



Organic Extracts of *Pelargonium graveolens*: Phenol Content, Anti-oxidant and Anti-bacterial Activities

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Authors' contributions

This work was carried out in collaboration between all authors. All authors had equal contribution in preparing the article. All authors have read and approved the final manuscript.

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ABSTRACT

Aims: The aim of this study was determination of antioxidant activity and total phenolic content and antibacterial activity of *Pelargonium graveolens* organic extracts.

Study Design: Different methods implemented for isolation and analysing of organic *P. graveolens*.

Place and Duration of Study: Department of Genetics, Osmania University, Hyderabad, India, between March 2012 to December 2014.

Methodology: The aerial parts were extracted by solvents (ethyl acetate and methanol) by two conventional methods to screen the ideal solvent for isolation of non-volatile extracts and calculate its phenolic content yield, level of anti-oxidant and anti-bacterial activity on bacterial strains.

Results: Our results show the extraction of crude from ethyl acetate and methanol by two conventional methods. Both organic extracts were shown the presence of total phenolic content 53 mg/dry mass. Methanolic maceration extract were showing highest EC₅₀ value (115.528 ug/mL) with low antioxidant level, whereas, Soxhlet process were exhibiting lowest EC₅₀ value (47.666 ug/mL) with higher free radical activity with the standard ascorbic acid. The Methanolic extract were exhibiting best zone of inhibition on *K. pneumonia* (13 mm) than ethyl acetate extract on *E. coli* (12

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mm) was determined at concentration 20 ug/mL. The most significant antioxidant properties were observed in Soxhlet method by negative correlation with total phenolic content.

Conclusion: The results from this study have proven the information on the total phenolic, anti-oxidant level and anti-bacterial activity of organic extracts of *P. graveolens*. Further studies should be performed on other strains to strengthen the research. We conclude on the value of search non-volatile compounds for the medicinal value in the health care system.

Keywords: Extraction; maceration; soxhlet; *Pelargonium graveolens*.

1. INTRODUCTION

Since from long time, the medicinal plants are using in treatment of illness and diseases. The use of such alternative medicines has become increasingly popular in the developing world. The medicinal plants are being using in different preparation of folk medicine in treat of varied infection and curing deadly conditions. According to the World Health Organization, over 80% of the world's population depend on traditional plant-based medicine to deliver them with primary health care. Even now they are economically important and being used in different pharmaceutical industries [1]. The present pharmaceutical drugs which are derived from the medicinal plants have fastening the world marketing in the clinical research to prevent the life threat illnesses. Since from the past decade, the demand of antimicrobial agents derived from the medicinal plants is increasing day by day due to resistant of clinical microbial strains to many antibiotics. The medicinal plants are the source of natural anti-oxidants and as acts good antimicrobial agents. In addition to that, the extraction, classification and utilization of natural antioxidant are extensively performed to find potent compounds in combating the ailments [2,3]. *Pelargonium graveolens* (Rose Geranium) is an erect, much-branched shrub. It is indigenous to various parts of Southern Africa it is often called Geranium [4]. This species has great importance in the perfume industry. *Pelargonium* extracts and its principles, commonly known as "Geranium Essential oil" [5] sold for aroma and massage therapy [6] and it is occasionally used as more expensive rose oils and as natural insect repellent's [7]. The benefits of Geranium Oil attributed to its properties like astringent, homeostatic, diuretic, spray, stimulant, vermiform and diabetic problems [8,9]. literature study limits to essential oil of *Pelargonium* sp, it shown many biological studied as: Anti-microbial, anti-fungal, anti-cancerous, anti-plasmodial and anti-insecticidal [10-12]. According literature report of Boukhris M, et al. supports the idea of pharmacological importance

of organic extraction of *P. graveolens* [13,14]. The most suggested studies are focused on the geographical variation of *pelargonium* species and on its non-volatile compounds for the identification and isolation of phytoconstituents. On other hand, with this evidence our research work was done on preliminary analysis of anti-microbial activity to find out the secondary metabolites present in the various diluents of *P. graveolens*. Consequently, Total phenolic content and anti-oxidant activity were calculated with crude extracts to identify the best quantities to explore the beneficial extract for isolation of crude further biological testing.

2. MATERIALS AND METHODS

2.1 Chemicals

The chemicals 1, 1-diphenyl-2-picrylhydrazyl (DPPH), Ascorbic acid, Quercetin, Gallic acid, Folin-Ciocalteu's Reagent (FCR) were purchased from Sigma Chemical and Merck. Chemicals, solvents and reagents are analytical and HPLC grade.

2.2 Plant Material

The full-fledged vegetation *Pelargonium graveolens* a rose scent geranium grown in Central Institute of Medicinal and Aromatic Plants (CIMAP) Hyderabad, under tropical climate in August 2014. The shrub material was sun-dried and made a suitable fine powder for experimental analysis. The analytical grade solvents methanol and ethyl acetate were used for extracting the crude samples.

2.3 Methods of Plant Extractions

2.3.1 Conventional soxhlet extraction

A ration of dried leaves powder (100 g) of *P. graveolens* was placed in soxhlet apparatus. Extraction was performed with 500 mL of an appropriate solvents (methanol and ethyl

acetate) with increasing polarity for 48 h at a temperature not exceeding the boiling point of the solvent. The crude extracts were filtered through 45 µm filters and concentrate by rotatory vacuum for experimental analysis.

2.3.2 Maceration extraction

The pulverized plant material (10 gm) was immersed in sufficient volume of each 100 mL of methanol and ethyl acetate in an air-tight flat bottomed vessel for three days with uneven shaking and stirring. The organic extracts were then filtered and rotatory evaporator to get final methanol and ethyl acetate crude extract [15,16].

2.4 Determination of Total Phenol content

Total phenol content in the *P. graveolens* leaves extracts was determined by Folin Ciocalteu's reagent method and was calculated in terms of mg/gm GAE (Gallic Acid Equivalent) based on calibration curve standard [17]. The experimental extracts obtained from Maceration and Soxhlet methods were used for preparation of stock solutions and sodium carbonate 20% and Gallic acid 10 mL was made in the 50% aqueous methanol and test sample were made up of 20 µl of 1 mg/mL ethanolic and methanolic extract were added to separately in 630 µL distilled water and 200 µL freshly prepared Folin–Ciocalteu reagent, and incubated in the dark for 5 min and then 150 µL of 20% sodium carbonate solution were added and samples were incubated in the dark for 30 min than the solution turned deep blue [18]. Gallic acid standards of 0.2, 0.4, 0.6, 1 and 2 mg/µL were reacted with the Folin-Ciocalteu reagent and absorbance were taken at 725 nm.

2.5 DPPH Free-Radical Scavenging Assay

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging effect were evaluated following the procedure described in a previous study [19]. Polyphenols are the natural compounds present in the plant system which are the inhibiting substance with the antioxidant activity and play a major role in the absorbing, decomposing the free radicals and neutralizing and as well as quenching [20]. DPPH at various concentrations of extracts were determined and compared with the standard Ascorbic acid. Aliquots (50 µL) of various concentrations (25, 50, 100, 200, 300 µg/mL) are made with methanol and were equalized to 3 mL of

methanolic solution containing DPPH radicals (6×10^{-6} M). After 30 min incubation at room temperature and in the dark, the absorbance was taken in opposition to the control at 517 nm [21]. The proportion of absorbance of DPPH with test extracts was calculated respectively by using the following.

$$\% \text{ Inhibition} = [(AB-AE)/AB] \times 100$$

Whereas AB = absorbance of the control; AE = absorbance of the test sample. The EC₅₀ (the microgram of extract to scavenge 50% Absorbance) values was calculated using linear regression analysis. Higher EC₅₀ values indicate lower effectiveness in antioxidant properties.

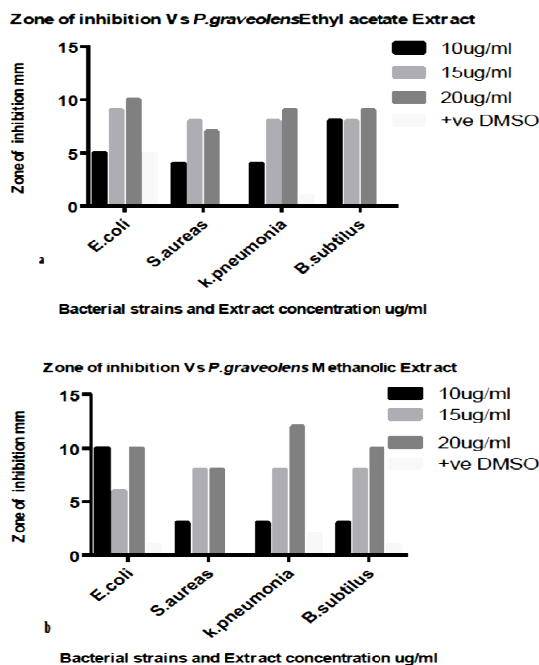


Fig. 1. The graphical bar representations a) and b) were showing antibacterial activity comparisons between the two different organic solvents of *P. graveolens* on gram negative and gram positive bacteria with three varied concentration with the zone of inhibitions mm

2.6 Antibacterial Activity

Antimicrobial activity was tested in both Gram-negative and Gram-positive bacteria and strains obtained from the Department of Microbiology, Osmania University, Telangana, India. The bacterial strains were cultured and maintained by periodic subculture on nutrient agar and

preserved at 4°C prior to use. They were grown overnight in 10 mL broth at 37°C, which was then centrifuged at 150 rpm and followed Disc Diffusion method for identification of anti-bacterial activity. The zones of inhibition were calculated with the reference to the antibiotics resistance on the both bacterial strains.

2.7 Statistical Analysis

Results are expressed as Mean ± SD. The Total Phenolic Content and EC₅₀ of DPPH anti-oxidant activity and one –way ANOVA followed by t test (p< 0.01). The difference mean were determined using the Tukey’s multiple comparison tests and Pearson’s correlation coefficients of *P. graveolens* was calculate regressions analysis by Graph pad prism 6.

3. RESULTS AND DISCUSSION

3.1 Total Phenolic Content

All experimental measurements were carried out triplicates. The results were statistically analysed by using Graph pad prism6 software. Our study

gives the detail comparative analyses of the samples are extracted by the two different organic solvents with two extraction methods.

The preliminary analysis of *P. graveolens* of organic extracts were shown the presence of different phytochemical like alkaloids, flavonoids, glycosides, phenol, sterol and lignin found in both in methanolic and ethyl acetate extracts [22]. Total phenolic content of *P. graveolens* organic extracts were determined using the FC reagent method, and absorbance are taken at 765 nm (Fig. 2). Most similar values were found to be with slight variance with ethyl acetate extract 53.218 ±0.80 (soxhlet) and 53.112±0.03 (maceration) mg/gm GAE for 5gms of dry mass, whereas methanolic extract values are shown in (Table 1) respectively. Linear Regression analysis are calculated with standard Gallic acid (Y= 0.0451x+2.2451) R-square valve 0.9880. Hence, two organic extracts of *P. graveolens* was found to be a significant in the presence of phenolic content and generally which directly relate to the antioxidant activity and consequently correlates to the antimicrobial activity.

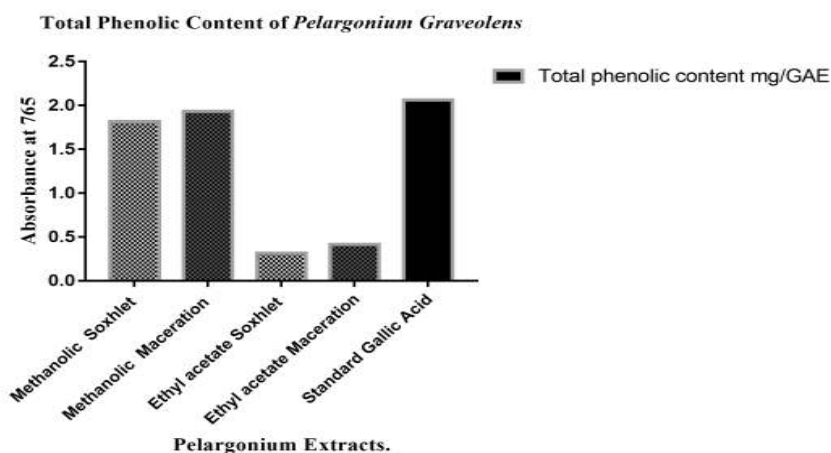


Fig. 2. Schematic representation showing response to the different extracts with two organic solvents of *P. graveolens* with linear regression analysis was done to calculate the total phenolic content Mean±SD in triplicates

Table 1. Non - linear regression analysis of DPPH assay of EC₅₀ with mean ±SD values and total phenolic content values are calculated by graph pad prism6

	Non-linear regression analysis of DPPH radical activity of pelargonium extract			
	Soxhlet extraction		Maceration extraction	
	DPPH EC ₅₀ (ug/ml)	Total phenolic mgGAE/g	DPPH EC ₅₀ (ug/ml)	Total phenolic mgGAE/g
Ethyl acetate	38.17±0.07	53.23±0.80	99.98±0.09	53.11±0.03
Methanol	47.67±1.5	51.83±0.06	115.53±0.03	51.60±0.09
Ascorbic acid	83.49±1.72	--	74.10±1.05	--
Gallic acid	---	125	--	125

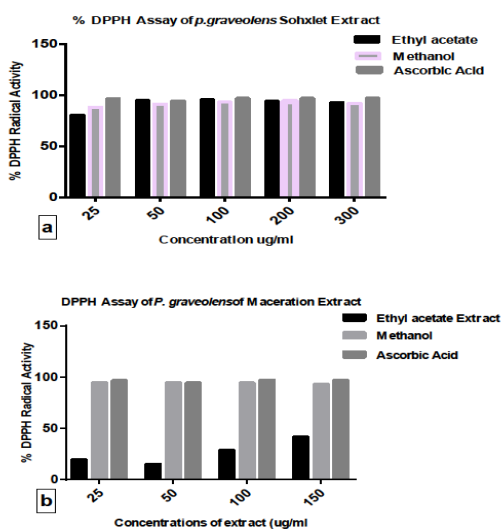


Fig. 3. The graphical bar representation showing between % DPPH radical activity Vs concentration of A) Soxhlet and B) maceration extraction in *P. graveolens*

3.2 Evaluation of DPPH Antioxidant Activity

The Free Radical Anti-oxidant activity of *P. graveolens* was determined by the DPPH assay. The DPPH radical has been used widely to test the potential compounds as free radical hydrogen donors and to investigate the antioxidant of plant extracts. All test samples were showing the best anti-oxidant activity to standard Ascorbic acid (Table 1 and Fig. 3). The extracts (maceration and soxhlet) reduced the concentration of DPPH into light coloured DPPH obtaining the 50% reduction with EC₅₀ value as

follows. The maximum scavenging effect (%) was found in methanolic maceration EC₅₀ (115.528 ug/mL) and least were in ethyl acetate (Fig. 4). However, the statistical significance was calculated by One-way ANOVA Tukey's multiple comparisons test to study the most significant concentrations among ethyl acetate Vs methanolic solvents, significant difference were showing (P<0.001) P value summary (***) and R squared 0.968 and obeying Bartlett's test in Maceration method than Soxhlet. Consequently, regression analysis showing negative correlation between Total phenolic content and antioxidant properties among soxhlet and maceration (Fig. 5) and Pearson correlation (r 0.084) R squared (r² 0.691) and p value was not much significant to EC₅₀ values of anti -oxidant activity of DPPH assay of organic extracts were calculated by Zheng chen et al. [23] and H.J. Motulsky [24]. (Fig. 3 & Fig. 4) Moreover, the previous literature study of *P. graveolens* essential oil was showed the significant results on antioxidant and biological activities [25]. Overview of detail research work of *Maria Dimitrova* study shows that ethanol extract was measured with high antioxidant activity with the negative TPC values in related to acetone and methanolic extract of *Geranium* sp [26]. Generally, DPPH free radical activity is directly relates to the content of phenolic in the extracts but some research studies are also giving the supportive information on negative correlation with antioxidant level, present literature studies clearly defines that they might be some other secondary metabolites playing major role in anti-oxidant activity and we are in search of non-volatile compounds medicinal activity in aromatic plants.

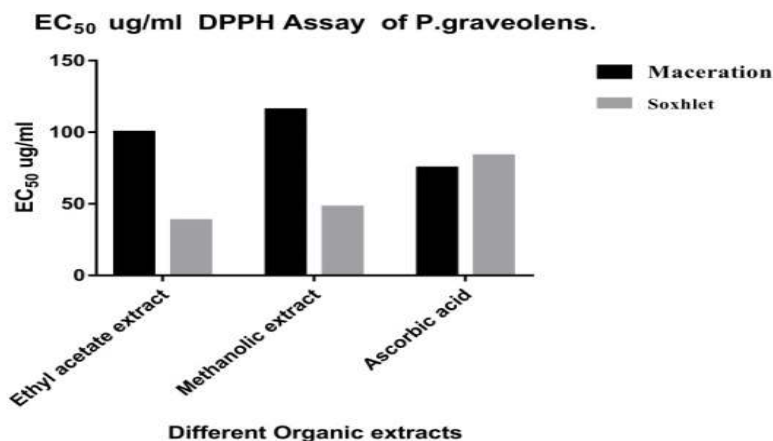


Fig. 4. Illustration showing the Ec50 values of DPPH assay of *P. graveolens* in maceration and soxhlet extractions in two organic solvents in related to standard ascorbic acid

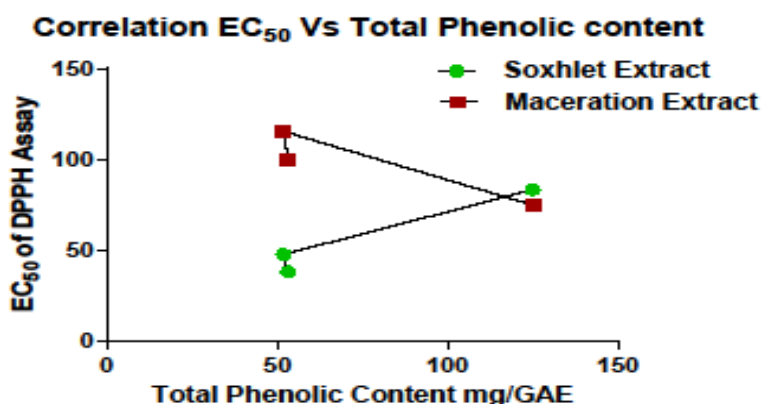


Fig. 5. The correlation analysis of EC₅₀ values of antioxidant of activity and total phenolic content of two extraction methods for negative relation

3.3 Antibacterial Activity

P. graveolens organic extracts exhibiting anti-bacterial activity on both Gram Positive and Negative bacterial strains with varied zone of inhibitions (Figs. 1a and b). Firstly, conventional extracts (Maceration and Soxhlet) with two different organic solvents (ethyl acetate and methanol) were showing the most active Zone of Inhibition (*S. aurea* 7 mm and 9 mm *B. subtilus*) and (*S. aurea* 8 mm and 10 mm *B. subtilus*) at 20 ug/uL. Whereas, ethyl acetate extract revealed positive inhibition on (*K. pneumonia* 8 mm and *E. coli* 5 mm) than the methanolic extract. Secondly, methanolic extract were also more active suppression on Gram Negative bacterial (*E. coli* 9 mm and *K. pneumonia* 10 mm). Geranium oil and organic extracts of pelargonium were exhibiting the most promising antibacterial effect on microorganism due to the presence of phenolic compounds in the extract was proven study [27]. Significant antibacterial activities on strains were also sensitive to the control antibiotics (amoxicillin and chloramphenicol). The antibacterial activity due to generally depend the amount of flavonoids and phenolic compounds and higher antioxidants properties present in the crude extracts.

4. CONCLUSION

Our research study providing the new information on the two conventional extractions of crude organic extracts from an oil yielding aromatic plant (*Pelargonium graveolens*). Illustrating the potential statistical analysis on preliminary studies with concern to organic extracts with the significant results on antioxidant and phenolic content properties and also showing positive

effects on anti-bacterial activity. In conclusion, This study gives supportive data on basic research of non-volatile compounds present in the crude organic extract does possess the therapeutic properties and which is the primary step to carry out the further work on active principle's.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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