

## ORIGINAL ARTICLE

## ANTI-INFECTIVE POTENTIAL OF A MEDICINAL PLANT *PIMPENELLA STEWARTII* USED TRADITIONALLY IN HAZARA

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**Background:** Medicinal plants have been used since long as folk medicines and also in Ayurvedic and Unani systems of treatment. They have importance to save human and animal lives because of their utilization in the herbal medicines. In all over the world plants are in constant use for curing of various kinds of diseases. The herbal medicines are effective due to presence of bioactive compounds along with vitamins and minerals in them. Moreover, plants produce photo-chemicals mainly secondary metabolites which have significant pharmaceutical activities like antioxidant, antimicrobial, pesticidal and anti-tumour activity. They are very effective and have little or no side effects. **Methods:** Five microbial strains including three fungal and two bacterial species were tested for the efficacy of plant materials by Agar diffusion method. The fungal species were *Aspergillus flavus*, *Aspergillus niger* and *Alternaria alternata* while bacterial species included *Arvenia caratovora* and *Xanthomans* spp. **Results:** Results obtained for methanolic extracts of *Pimpenella stewartii* against *Aspergillus flavus*, *Aspergillus niger* and *Alternaria alternata* showed significant antifungal activity. The antibacterial screening of Methanolic extracts of *Pimpenella stewartii* were evaluated against *Arvenia caratovora* and *Xanthomans* spp. The best zone of inhibition was found against *Xanthomans* spp (97.33±2 mm) at 250 ppm concentration followed by *Arvenia caratovora* (49.7±14 mm) at same concentration.

**Keywords:** *Pimpenella stewartii*, *Aspergillus niger*, *Xanthomans* spp, Methanolic extract

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### INTRODUCTION

An antimicrobial is a compound that kills or inhibits the growth of microbes and many medicinal herbs are a rich source of antimicrobial agents.<sup>1</sup> A wide range of medicinal plant parts are used for extract as raw drugs and possess varied medicinal properties.<sup>2</sup> Primitive people learned by trial and error to distinguish useful plants with beneficial effects from those that were toxic or non-active and also which combinations or processing methods had to be used to gain consistent and optimal results. This reliance on herbal medicine has proven to be effective in the treatment of long term illness namely diabetes, malaria and pneumonia where it is seen to have lesser side effects and a cheaper form of medicine and preventive measure against diseases.<sup>3</sup> In spite of the great advances observed in modern medicine in recent decades, plants still make an important contribution to health care.<sup>4</sup>

Antimicrobial screening of plant extracts and phytochemicals represents a starting point for antimicrobial drug discovery. Phytochemicals studies have attracted the attention of plant scientists due to the development of new and sophisticated techniques. These techniques played a significant role in the search for additional resources of raw material for pharmaceutical industry.<sup>5</sup> The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. Many plants have been used because of

their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant.<sup>6</sup>

The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phyto-medicine to act against microbes; as a result, plants are one of the bedrocks for modern medicine to attain new principles.<sup>7</sup> Plant based antimicrobials represent a vast untapped source of medicine. Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. Further continued exploration of plant derived antimicrobials is needed today.<sup>8</sup>

The medicinal plants around the world contain many compounds with antibacterial activity. Many efforts have been made to discover new antimicrobial compounds from various sources such as microorganisms, animals, and plants. Systematic screening of them may result in the discovery of novel effective antimicrobial compounds.<sup>9</sup> The use of botanical medicines is generally on the rise in many parts of the world. The screening of plant extracts and plant products for antimicrobial activity has shown that plants represent a potential source of new anti-infective agents.<sup>10</sup> Numerous experiments have been carried out to screen natural products for antimicrobial property.<sup>11</sup>

Considering the above, it can be stated that

plants are valuable sources for new compounds and should receive special attention in research strategies to develop new antimicrobials urgently required in the near future. In the present study, methanolic extracts of *Pimpenella stewartii* were screened for antimicrobial activity against selected human pathogenic strains. *Pimpenella stewartii* is a member of family Apiaceae. According to the taxonomic features this plant is found in almost all areas of Hazara division and its fruits are used as carminative and for treatment of other stomach diseases.<sup>12</sup> *Pimpenella anisum* L. have been tested already for antimicrobial activities.<sup>13</sup>

## MATERIAL AND METHODS

The botanicals of *Pimpenella stewartii* were used in the study. The verification of the plant collected from different locations of Hazara region during March to August 2013 was done by the Botanist at Hazara University, Pakistan. The plant is registered in Hazara University Herbarium with Voucher No. HU-5293. The seeds of the authenticated herbal plant were collected and air-dried for two weeks to obtain constant weight. The dried sample was cut into smaller pieces and ground into fine particles with a grinder at the Department of Biochemistry, Hazara University, Mansehra. The powdered sample was bagged in black plastic bags and stored in an air-tight container for further work.

A sample of the powdered seeds weighing 30 g was extracted with 160 ml of methanol. The extraction was carried out in 500 ml flask. Four extractions were done in organic solvent used and the extracts were concentrated to about one-sixth of the original volume at 60°C under reduced pressure using a rotary evaporator. The extracts were air-dried for three weeks to a constant weight and kept in air-tight containers for further work.<sup>14</sup>

Five microbial strains including three fungal species and two bacterial species were tested for the efficacy of plant materials. The fungal species were *Aspergillus flavus*, *Aspergillus niger* and *Alternaria alternata* while bacterial species include *Arvenia caratovora* and *Xanthomans* spp.

Nutrient agar (7 g/250ml H<sub>2</sub>O) was used for culturing of bacteria while sabouraud dextrose agar (6 gm/250 ml H<sub>2</sub>O) was used for fungal strains. Both media were suspended in 250 ml of distilled water in a 1L flask, stirred, boiled to dissolve and then autoclaved for 15 minutes at 121 °C. The pH of the nutrient agar was set between 7.0 and 8.0.

Streptomycin was selected for Positive Control and 2% DMSO Aqueous solution was used for Negative Control as the reference for all bacterial species used. In case of fungal strains DithenM45 used as Positive Control and 2% DMSO Aqueous

solution as Negative Control. The control experiment consists of a plate of solidifying agar onto which was inoculated pure solvent with microorganism mixed in a 1:1 portion.

Bioassay tests were performed on seed extracts of *Pimpenella stewartii* to ascertain their activity against selected bacterial and fungal strains. In the test tube, 20 ml nutrient agar was melted at 100 °C and stabilized at 45 °C for about 15 minutes. About 0.1 ml inoculums were added from culture tubes to the agar in the test tube by the use of a loop. The test tube containing the agar and the inoculums was then rolled in between the palms gently to mix the inoculums thoroughly with the agar. The loop was flamed before it was used each time.

The content of the test tube was poured into a Petri dish and allowed to set. The Petri dishes were then labelled with the respective organism (inoculums) and date. By means of a 6 mm cork borer, four cups were bored, well separated and equidistant from each other in the agar. The cups were labelled with the four crude extracts. Each cup was filled with its corresponding concentration of extract to about three-quarters full. They were kept on a bench at room temperature for about 60 minutes (for the extracts to diffuse into the agar). The plates were then incubated aerobically at 37 °C and examined for any zone of inhibition after 24 hours. The same procedure was repeated with the references using the chosen antibiotics. The reading was done against a dark background under reflected light. The diameters of the zones of growth of inhibition were measured with the help of Hi Antibiotic zone scale (range 1–35 cm or 10–400 mm) from the underside of the covered plates for spots with inhibitions. The average of the diameters was taken. The actual zones were calculated by subtracting the diameter of the cups (6 mm) from the total zone of growth.

The antimicrobial activities of different concentrations against selected microbes were obtained by measuring the diameters of the inhibition zones and compared them with that of the control drugs. Antibacterial activity was expressed as the mean zone of inhibition diameters (mm) produced by the herb extracts.

Results obtained in this study were expressed as mean inhibition zone (mm)±S.D of three replicates. The mean and the S.D of each herbal extract were used to compute the calculated t-value. Differences between the critical t-value and calculated t-values of the diameter of the inhibition zones of the herbal extracts on selected microbes at  $p=0.05$ .

## RESULTS

Results obtained for Methanolic extracts of *Pimpenella*

*stewartii* against *Aspergillus flavus*, *Aspergillus niger* and *Alternaria alternata* showed significant antifungal activity. The comparative measurement of zones of inhibition revealed that all prepared concentrations have positive antifungal activity. A well-marked variation in zones of inhibition was observed for different concentrations used against all fungal strains. The *Aspergillus niger* showed maximum zone of inhibition (98.67±3 mm) followed by *Aspergillus flavus* (90±4 mm) and *Alternaria alternata* (80.5±1 mm) at a concentration of 250 ppm while at a concentration of 500 ppm *Aspergillus flavus* showed maximum zone of inhibition (48.7±3mm) followed by *Alternaria alternata* (45.75±3 mm) while *Aspergillus niger* was with lowest zone of inhibition (38.67±0.9 mm). It was also observed that *Alternaria alternata* again showed least zone of inhibition at concentration of 1000 ppm (Table-1).

The results of present investigation clearly indicate that zone of inhibition reduced with an increase in concentration of plant extract which means an inverse correlation between zone of inhibition and concentration. The DMRT analysis of zone of inhibition showed a significant correlation among all variables as shown in table-1. The antibacterial activity of Methanolic extracts of *Pimpinella stewartii* was evaluated against two bacterial species, i.e., *Arvenia caratovora* and *Xanthomans* spp. in triplicate with three concentrations (250, 500 and 1000 ppm). The mean results of triplicate experiment are given in table-2. The best zone of inhibition was found against *Xanthomans* spp (97.33±2 mm) at 250 ppm concentration followed by *Arvenia caratovora* (49.7±14 mm) at same concentration. In present investigation it was noted that *Xanthomans* spp. is more sensitive as compared to *Arvenia caratovora* (Table-2). DMRT analysis of variables revealed a positive and significant correlation.

Indigenous use of medicinal plant preparation mainly utilizes water for extraction procedures. Researchers, however, often focus on organic solvents for laboratory testing. One needs to always consider the traditional mode of administration when validating efficacy. Where the plants are directly applied to inhibit microbial population, alternate methods should be considered for assaying efficacy.

**Table-1: Antifungal activity of Methanolic extract of *Pimpinella stewartii***

Plant Extract (ppm)	<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>	<i>Alternaria alternata</i>
1000	26±3a	32.67±1b	15.25±3b
500	48.7±3c	38.67±0.9b	45.75±3c
250	90±4b	98.67±3c	80.5±1a
Dithen M45	7±1d	11.67±1a	12.5±3a
2% DMSO Aqueous	103±1d	102.7±4a	86±5d

Zone of Inhibition (mm)±SEM, DithenM45 as Positive Control and 2% DMSO Aqueous as Negative Control. MRT at  $p=0.05$  is significant among variables

**Table-2: Antibacterial activity of methanolic extract of *Pimpinella stewartii***

Sample (ppm)	<i>Arvenia caratovora</i>	<i>Xanthomans</i>
1000	11.67±3a	31±2b
500	26.33±7b	32±1b
250	49.7±14c	97.33±2c
Streptomycin	2.33±1a	2±0.5a
2% DMSO Aqueous	94±6d	127.33±3d

Zone of Inhibition (mm)±SEM, Streptomycin as Positive Control and 2% DMSO Aqueous as Negative Control. DMRT at  $p=0.05$  is significant among variables.

## DISCUSSION

The result of this study showed that *Pimpinella stewartii* extracts have varied antimicrobial activities against the tested organisms. This suggests that the extracts of these plants are broad spectrum in their activities. This correlates with the observation of previous workers that plants contain substances that are antimicrobial (Olukoya, 1986). Extracts from the dried powder of different botanicals of *Pimpinella stewartii* showed active inhibition of selected microbes. Present investigation was carried out to evaluate the in vitro efficacy of different concentrations of methanolic extracts of *Pimpinella stewartii*. Efficacy of plant materials against different fungal strains revealed positive results with all concentrations. The inhibition pattern of different concentrations was very obvious not only within species but also with lowest concentrations. The same pattern of inhibition was also found in many previous studies conducted with different plant extracts against fungal species (Ellof, 1998; Adesanya, 2005). In case of antibacterial evaluation it has been observed that the similar pattern was found. The plant extract with 250 ppm concentration was found more active than high concentration.

The results of present investigation coincide with the findings of many previous studies in terms of inhibitions and concentrations. In vitro Antibacterial activity of *Pimpinella anisum* fruit extracts against some pathogenic bacteria was conducted by Akhtar *et al.* in India and they revealed the inhibition of bacterial strains with the same pattern. The results of present investigation clearly indicated that the efficacy of plant extracts of *Pimpinella stewartii* is variable against selected fungal and bacterial strains but whereas concentrations are concerned it has the similar pattern of action. This study does not only show the scientific basis for some of the therapeutic uses of this plant in traditional medicine, but also confirms the fact that ethno botanical approach should be considered when investigating antimicrobial properties of plants. The implication of the broad spectrum action of some of these extracts is that they can be useful in antiseptic and disinfectant formulation as well as in chemotherapy if the active principle can be isolated.

## CONCLUSION

The methanolic extracts of *Pimpinella stewartii* have antimicrobial properties. The antimicrobial activity profiles of all prepared concentrations, against selected microbes were significantly correlated with inverse proportion by applying DMRT. It was also observed that different concentrations showed different sensitivity level for selected strains without a uniform pattern of zones of inhibition.

## AUTHORS' CONTRIBUTION

The present investigation is a part of Doctoral research work of Principal Author FG and reflects little outcomes of investigation on very specific aspects from a broad research work. MH Supervised the entire research work and contributed his expertise and experience being the Dean of faculty of Health Sciences, Hazara University, Mansehra.

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