

QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL ANALYSIS AND BACTERICIDAL ACTIVITY OF *PELARGONIUM GRAVEOLENS* L'HER

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ABSTRACT

Objective: The present study investigates the qualitative and quantitative phytoconstituents and bactericidal effect of medicinally important *Pelargonium graveolens* L' Her leaves extracts.

Methods: Preliminary phytochemical screening analyses were determined using standard protocol methods. In addition, antibacterial activities of the *P. graveolens* leaves extracts were evaluated by disc diffusion method against Gram-positive and Gram-negative bacteria.

Results: The results revealed that ethanolic extract of *P. graveolens* was found to be the predominant occurrence of phytochemicals (9/11) which contains flavonoid, phenol, tannins, saponins, reducing sugar, glycosides, terpenoids, anthraquinone, and phlorotannins while starch and steroids were absent. In quantitative estimation of bioactive phytoconstituents showed carbohydrates (74 mg/gdw), protein (41.25 mg/gdw), chlorophyll (2.2±0.05 mg/gdw), lipids (0.07 mg/gdw), tannins (135.3 gm/gdw), phenolic compounds (123.75 mg/gdw) and flavonoids (50 mg/gdw) were found to have higher amount in ethanolic extract followed by acetone, methanol and aqueous extract and also showed an inhibitory action on growth of tested bacteria.

Conclusion: Ethanolic extract of the *P. graveolens* leaves hold promises as a potential source of pharmaceutically important phytochemicals and also have strong antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*.

Keywords: Phytochemical screening, Primary and secondary metabolites, Antibacterial screening, *Pelargonium graveolens*

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INTRODUCTION

In recent years, many of the plant species have been scientifically evaluated for their possible medicinal applications. The traditional system of herbal medicine has become a topic of global importance since they are considered as rich sources of lead compounds and quietly safe for both human use and environment-friendly. The therapeutic value of plants lies in some phytochemical constituents present in it that may be useful for healing of human diseases [1, 2]. Phytochemicals are primary and secondary metabolites, which are naturally occurring in the leaves, vegetables, and roots that have defense mechanism and protect from various diseases. Primary metabolites are proteins, carbohydrates, chlorophyll, lipids and common sugars, which are synthesized during photosynthesis, and these organic compounds are essential for plant life and growth and development [3]. Secondary metabolites are tannins, flavonoids, phenolics, saponins and alkaloids, which are synthesized by the plant during development and are time, tissue and organ specific [4].

Screening of these phytoconstituents can be covered by nitrogenous compounds, acetogenins compounds, and isoprenoids compounds. These organic compounds allow easy transport across the cell membrane to induce different biological activities and physiological on the human body [5]. Nitrogenous compounds like alkaloids and amino acids which are critical to life, and have many functions in metabolism. These are commonly used in food technology and industry [6, 7]. Acetogenin screening included phenolics, flavonoids, tannins, coumarins, emodins, anthocyanidins, anthocyanins, anthraquinones, anthracene derivatives and fatty acids which are known to exhibit antioxidant, anti-inflammatory immuno-modulating, anti-tumor and antibacterial activities, etc. [8]. Screening of isoprenoids was cramped to saponins, cardiac glycosides, iridoids, steroids and carotenoids which are possess anti-inflammatory activity [9]. In the last few years, plant extracts have been developed and proposed for to target biologically active compounds, isolated for the elimination of pathogenic microorganisms, because of the resistance that microorganisms have built against antibiotics [10].

India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants [11]. Geranium

is a native of Cape Province in South Africa, and it was introduced to India in 20th century and since then its cultivation and oil production remained restricted to the high altitude regions of Southern India. In India, Maharashtra, Andhra Pradesh, Tamil Nadu, Jammu and Kashmir, Himachal Pradesh and Kerala are suitable for its cultivation [12].

Pelargonium graveolens L'Her. generally known as rose scented geranium belongs to the family Geraniaceae. It is a suffrutescent bushy aromatic perennial shrub with cylindrical stem, which is commonly growing in Kodaikanal hills, Tamilnadu, India, because it grows successfully at an altitude of 1000-2100m, mild climate with low humidity, warm winter and an annual rainfall of 1000-1500 mm which is ideal for this crop [13]. It's grown for the production of high-value essential oil that finds extensive use in flavoring, fragrance and aromatherapy for the treatment of gastrointestinal diseases, throat infections, and bleeding. *P. graveolens* leaves are popular constituent for antibacterial [14], antifungal [15] and also some other pharmacological properties such as anti-inflammatory [16] and hypoglycemic effects [17]. It is also useful in treating acne and other skin infections like burns, broken capillaries, cuts, dermatitis, ringworm, ulcers and infected wounds [18]. Therefore, investigation of phytochemical compounds of this plant has become desirable within the medicinal plants. Hence, it is essential to establish the scientific basis for their therapeutic actions, which may serve as the source for the development of effective drugs [19]. Keeping this in view, efforts are underway to search for better understanding of qualitative, quantitative chemical composition and to explore the antibacterial activity of organic solvent extracts of *P. graveolens* leaves.

MATERIALS AND METHODS

Collection of plant

Fresh plant leaves of *P. graveolens* were collected from Botanical garden of Mother Teresa Women's University, Kodaikanal, India. The leaves were washed thoroughly with normal tap water followed by sterile distilled water. Then leaves were dried under shaded condition at room temperature. Leaves were crushed to a powder using grinding machine. Powder was stored at 4°C in tight air container bottle.

Preparation of solvent extracts for qualitative phytochemical screening

The ground material of *P. graveolens* (20g) was filled in the thimble and extracted successively with 250 ml of ethanol, acetone, methanol using soxhlet extractor for 32 h at a temperature not exceeding boiling point of the solvent for each extraction. For aqueous extraction the content were kept in shaker at 37 °C for 24hours. All the extracts were filtered using Whatman No. 1 filter paper and then concentrated in vacuum at 40 °C using rotary evaporator.

Qualitative analysis of phytochemical screening

The extracts were subjected to phytochemical screening to test presence of metabolites such as flavonoids, phenol, tannins, saponins, reducing sugar, glycosides, terpenoids, anthraquinone, phlobatanins, starch and steroid were qualitatively analyzed [20-23].

Quantitative analysis of metabolites

Primary metabolites are the compounds synthesized in plants and directly involved in normal growth, development and reproduction which provide an idea of the nutritive potential of the plant parts. Primary metabolites like carbohydrate [24], protein [25], chlorophyll [26] and lipids [27] were done according to standard procedures. Secondary metabolites produced by plants to test their properties and to evaluate their possible use in the industry. Secondary metabolites like phenols, flavonoids and tannins were quantified in all the individual extracts. The total content of phenolics was determined using the Foline Ciocalteu method [28], flavonoids were estimated according to the procedure by Aluminium chloride colorimetric method [29] and estimation of tannin was carried out according to the method described by Schanderl [30].

Antibacterial activity

The efficiency of extracts of *P. graveolens* leaves were evaluated against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas*

aeruginosa and *Klebsiella pneumonia* using disc diffusion method. Muller-Hinton Agar plates were inoculated with different bacterial strains and sterile Whatman filter paper discs (3 mm) were containing different extracts of *P. graveolens* (100µl). Sterile discs were placed on the plates and the plates were incubated at 37 °C for 24 h in an incubator and observe zone of inhibition [31].

Statistical analysis

The results were expressed in mean±Standard Deviation. The statistical analysis was performed using GraphPad Prism 6.0. All the assays were performed in triplicate.

RESULTS

Qualitative phytochemical screening

In the present study primary metabolites of different solvent extracts of *Pelargonium graveolens* leaves were qualitatively analyzed. The extracts of *P. graveolens* showed diverse phyto-profiles with reference to the solvents. Out of four extracts (ethanol, methanol, acetone and aqueous) ethanolic extract demonstrated the maximum occurrence of phytoconstituents (9/11) such as flavonoids, phenol, tannins, saponins, reducing sugar, glycosides, terpenoids, anthraquinone and phlorotannins and absence of starch and steroids were observed. In the case of methanolic extract flavonoids, phenol, tannins, saponins, reducing sugar, glycosides, terpenoids and anthraquinone were present and phlobatanins, steroids and starch were absent. Followed by acetone extract showed presence of flavonoids, phenol, saponins, steroids, glycosides, terpenoids and phlobatanins and absence of tannins, starch, anthraquinone and reducing sugars, whereas aqueous extract showed presence of flavonoids, phenol, tannins, saponins, steroids, glycosides, terpenoids and reducing sugar and absence of tannins, phlorotannins, anthraquinone and starch (table 1). The presence and absence of the phytoconstituents depends on the solvent medium used for extraction and the physiological property of individual taxa.

Table 1: Phytochemical analysis conducted on different extracts of *P. graveolens*

S. No.	Phytochemicals	Ethanolic extract	Methanolic extract	Acetone extract	Aqueous extract
1.	Tannins (Braymer's test)	+	+	-	-
2.	Flavonoids (Alkaline reagent test)	+	+	+	+
3.	Phenolics (Ferric chloride test)	+	+	+	+
4.	Saponins (Foam test)	+	+	+	+
5.	Starch(Iodine test)	-	-	-	-
6.	Terpenoids (Salkowki's test)	+	+	+	+
7.	Steroids (Liebermann-Burchard test)	-	-	+	+
8.	Glycosides (Liebermann's test)	+	+	+	+
9.	Anthraquinone (Borntrreger's test)	+	+	-	-
10.	Reducing sugar (Benedict's test)	+	+	-	+
11.	Phlobatanins (Precipitate test)	+	-	+	-
	No. of phytochemicals	9	8	7	7

Table 2: Quantification of primary metabolites of *P. graveolens*

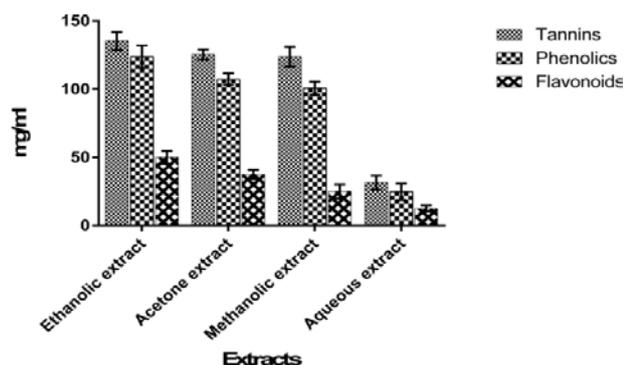
Primary metabolites	Ethanolic extract	Acetone extract	Methanolic extract	Aqueous extract
Carbohydrates	74±8.27	66±3.16	41.25±6.18	57.75±3.12
Protein	41.25±0.49	36.3±1.25	24.75±0.56	8.25±0.18
Chlorophyll	2.24±0.05	1.512±0.03	0.925±0.04	0.882±0.03
Lipids	0.04±005	0.07±0.004	0.02±0.001	0.01±0.001

Quantitative analysis of metabolites

Many primary metabolites lie in their impact as precursors or pharmacologically active metabolites in pharmaceutical compounds [30]. In the present investigation, primary metabolites like carbohydrates, protein, chlorophyll and lipids were quantitatively analyzed. The higher amount of carbohydrates present in the ethanolic extract (74±8.27 mg glucose equivalent/gdw) followed by acetone extract (66±3.16 mg/gdw) and methanolic extract (41.25±6.18 mg/gdw) and lower level of carbohydrates was found in the aqueous extract (57.75±3.12 mg glucose/gdw). Maximum

yield of protein was estimated in ethanolic extract i. e 41.25±0.49 mg BSAE/gdw followed by acetone extract (36.3±1.25 mg/gdw) and adequately presents in the methanolic extract (24.75±0.56 mg/gdw) and minimum amount was expressed in the aqueous extract (8.25±0.18 mg/gdw). Determination of chlorophylls in the ethanolic extract was 2.24±0.05 mg/gdw which was much higher than acetone (1.512±0.03 mg/gdw) and methanolic extracts (0.925±0.04 mg/gdw) and the minute amount was present in the aqueous extract (0.882±0.03 mg/gdw). A Higher level of lipid was observed in acetone extract (0.07±0.004 mg/gdw) and a considerable amount of lipids found in ethanol (0.04±005 mg/gdw) and methanolic extract

(0.02 ± 0.001 mg/gdw) and traces of lipids present in the aqueous extract (0.01 ± 0.001 mg/gdw).



Graph 1: Quantification of secondary metabolites of *P. graveolens*

Secondary metabolites like tannins, phenolics and flavonoids were estimated. The total level of tannin was found to be maximum in the

ethanolic extract (135.3 ± 6.95 mg/gdw), whereas acetone extract showed 125.4 ± 3.77 mg/gdw and for methanolic extract 123.75 ± 7.31 mg tannic acid equivalent/gdw and a minimum level of tannin found in the aqueous extract (31.35 ± 5.29 mg/gdw). Phenolic content of *P. graveolens* leaves was calculated as gallic acid equivalent and showed in the range of 24-123 mg/gdw. Ethanolic extract was showed the abundant occurrence of phenolics (123.75 ± 8.25 mg/gdw) when compared to other extracts like acetone 107.25 ± 4.25 mg/gdw, methanol 100.65 ± 4.90 mg/gdw and the miniature amount in aqueous extract 24.75 ± 5.62 mg/gdw. The best results of flavonoids showed in the ethanolic extract (50 ± 4.70 mg catechin equivalent/gdw), and the rest of the extracts showed 37.5 ± 3.33 mg/gdw for acetone extract, 25 ± 5.16 mg/gdw for methanolic extract and 12.5 ± 2.21 mg/gdw for aqueous extract.

Bacterial resistance of *P. graveolens*

The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Antimicrobial activities of the plant extracts (aqueous, methanol, acetone, and ethanol) showed inhibitory activities against *S. aureus*, *E. coli*, *P. aeruginosa* and *K. pneumonia* with significant effects depending not only on the tested microorganisms but also on the solvents used for extraction. The ethanolic extract exhibited highest inhibition zone varied from 9.02 to 16.20 mm against *Escherichia coli*.

Table 3: Minimum inhibitory concentrations (MICs) of different solvent extracts of *P. graveolens* leaves

Tested organisms	Zone of inhibition (mm)			
	Aqueous extract	Methanolic extract	Acetone extract	Ethanolic extract
<i>S. aureus</i>	-	14.7	11.8	10.1
<i>E. coli</i>	9.2	11.6	14.4	16.2
<i>P. aeruginosa</i>	9	15.1	14.5	13
<i>K. pneumonia</i>	8.5	13.6	13.9	12.4

DISCUSSION

Preliminary phytochemical screening on medicinal plant an important in the detection of bioactive principles which is a new source of therapeutically and industrially valuable compounds that may lead to drugs discovery and development [31]. Extraction is the vital step to extract the desired chemical components from the plant materials using polar and non-polar solvents [32]. Therefore underscores the plant composition we tried as much solvents as possible in the screening of phytochemicals. Water and alcohol were selected as the extraction solvents for quantitative analysis since both are more harmless when compared with other solvents. However, ethanolic extract was scrutinized owing to a maximum number of phytochemicals present in it. Since previous studies reported better extraction rates for several bioactive compounds in the ethanolic extracts [33-35].

The quantitative evaluation is key parameters in setting the standard for crude drugs. The ideals of solvent extractives can be a means of providing preliminary information on the quality of the drug. Carbohydrates are macromolecules, and it's composed of the elements of water and carbon. All carbohydrates are polar it can be readily converted into glucose which is used as an ultimate source of energy [36]. Proteins are also derived partly from carbohydrates through the formation of amino acids. Proteins occur throughout the plant cells, as conjugated forms. In many plant species the exhibited biological activity due to the proteinaceous substances present in their tissues [37]. As a result studies on the quantity of proteins have been undertaken in this plant. Lipids are fatty substances with long hydrocarbon chains and often ester linkages somewhere in the molecule. Plant lipids can be used as essential oils, spice oleoresins, food color [38]. Thus, higher levels of lipids in the selected plant species indicate their usefulness of essential oil present in it. The essential oil fraction of this plant is extensively used as skin care oils in the cosmetic industry because it is good in opening skin pores and cleaning oily complexions [39]. Chlorophylls are the main photosynthetic pigments in the green plants and hence, their determination is frequently required in most of the plant analysis

[40]. Tannin is considered as a food product in plant vegetable. Tannins decrease the bacterial proliferation by blocking key enzymes at microbial metabolism [41, 42]. Hence, this plant has a higher level of tannins which could act as an efficient antimicrobial drug. Phenolics are well-documented fact that most of the medicinal plants are enriched with phenolic compounds which have revealed biological and pharmacological properties like antimicrobial, antiviral, antioxidant, anti-inflammatory and cytotoxic activity which could validate the use of the plant in ethnomedicine [43]. The presence of flavonoid compounds which are known to elicit a wide of therapeutic activities like antihypertensive, antirheumatism etc., Thus, it is anticipated that flavonoid was adequate in this plant leaves which can be used to treat healing wounds since antiquity [44]. Antimicrobial properties of medicinal plants are increasingly reported from different parts of the world. The results of the ethanolic extract of *P. graveolens* leaves also indicated that scientific studies carried out on medicinal plant having traditional claims of effectiveness might warrant fruitful results. Thus, this plant could be utilized as an alternative source of useful antimicrobial drugs [45].

In essence, ethanolic extract was found to have the highest amount of total carbohydrates, protein, lipids, phenolics, tannin and flavonoid amounts, while aqueous one presented the lowest contents of primary and secondary metabolites due to low hydro-solubility of the molecule. On the other hand, acetone extract contained significantly higher levels of carbohydrates, protein, tannins, phenolics and flavonoids.

These compounds showed differences in their total contents depending on solvents polarities (EtOH>Acetone>MeOH>aqueous extract). The differences of the phytochemical composition are not only depending on solvents but also include edaphic and climatic conditions of the plant. Indeed, an Indian study reported that ethanolic extract of *P. graveolens* growing a medicinal plant in Kodaikanal, showed a higher level of potential phytol compounds which slightly differs from *P. graveolens* extracts studied in Tunisia [46].

CONCLUSION

Thus, the results are encouraging and contribution to a better valorization of *P. graveolens* since it has more active principles like carbohydrates, protein, lipids, phenol, flavonoid and tannin. These phytoconstituents seemed to have a potent drug for various diseases. This study would provide the preliminary scientific evidence for the ethnobotanical and traditional use of *P. graveolens*. Furthermore, biological tests are needed for to search active fractions of ethanolic extract of this plant and to characterize the eventual activities and assess toxicity.

CONFLICTS OF INTERESTS

Declare none

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