activities of medicinal plants are qualified due to the presence of alkaloids and flavonoids[12]. Presence of flavonoids, alkaloids in different extract of *P. vulgaris* confirm its prospective against all selected pathogenic bacterial strains [8]. The present study suggests that the leaves and roots extracts of *P. vulgaris* have a board spectrum of antimicrobial activity, although the degree of susceptibility could differ between diverse microorganisms. The antibacterial activity found in this current conducted study may be attributed to the presence of secondary metabolites either individually or in combination of various types of chemical composition present in the plant constituents.

Zones of inhibition of leaves and roots (methanol and cold water) extracts of *P. vulgaris* plant against *E. coli*

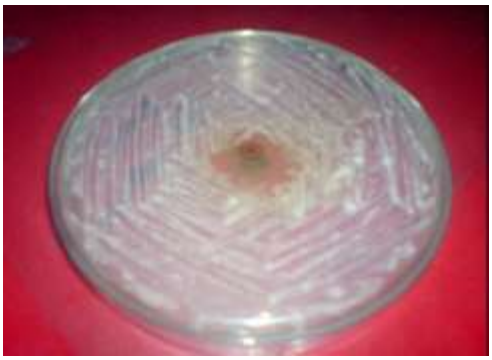

Fig. 1: Zone of inhibition (18 mm) of leaves extract in methanol against *E. coli*

Fig. 3: Zone of inhibition (13 mm) of root extract in methanol against *E. coli*

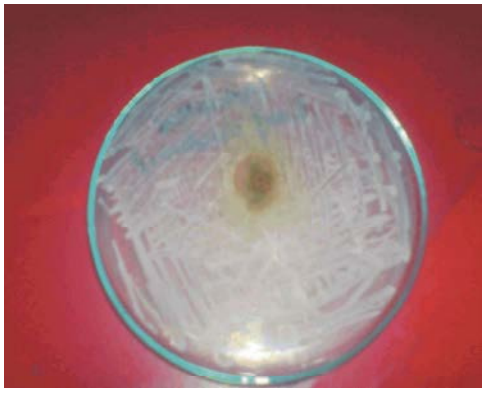

Fig. 2: Zone of inhibition (14 mm) leaves extract in cold water against *E. coli*

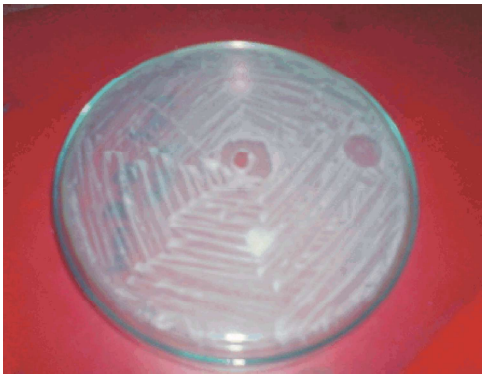

Fig. 4: Zone of inhibition (16 mm) of root extract in cold water against *E. coli*

Zones of inhibition of leaves and roots (methanol and cold water) extracts of *P. vulgaris* plant against *P. aeruginosa*:

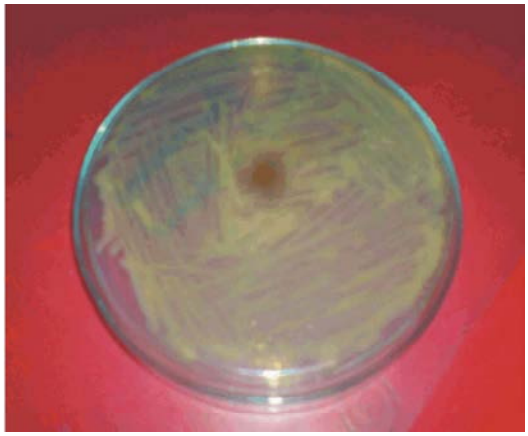

Fig. 5: Zone of inhibition (14mm) of leaves extract in methanol against against*P.aeruginosa*

Fig. 7: Zone of inhibition (13mm) of root extract in methanol against *P. aeruginosa*

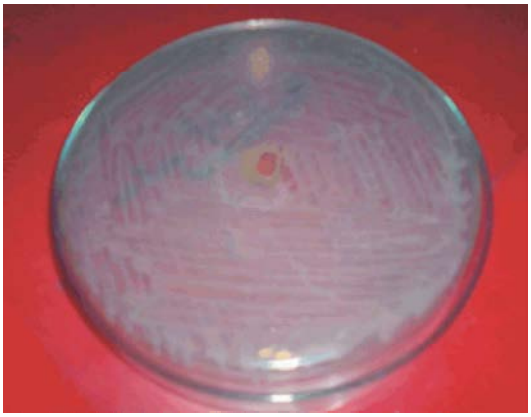

Fig. 6: Zone of inhibition (13mm) of leaves extract in cold water against *P.aeruginosa*

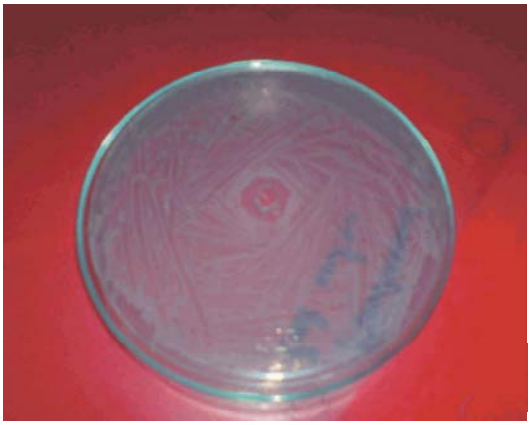

Fig. 8: Zone of inhibition (14mm) of root extract in cold water against *P. aeruginosa*

Zones of inhibition of leaves and roots (methanol and cold water) extracts of primula plant against *S. aureus*:

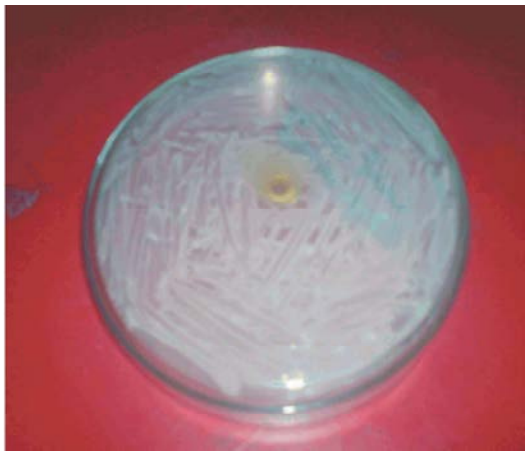

Fig. 9: Resistance of *S.aureus* to methanol leaves extracts

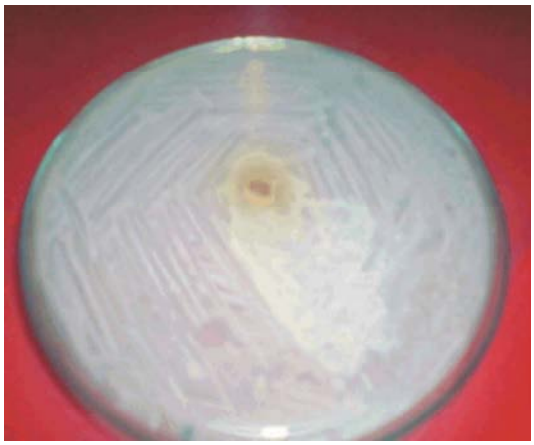

Fig. 10: Resistance of *S. aureus* to cold water leaves extracts

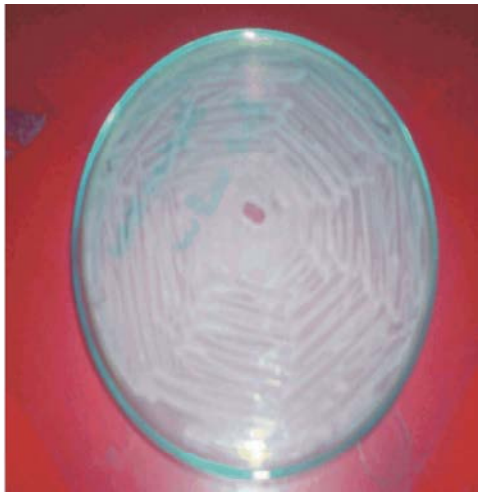

Fig. 11: Resistance of *S. aureus* to methanol root extracts

Fig. 12: Resistance of *S. aureus* to cold water root extracts

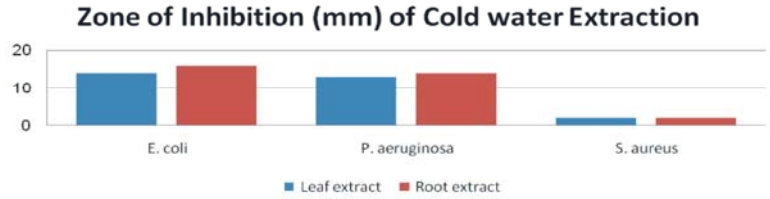

Fig. 13: Zone of inhibition of Cold water extract to selective Human Pathogenic Bacterial strains.

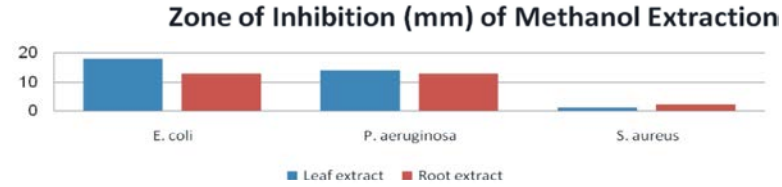

Fig. 14: Zone of inhibition of methanol extract to selective human pathogenic bacterial strains.

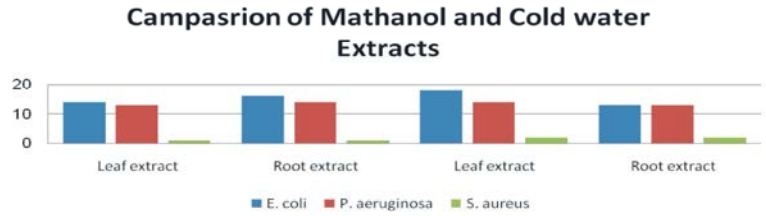

Fig. 15: Comparison of methanol and cold water extracts against selected bacterial strains.

Results of this study showed that the potential usefulness of *P. vulgaris* in the treatment of a variety of pathogenic bacterial strains as it may help in the novelty of new chemical classes of drugs or antibacterial that could provide as selective agents for the defense of human health and may provide life tools for the study of bacterial infection or diseases.

Results revealed that *P. vulgaris* leaves extractexhibit excellent inhibitory effects against *E. coli* that showed zone of inhibition 18 mm and 14 mm with methanol extract and cold water extract respectively, in addition *P. aeruginosa* showed zone of inhibition 14 mm with methanol extract and 13mm with cold water extract. Roots extract of *P. vulgaris* also exhibited inhibitory effects against *E. coli* as it showed zone of inhibition 13 mm with methanol extract and 16mm with cold water extractsand *P. aeruginosa* showed 13mm inhibition zone with methanol extract and 14mm with cold water extract while *S. aureus* did not show zone of inhibition with leaves and roots (methanol and cold water) extracts.

There are limited antibacterial agents that are currently available in the market due to their toxicity, low efficiency and prove costly in case of prolonged treatment. The discovery of plant origin a potent remedy will be a great development in microbial infection therapies. Therefore, there is desired to develop new antibacterial agents which can satisfy the present demand.

CONCLUSION

This research summarized that leaves and roots extracts of *P. vulgaris* contains a brought range of antimicrobial activity against the microorganism. Advance studies should carry out to find out the precise method of action by which extracts exert their antimicrobial effect to recognize which can be used in drug development.

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