



Antibacterial Activity of Different Extracts of Medicinal Plant *Antirrhinum orontium*

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Abstract

Antirrhinum Orontium is belonging to the family Plantaginacea commonly known as lesser snapdragon which is a flowering plant belongs to genus *Antirrhinum*. It was traditionally used in treatment of Tumor, Eye inflammation and Ulcer. *Antirrhinum Orontium* contains mostly tertiary alkaloids bases one of them is identified as 4-methyl-2, 6-naphthyridine and Choline is identified as quaternary base. The recent study is to investigate the antibacterial activity of Ethyl acetate, Dichloromethane, Hexane and Water extracts of *Antirrhinum Orontium*, against the pathogen like *Escherichia coli* and *Staphylococcus aureus*. Both the Soxhlet and Maceration water extract were found more active from the other extract against *Escherichia coli* and *Staphylococcus aureus* by using agar well diffusion method. The measured zone of inhibition for Soxhlet water extract is 21 ± 0.05 mm and Maceration water extract is 20 ± 0.05 mm against gram positive *Staphylococcus aureus*, while for gram negative *Escherichia coli* 23 ± 0.05 mm is measured for Soxlet and 21 ± 0.05 mm measured for Maceration water extracts.

Keywords: *Antirrhinum Orontium*, Antibacterial, Plantaginaceae, *Escherichia coli*, *Staphylococcus aureus*

Submitted: 08 October 2019

Accepted: 16 December 2019

DOI: <https://doi.org/10.25026/jtpc.v5i2.221>

■ Introduction

The word herb is derived from Latin's word "herba", which means all non-woody plant.

Medicinal plants are also known as herbs, which have been used in traditional medicine practices since earliest times[1]. Herbal medicine is the oldest method of medicine known to mankind.

Each population in the world developed its own herbal medicinal knowledge and experience, advanced studies showed that plants are an important source of secondary metabolites, which are used as pharmaceuticals, agrochemicals, flavor, pesticides and other food additives. Plants prepare hundreds of chemicals compounds for function including defense against insects, fungi and other diseases [2]. These plants are either "wild plant species" those growing spontaneously in natural ecosystem and could exist independently of human activities or the differentiating "domesticated plants species" those that have emerged through human activity such as breeding and depend on management for their existence [3]. For large world's population medicinal plants continue to show important role in the health care system. The herbal medicine has a continuous history of long use, these plants act as important curing agent which gives a variety number of modern medicine. The history tells us that ancient peoples used these medicinal plants for treatment of different chronic illness, wounds, injuries and terminal illness. Since long long ago, plants with curing properties have secured an important role in treatment of diseases. Now days in advance countries, traditional medicine is still used for health care and most of the drugs are obtained from natural resources such as plants. The world's large population accepts herbal medicines because they are nontoxic and have no side effects. In case any adverse effect occurs they are much lesser than caused by chemically synthesized medicines. All over the world three quarter of population use plants and plant extract for their health care. More than 30% plants species are used for medicinal purposes. The ancient people believed in the herbal medicine. These herbal medicines provide treatment of numerous internal diseases, which are hard to cure. These plants would be used as a source of medicine and nutrients apart from these using as food, natural dye, pest control and perfumes. It has been recorded that about 450 to 500 plants growing in Bangladesh have curing value, which are used in different modern medicine [4]. Recently WHO (world health organization) estimated 70% people use herbal medicinal for primary health care, and some 3500 to 7000 species have been used as medicine. It is estimated that 250,000 plants species on the earth are present, in which 35-70% of all species are

used worldwide. Native America used 2564 medicinal plants. According to ethno botanist Daneil Moerman, Native America used about 9% of plants for medicine and majority of plants still remain unknown [5]. About 500 plants species with medicinal uses are mentioned in former books and 800 plants have been used in local system of medicine. 80,000 types of plants are being used throughout the world. But more than 500 conventional communities use 800 plants species for treatment of different diseases [6]. The herbal medicine use is wide spread in Bangladesh, Pakistan, India, China and Japan. Pakistan has centuries old knowledge about traditional uses of plants occurring in different areas. These plants are used to treat almost much kind of diseases like Headache, Stomach problem and wounds etc. About 500 species of flowering plants being used as medicine [7]. Now a day's herbal medicine is still commonly used in China. More than half population uses regularly herbal medicines which are highly common in rural areas. About 500 herbal medicines are available in china; they account for approximately whole Chinese Pharmaceutical market [8].

Antirrhinum Orontium is a herbaceous annual plant of the family Plantaginaceae (earlier Scrophulariaceae), native distribute in Europe [9]. Plant height is 10–60 cm, leaves size 15–40 mm long × 1.2–6 mm wide, corolla color pink, seed size 0.9–1.1 mm and widely distributed plant [10]. Flowering period is spring and grow in Summer and dormant in Winter seasons[11]. *Antirrhinum Orontium* is widely distributed in world, four species studied from Asia, three from European forms. The local species are not only morphological different from European counter parts but also genetically different [12]. It is herbaceous plant which is used as fodder [13] and it is traditionally used for medicinal purposes specially seeds used for treatment of Ulcer, eye inflammations Tumor and as a good Stimulant [14, 15]. *Antirrhinum Orontium* found to contain seven tertiary alkaloids bases one recognized as 4-methyl-2,6-naphthyridine and second has been assigned the structure $C_{15}H_9N_3O$ [16] and also Choline has been identified as quaternary base [17].

■ Experimental

Plant collection

The entire plant was gathered from Madahella; a village in tehsil Razzar; district Swabi; KP, Pakistan in the month of March 2019.

Identification

Mr Miraj Ali (lecturer Govt: Degree College Shewa Swabi KP) and Mr Muhammad Israr (lecturer Department of Botany Govt: Post Graduate College Mardan KP) identified and characterized the plant to be *Antirrhinum Orontium*.

Grinding and Drying

The whole plant is clean and washed with tap water three times and kept for one week at room temperature for drying. The dried plant material (root, stem and leaves) were crushed into fine powder.

Extraction

The extraction is the separation of active parts of plants or animal tissues utilizing specific solvents through standard methodology. Ethanol was utilized as solvent which was discovered simpler to enter the cell membrane to separate intracellular elements for the plant materials. The extract obtained from the plant is complex mixture metabolites. The extraction is performed by two different methods.

Maceration:

This method is the oldest and non-heat method used. The plant powder 40g is soaked in 350ml ethanol placed in close vessel; allowed for 12 days with daily shaking at room temperature.

Soxhlet:

In this method heat and Soxhlet apparatus are used for extraction. Plant ingredients 15g are placed in "thimble"(made up of filter paper) and thimble is loaded into the main chamber of Soxhlet extractor. The Soxhlet extractor placed at top of flask. Condenser ensures the vapor cool and drips back to extractor chamber on plant ingredients. The condenser fits at the top of extractor chamber. The extractor chamber slowly fills with warm solvent, the desired compounds are

dissolved in warm solvent. The resulted extracts from Maceration and Soxhlet crude extract are divided into various fractions of distilled water, ethyl acetate, DCM and N-hexane by using liquid-liquid extraction.

Test Organisms

For Antibacterial activity gram negative *Escherichia coli* bacteria and gram positive *Staphylococcus aureus* bacteria was used. Nitrofurantoin, Cefoperazone, Moxifloxacin and Linezolid antibiotics were used as standard, while dimethyl sulphoxide (DMSO) was used as negative control against bacteria. All the bacterial strains were obtained from the Micro lab Bacha Khan Medical College Mardan, KP, Pakistan.

Antibacterial assay

The effect of plant extract was examined by various bacterial strains by Agar well diffusion method. Nutrient Agar is utilized as culture media. Nutrient Agar medium was prepared by dissolving 35 g of available medium in 1000 ml of distilled water. The medium was autoclaved at 15 lbs pressure at 121°C for 15 min. The autoclaved medium was blended well and poured onto 100mm petridishes (20-25 ml) in liquid state. Four wells were made in those plates at required distance, with the diameter of 7mm. With help of micropipette 30µL of DMSO soluble extract was utilized in well of each petridishes. The plates were kept for incubation at 37°C for 24 h. At the end of incubation, inhibition zones estimated with straight forward ruler in mm. The investigations were repeated three times.

Statistical calculation

The zone of inhibition is measured as Mean \pm SEM for each fraction shown in **Error! Reference source not found.** and Table.2..

■ Results and Discussion

In the present investigation, different extracts of *A. Orontium* was evaluated for exploration of their antibacterial activity against Gram negative and Gram positive bacteria which was regarded as human pathogenic microorganism. Our investigations show that water extracts of both Soxhlet and Maceration show bitter activity against the human pathogenic living organisms.

Water extracts of plants shows significant activity against *Staphylococcus aureus* and *Escherichia coli*. [18, 19] The various extract Antibacterial result of *Antirrhinum Orontium* is appeared in **Error! Reference source not found.**, Table.2, **Error! Reference source not found.** and **Error! Reference source not found.** and **Error! Reference source not found.**. Among all those extracts the Soxhlet water extract fraction is more active against the gram negative pathogen *Escherichia coli*, which have maximum zone of inhibition 23 ± 0.05 mm as compare to standard Antibiotics Nitrofurantoin and Cefoperazone, while zone of inhibition for gram positive pathogen *Staphylococcus aureus* is 21 ± 0.05 mm shown in **Error! Reference source not found.** and **Error! Reference source not found.**. Maceration water extract measured zone of inhibition 21 ± 0.05 mm against the gram negative pathogen *Escherichia coli* showing parallel result with standard Nitrofurantoin and as an intermediate to Cefoperazone, while zone of inhibition for gram positive pathogen *Staphylococcus aureus* is 20 ± 0.05 mm shown in Table.2 and **Error! Reference source not found.**.

Table.1. Inhibited zone measured (in mm) for different Soxhlet extracts against bacteria *Escherichia coli* and *Staphylococcus aureus*

Fractions	Concentration (μL)	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
Dichloromethane	30	7.33±0.12	7.2±0.12
Ethylacetate	30	7.4±0.11	7.4±0.08
Hexane	30	7.13±0.08	7.4±0.12
Water	30	23±0.05	21±0.05
Nitrofurantoin		21	-----
Cefoperazone		22	-----
Linezolid		-----	23
Moxifloxacin		-----	24

Table.2. Inhibited zone measured (in mm) for different Maceration extracts against bacteria *Escherichia coli* and *Staphylococcus aureus*.

Fractions	Concentration (μL)	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
Dichloromethane	30	7.4±0.11	7.2±0.08
Ethylacetate	30	7.33±0.12	7.6±0.08
Hexane	30	7.43±0.18	7.4±0.05
Water	30	21±0.05	20±0.05
Nitrofurantoin		21	-----
Cefoperazone		22	-----
Linezolid		-----	23
Moxifloxacin		-----	24

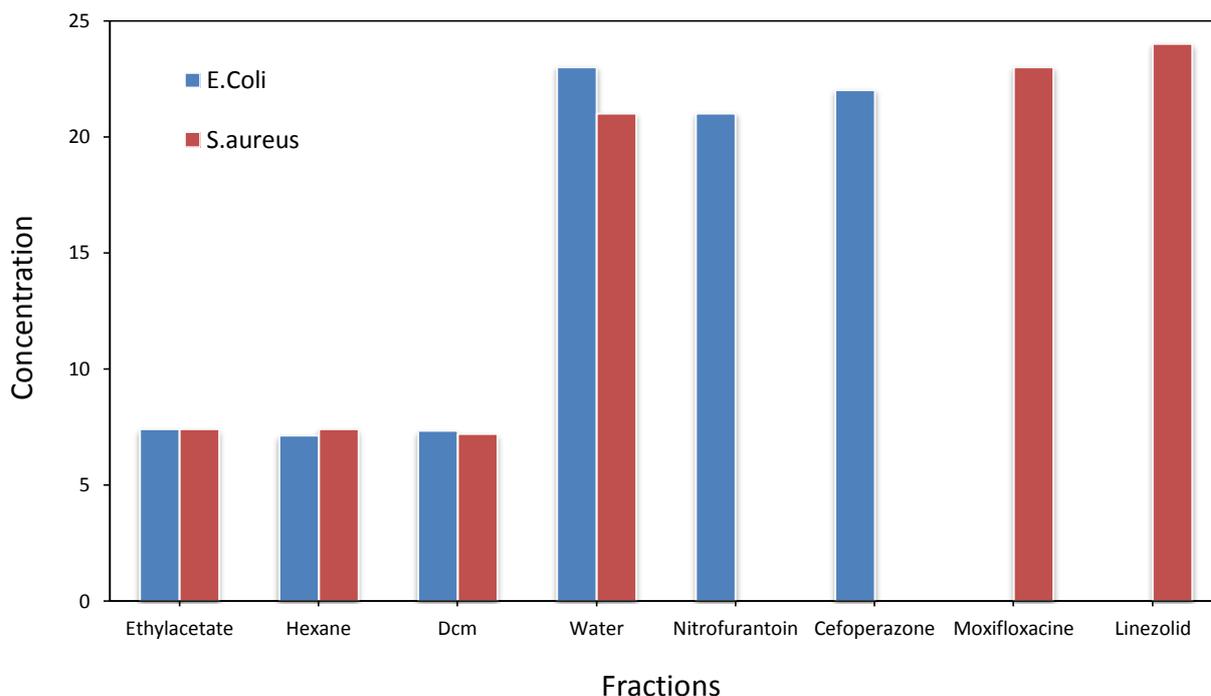


Figure.1 Antibacterial activity of different Soxhlet extracts of *Antirrhinum Orontium*.

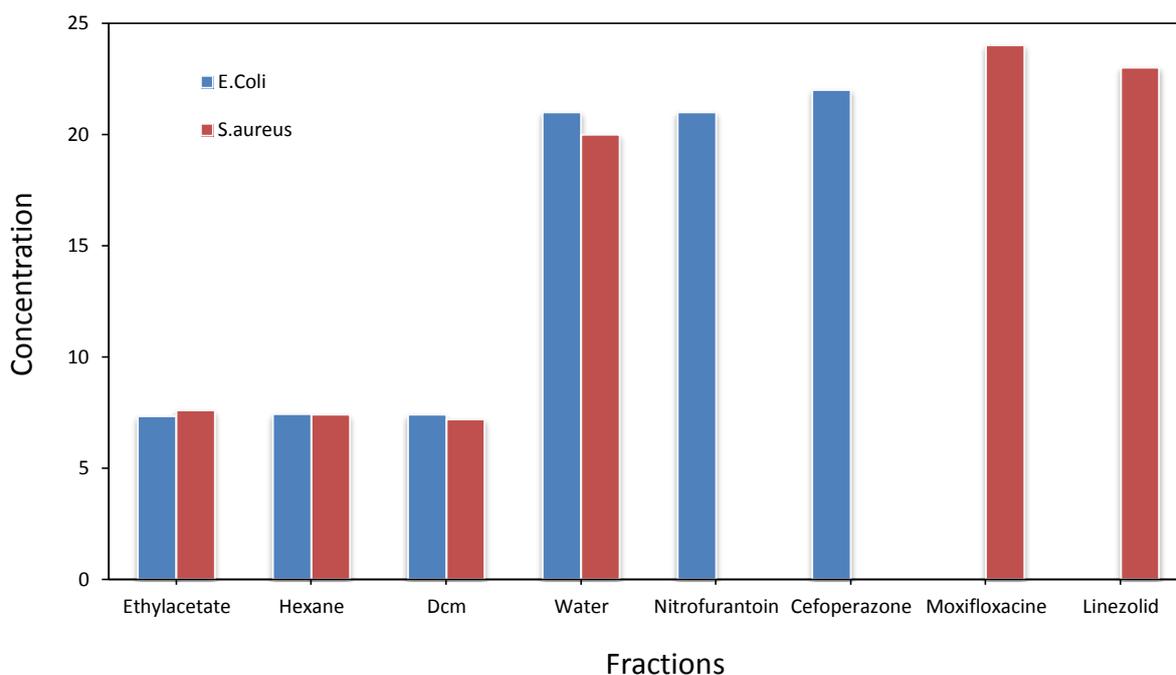


Figure.2- Antibacterial activity of different Maceration extracts of *Antirrhinum Orontium*

■ Conclusion

From the antibacterial activity result, it is concluded from several fractions water extracts of both Soxhlet and Maceration show activity against pathogen *Staphylococcus aureus* and *Escherichia coli*, but among those extracts the water extract of Soxhlet shows greater zone of inhibition than standards Nitrofurantoin and Cefoperazone. The water extracts of both Soxhlet and Maceration should contain some active compounds which are active against gram positive and gram negative bacteria. From this work *Antirrhinum Orontium* water extracts is more effective and can be used for the future against pathogens.

■ Acknowledgments

Our special thanks to Mr Syed Akif Shah Musarrat microbiologist, Mr Asif Ali and Mr Miraj Ali for providing basic equipment and for giving such a fruitful advices which helped us in our research. We are gratefully acknowledging the various suggestions gives by our class fellows and other friends on various occasions.

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