



Original Research Article

Evaluation of Qualitative Analysis and Antibacterial Activity of *Pelargonium graveolens* L'Herit.

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Abstract

Medicinal plants are an important source of phytochemicals that offer traditional medicinal treatment of various ailments and one of the plants is *Pelargonium Graveolens* which was grown in Hyderabad province. The preliminary screening of their aerial leaves showed best results the presence of different phytochemical like alkaloids, flavonoids, glycosides, phenol, sterol and lignin found in Methanolic and Ethyl acetate extracts. However Chloroform extract revealed the absence of alkaloids and sterols, where as in Water extract flavonoids, phenol, sterol and lignin. The best resulted extracts from preliminary screening test were subjected to Antimicrobial studies on gram positive and gram negative bacterial strains which exhibited a significant effect. The both ethyl acetate and methanolic extracts were shown more active suppression *gram positive* bacteria (*S.aurea* and *B.Subtilus*) than the zone of inhibition on *gram negative* bacteria (*k. pneumonia* and *E.coli*) in comparison with the standard antibiotic.

Keywords: Hyderabad, chloroform, methanolic, ethyl acetate, water and antibacterial test.

Introduction

Medicinal plants are effective sources of phytochemicals and which are non –nutritive plant chemicals that have protective therapeutic properties. These phytochemical not only protect themselves and also against the deadliest diseases in the animal and plant systems. Medicinal herbs are rich source of natural products for antimicrobial and antioxidant assets. The phytochemicals are categorising into two different groups based on the function in plant absorption. The primary metabolites are grouped into a amino acids, carbohydrates and proteins and the secondary metabolites are well known phytochemicals which are protective functions against living organisms like: alkaloids, flavonoids, glycosides, tannins, and terpenoids, anthraquinones and steroids [1, 2].

Geranium is well known medicinal plant of African which has been used in the derived form of Essential oil(s) for cure various ailments like the balancing effects of anxiety, worry, high blood pressure constipation, insomnia, restlessness, nervousness, anger, weight loss [3]. According to the previous literature studies, the available data retrieved on Geranium oil belonging to the genus *pelargonium* are noted for their good anti-bacterial activity such as *P. glutinosum*, *P.pseudoglutinosum*, *P.scabrum*, *P.sublignosum*, *P. denticulatum* and *P. ficifoliu* [4]. The important geranium species which has been so far work out on essential oil such mainly *P.reniforme* and *P.graveolens* and extracts of roots and leaves which shown the significant antimicrobial activity, antifungal, antimalarial, antiemetic

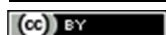
activity, anthelmintic activity [5,6,7,8]. The phytochemical constituents are playing a significant role in the identification of crude drugs and there is widespread interest in evaluating drugs derived from plant sources. The main objective of the present study is to investigate the presence of various phytochemicals screening and antimicrobial activity in *Pelargonium graveolens* by organic solvents.

Plant material

The matured leaves of *pelargonium graveolens* was collected from Central Institute of Medicinal and Aromatic Plants (CIMAP) in Hyderabad, August 2014. Authenticated by Dr. Shashikanth, Taxonomist, Department of Botany, Osmania University, Hyderabad-7. Plant materials were used for extraction and identification of active compounds from analytical grades solvents like Methanol, Ethyl acetate, Chloroform and Water.

Preparation of Crude Extracts

Extraction: *Pelargonium graveolens* was grown and maintained Hyderabad climatic conditions in CIMAP and leaves were collected and used for experimental analysis. The plant material *Pelargonium graveolens* was shade dried or in an oven at reduced temperature (< 60°C) to make suitable for grinding. 1g of powdered plant material was submerged in (50ml) sufficient volume of Methanol and Ethyl acetate, Chloroform and Water in an air-tight flat bottomed container for three days, at room temp with occasional shaking and stirring.



The organic phases were then filtered and rotatory evaporated and lyophilized for experimental analysis.

Preliminary Phytochemical Screening Test of Plant Extracts

The preliminary phytochemical screening of *Pelargonium graveolens* was carried out for the identification of various Phytochemicals using according standard procedures [9]. The following solvents were used for the analytical study such as water, ethyl acetate, chloroform and methanol. In this process, the extracts were filtered and used for preliminary phytochemical screening for alkaloids (Iodine, Wagner, and Dragendorff's test), flavonoids (Pew's, Shinoda and NaOH tests), glycosides (Keller-killani, Conc. H₂SO₄, and Molisch tests), tannins (Lead acetate test), Lignin (Labat), Phenols (Ellagic acid and Phenol tests) were carried out [10] results are framed up in table 1.

Test Microorganisms

Antibacterial activity of *pelargonium graveolens* was carried out according the best resulted preliminary work from the organic extracts and studied was done further by using four Lyophilized microorganisms (MOs) *E.coli*, *S.aureas*, *K.pneumoia* and *B.Subtilis* were grown on nutrient broth at 30°C and were subcultured and maintained in nutrient broth at 40C.

Evaluation of Antibacterial activity

The antimicrobial activity analysis was determined by an in vitro screening method such as Disc Diffusion method. Briefly, 100 µl of the test culture bacteria were grown in 10 ml of fresh media until they reached a turbid growth of approximately 10⁵ cells/ml. 50 µl of the microbial suspension was spread onto nutrient agar plate.

Organic extracts are selected from the results obtained (table 1) from the preliminary analysis. The best two Ethyl acetate and Methanol organic extracts were used for bacterial activity. The test sample (1µg/ul) by dilution in DMSO for bacterial activity and by using 6 mm sterilised filter paper discs were used and placed on the overgrown nutrient agar plates the test samples with three different volumes of 10 ul, 15µl and 20ul and plates were incubation at 37°C for 24 hrs. Negative controls were 20µl of DMSO. The concentration of microbial suspension was determined spectrophotometric ally 580 nm; sterile paper discs (6 mm) and Amoxicillin (25 µg/disc) used as a positive control to determine the sensitivity of microbial strains and after pre-incubation (60 min at 4°C), infected plates were incubated at 37°C for 24 h. Antimicrobial activity was assessed by measuring the inhibition zone on the surface of plates and the results were reported as Mean ± SD after three repeats

Results and Discussion

The present study evaluated about the preliminary phytochemical screening of *pelargonium* extract and its individual extracts were subjected to the qualitative analysis for the presence of chemical constituents.

Table 1: Preliminary qualitative phytochemical screening of organic solvent of *pelargonium graveolens*.

S.No	Plant constituents	Water	Ethyl acetate	Chloroform	Methanol
1	Alkaloids:				
	Iodine Test	+	-	-	-
	Wagner's Test	+	+	-	+
2	Flavonoid's				
	Pew's Test	-	+	+	+
	NaoH Test	-	+	-	+
3	Glycosides				
	Keller kiliani Test	+	+	-	+
	Conc.H ₂ So ₄	+	+	+	+
	Molisch's Test	-	+	+	-
4	Lignins				
	Labat Test	-	-	+	-
5	Phenols				
	Ellagic acid test	-	+	+	+
6	Sterol				
	Salkowski test	-	+	-	+
7	Tannins				
	Lead-acetate	-	+	+	+

(+indicates positive test results; - indicates negative test results)



Table2:-Antimicrobial activity of *Pelargonium graveolens* in selective organic extracts.

Bacterial strains	Ethyl acetate extract Diameter of inhibitory zone				Methanolic extract Diameter of inhibitory zone			
	10 ul	15ul	20ul	+ve DMSO	10 ul	15ul	20ul	+ve DMSO
<i>E.coli</i> (-gram)	5mm	9mm	10mm	5mm	3mm	6mm	10mm	1mm
<i>S.aureas</i> (+gram)	4mm	8mm	7mm	--	3mm	8mm	8mm	-
<i>K. pneumonia</i> (-gram)	4mm	8mm	9mm	1mm	3mm	8mm	12mm	2mm
<i>B.subtilus</i> (+gram)	8mm	5mm	9mm	--	3mm	8mm	10mm	--

However; all these compounds were not extractable in the one solvent. Alkaloids and Glycoside were present in the Water extract. Alkaloids, Glycosides, Flavonoids, Phenols and Tannins were found in the Ethyl acetate and Methanolic extract. Where as, Chloroform extracts showed presences of Glycosides, Flavonoids and Phenols. The Methanolic and Ethyl acetate extract were found to be containing more flavonoids and Phenols(table1).

Earlier work was reported on preliminary screening on various species with different methodology done by the co-workers. The present experimental research was carried on the bacterial strains based on the preliminary analysis data (table1). The screened organic extracts were compared to the presences phytochemicals (table1) involved in the suppression bacterial growth and to justify the best crude activity. We concluded the result obtained from the anti-bacterial activity of two different organic extracts of *Pelargonium graveolens* were exhibited varied growth zone of inhibitions (table2). Ethyl acetate extract were showing the Zone of inhibition (*S.aurea* 7mm and 9mm *B.Subtilus*) and Methanolic extract (*S.aurea* 8mm and 10mm *B.Subtilus*) 20ul of test sample on Gram positive bacteria. Whereas, Ethylacetate extract revealed positive inhibition on (*k.pneumonia* 8mm and *E.coli* 5mm) for 20ul of sample. When compare to methanolic extract which was more active suppression on Gram negative bacterial (*E.coli* 9mm and *K.pneumonia* 10mm). the significant anti-bacterial effect were examined on Gram positive and Gram negative bacteria under investigation in comparison with the standard antibiotic. The current research work showing the justification of best organic solvent extraction for species

pelargonium for the identification active phytoconstituents and antimicrobial activity which is required in the medicinal plant research for drug development.

Conclusion

Qualitative analysis of *Pelargonium graveolens* studies showing results of the presence of Alkaloids, Glycosides, Flavonoids and Phenol were more in the Ethyl acetate extract as well as in Methanolic extract with comparison of other organic solvents which in turn showed the best antimicrobial activity on Gram positive than the Gram negative bacteria.

Further studies are needed in this specie to isolate, characterize and elucidate the structure of the bioactive compounds which are responsible for the antimicrobial activity and other medicinal value.

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