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Research Article

Preliminary Phytoconstituents Screening and Antibacterial Activities of *Pelargonium graveolens* Leave Extract

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Abstract

The Phytoconstituents of ethanolic/aqueous crude extract of leaves of *P. graveolens* was, screened qualitatively and quantitatively as well tested against some sorts of pathogenic bacteria. And the revealed results were by the Qualitative detection of those components such as Tannins, Saponins, Terpenoids, Volatile Oils, Flavonoids, Phenols, Aryl-amines, Alkaloids, Reducing Sugars, Glycosides and Amines, are varied in its presence in the extract between abundance, medium and few in existence. As for the Quantitative detection of the extract, the results were for flavonoids, saponins, alkaloids and polysaccharides 63.4, 60.3, 53.2 and 48 %, respectively. Besides, the bioactivity of ethanolic/aqueous crude extract of leaves of *P. graveolens* was tested against each of *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis* and *Staphylococcus aureus* and results revealed 17, 14, 12 and 15 mm respectively.

Keywords: Phytoconstituents; *Pelargonium graveolens;* Qualitatively and Quantitatively Screening; Antibacterial

Introduction

The use of herbal medicines in traditional treatment in numerous developing countries is a constant consideration of its historical and cultural relationship, as it is inherited from one generation to another. *Pelargonium graveolens (P. graveolens)* is utilized as the folk medication in diverse nations. And wherein, it has flavour to use as a treatment for several diseases such as diabetes, anti-cancer, anti-inflammatory and haemostatic. The *P. graveolens* plant is an herbaceous perennial. Its leaves have a strong aromatic smell, and therefore it is used as flavouring for tea in Libya. This herb is present in the regions of the Mediterranean Sea, and it is widely cultivated in the regions of Libya, and other countries, in-home gardens and verandas for its beautiful smell and breathtaking view, and its use is not limited to decoration only, but has many uses in the kitchen, and has great benefits for the body. Whereof the taxonomy or some of its scientific classification as the following: Kingdom: Plantae; Order: Geraniales; Family: Geraniaceae; Genus: *Pelargonium*; Species: *P. graveolens* and Binomial name: *Pelargonium graveolens* [1]. *P. graveolens* is an edible and medicinal plant, where this plant grows in the wild areas of Libya and likewise in most areas of the Mediterranean basin. It is also used as an aromatic plant to add a distinctive flavour to drinks, especially hot ones, as well traditionally, from old time until nowadays in Libya, *P. graveolens* is used as an aromatic component or to better or qualify sweets, food flavour, beverages, also as an antiseptic and antidepressant remedy. And on account of the demand for natural aromatic chemicals which includes increased in order to the great benefit from them in many areas of industry such as food, medicine, perfume and cosmetics, forth, because they are safe to use. Furthermore, they contain natural preservatives combinations because of the thorough chemical composition which possess them, compounds like Phenolics, Carotenoids, Terpenes

and Terpenoids [2,3]. Likewise, from the conventional use of the *P. graveolens* plant in the therapy such as Respiratory Tract Infections, Gastroenteritis, Diarrhoea, Wounds and Liver Complaints, or as Ornamental Plants [4-6]. In addition, given the importance of such a plant in various fields, including medical and others, our goal was to present a research study on the initial detection of some phytoconstituents from products of secondary metabolism, and also shed light on some of its biological effectiveness against some types of human pathogenic bacteria.

Materials and Methods

Collection and preparing of the plant material for extraction

The leaves of the *P. graveolens plant* were collected from one of the farms located in the Alkhums region, where the fresh and healthy leaves separated and washed well with tap water, then with distilled water, dried with a towel and spread on dry paper in the shade for three days, with stirring from time to time, and completed drying in the oven at a temperature of 40 °C for two days, after that, the leaves were crushed and a fine powder was obtained has a green coloured. The fine powder was kept in opaque plastic bottles in a cool and dry place away from light until use.

Extraction

The process of extracting the phytoconstituents from the leaves of the *P. graveolens* was carried out by soaking 20 grams of fine powder of the plant in 400 ml (w/v) of the appropriate solvent (Ethyl alcohol/distilled water, 1:1 v/v) for three days. Thereafter, the extract was separated using a rotary evaporator, at a temperature of 43°C and a rotation of 90 rpm. Where the extract was obtained in a dark brown colour for the crude extract, and the productivity of obtained extracts was calculated and kept in the freezer until use.

Qualitative phytochemical screening

Thin liquid chromatography TLC

Thin-layer chromatography plates were used to detect some phytoconstituents contents of the crude extract of *P. graveolens* leaves. Hence, that the spots from the crude extract were placed on the TLC plates and dried well (chromatogram plate). After this, the plates were transferred to the chamber which was filled with 2-3 ml of the desired solvent system (Ethyl Alcohol (EtOH) 20% and Dichloromethane (D C M) 80%, 1:1, v/v) and the chamber was covered. Once the desired solvent reached about 90% of the way up the plate, That is, after a while on a period of time they were taken out and dried. Then it was used to detect by spraying on it with the prepared reagents, separately, and checking the colour change using an ultraviolet lamp at a wavelength of 365 nanometers, (UV-Lamp) [5-8].

Detecting of flavonoids: (Aluminum Chloride Reagent)

The chromatogram plate with crude extract spot was sprayed with an ethanolic solution of aluminium chloride 1%. Then the plate was displayed under UV-lamp (long-wavelength, 365 nm). The appearance of the yellow-fluorescence colour is evidence of the presence of flavonoids in the crude extract.

Detecting of phenols and aryl amines: (Emerson reagent)

Preparation of Emerson reagent

- **Solution I:** 4-aminophenazone 1g was dissolved in 100 ml of Ethanol solution 80 %.
- **Solution II:** Potassium Hexacyanoferrate (III) 4g was dissolved in distilled water 100 ml.
- **Method:** The chromatogram plate was sprayed with a solution (I) then gently dried using warm air for 5 minutes: The chromatogram plate with crude extract spot was placed in a chamber with vapor from ammonium solution 25%. The appearance of Red-Orange with pink spots colours is evidence of the presence of phenols and aryl-amines in the crude extract.

Detecting of reducing sugars: (Aniline phthalate)

- **Preparation of reagent:** The precise weight of each 1.66g O-Phthalic acid and 0.93g of aniline was dissolved in 100 ml of the saturated solution (n-butanol - water).
- **Procedure:** The chromatogram plate with crude extract spot was sprayed with the aniline phthalate reagent, dry using warm air for 2 minutes and then heated in an oven to 105 °C for 10 minutes. The appearance of spots with various colours of them gives fluorescence at 365nm, an evidence presence of reducing sugars.

Detecting of glycosides: (Diphenylamine Reagent)

- **Preparation of reagent:** 10 ml of Diphenylamine (10%) was diluted in 90 ml of ethanol, then 100 ml of hydrochloric acid was added, thereafter 80 ml of glacial acetic acid was also added, mixed well on a shaker for a while.

- **Procedure:** The chromatogram plate with crude extract spot was sprayed slightly, covered chromatogram plate with another clean glass plate, and then was heated in an oven at 110°C for 45 minutes until appeared of blue spots which were evidence of the presence of the Glycosides.

Detection of alkaloids: (Dragendorff's reagent) (Nitrogen Compounds)

- **Solution (I):** Tartaric Acid 20g and Bismuth Nitrate 1.7g was dissolved in 80 ml of Ethanol solution 80 %.
- **Solution (II):** Potassium iodide 16 g was dissolved in distilled water 40 ml.
- Mix both Solutions I and II, Keep them as stoke solution in a refrigerator to be stable for weeks.
- **Stock solutions:** Mix an equal amount from both Solutions I and II each time, directly before use.
- **Procedure:** 5 ml of the stock solutions were mixed very well for ten minutes with each of Tartaric acid 10g, distilled water 50 ml, and then placed on the shaker for 10 minutes, after that sprayed by it the chromatogram plate with crude extract spot. Formation of an Orange coloured evidence of the presence of the Alkaloids.

Detection of amines: (Ninhydrin Reagent)

The chromatogram plate with crude extract spot was sprayed with a solution of Ninhydrin reagent (0.2 g of Ninhydrin was mixed with 100 ml of Ethyl alcohol) and then the chromatogram was heated at 110 ^{0}C tell the appearance of reddish spots which evidence of the presence of amines.

Detection presence of phytoconstituents by using different reagents

Where various reagents were prepared and employed to detect the existence of the secondary metabolites phytoconstituents compounds in the crude extract.

Detection of tannins and polyphenols

10 ml of the crude extract solution was mixed in a test tube with 2-3 drops of Ferric Chloride solution (Fe Cl_3). The appearance of blue-black or blue-green colouration revealed the existence of the Tannins and Polyphenols [9-11].

Detection of saponins: (Foam Test)

10 ml of the crude extract solution was mixed in a test tube and shaken vigorously for 1 minute. The formation of stable foam after 1 minute was revealed as evidence of the existence of saponins [9-11].

Detection of terpenoids

10 ml of the crude extract solution in a test tube was mixed 2 ml of chloroform and warmed in a water bath until dryness. Then, was added 2 ml of concentrated (H_2SO_4). The appearance of a reddish-brown colouration at the interface revealed the existence of terpenoids [9-11].

Detection of volatile oils

10 ml of the crude extract solution was mixed with 5 ml of sodium hydroxide solution (NaOH) in a test tube then was shaken vigorously for a while. The formation of white precipitate was revealed as evidence of the existence of volatile oil [12].

Quantitative determination of some phytoconstituents

The phytoconstituents such as Flavoring, Saponins, Alkaloids and Polysaccharides were detected in the *P. graveolens* leaves fine powder [13-15].

Detection of flavoring's contents

In it, the flavonoids were re-extracted three times, weighing 10 grams of raw plant powder and then mixed with 80% methanol solution at room temperature and soaked for 3 hours. After this, the mixture was filtered using Whatman filter paper No 42 (125 mm). The filtrate was taken into a beaker that was placed in a hot water bath and the solvent was evaporated, and the result was calculated after obtaining a constant weight [13].

Detection of saponins contents

15 g of the crushed fine powder was placed into a conical flask and 75 ml of aqueous ethanol 20% were added. After that was heated at 55°C, in a water bath for 4 hr., with continuous stirring. The combination was filtered and the residue was re-extracted 2 times with another 125 ml ethanol 20% over water bath at about 90°C the combined extracts were concentrated to 25 ml. Then was transferred into a separatory funnel and then 15 ml of Diethyl Ether was added and shaken vigorously, the ether layer was discarded while the aqueous layer was re-gained. After

the purification procedure was repeated, 20 ml of n-butanol was added. The combined n-butanol extracts were washed double with 5 ml aqueous sodium chloride solution 5%. Consequently, in a water bath, the remaining solution was heated. Behind evaporation the sample was dried in the oven until obtained constant weight; the saponins content was calculated as a percentage [14].

Detection of alkaloid's contents

Accurately, 10g of fine plant powder was weighed and placed in a beaker with a capacity of 500 ml, and 400 ml of 10% acetic acid solution was added to it, covered and allowed for 4 hours. After this, the mixture was filtered and concentrated in a water bath to a quarter of the quantity, then the ammonium hydroxide was added through dropping wise until the precipitate was finished and left for a period of time. After this, the mixture was filtered and the residue was washed with ammonium hydroxide, dried and weighed the alkaloids yield [15].

Detection of polysaccharide's contents

Determination of the crude of polysaccharides from the crude powder of *P. graveolens* leaves were 20 grams of the crude powder of *P. graveolens* were placed in a boiling flask with a capacity of 250 ml, 200 ml of distilled water was added to it, and the flask was connected with a suitable condenser and heated at a temperature of 100°C for 20 minutes. The flask was cooled and the mixture filtered. After the filtration was centrifuged, the supernatant was obtained, ethyl alcohol was added to it, and the mixture was placed on the vibrator for 24 hours at room temperature. The crude polysaccharides result was calculated as the following:

(C P) Percentages Yield % = C_0/C X 100

Where: C_0 (g) is a crude polysaccharide, and the C is a crude powder (g) of *P. graveolens*

Antibacterial activities

Measurement of the bacterial activity of the crude extract of *P. graveolens* leaves

Bacterial activity measurements were performed for the crude extract of powdered leaves against the 4 sorts of bacteria (*Bacillus subtilis* and *Staphylococcus aureus* as two gram-positive, and *Escherichia coli* and *Pseudomonas aeruginosa* as gram-negative bacterial strains, were carried out to detect the potency of the *P. graveolens* extract against the appropriate pathogenic bacterial sorts.

Disk diffusion method

The disk diffusion method was employed to evaluate the antibacterial activity. The bacteria were grown in Petri Plates containing nutrient broth medium, obtained from fresh bacteria culture, a few colonies were aseptically planted in 0.9% sodium chloride solution, at a denseness of 0.5 McFarland (107 to 108 C F U/mL). Afterwards were soaked in 40 µl of *P. graveolens*, 6 mm diameter disks were put on the surface of petri plates. Also, disks for each of the appropriate solvents (solvent employed in extraction) were used as a negative control and the antibiotic Amoxicillin (30 µg/disc) was used as a positive control Subsequent, The petri plates were incubated at 37 0C for 24 hours. The inhibition zones were measured and these growth inhibition zones were defined in mm [16-19].

Results and Discussion

Qualitative detection of phytoconstituents by using TLC

Phytoconstituents	Tannins	Saponins	Terpenoids	Volatile Oils
P. graveolens Leaves	+	+	+	+

Table 1: The Qualitative results of phytoconstituents by using TLC.

Key: +++ = Exist in abundant.

Qualitative detection of phytoconstituents by standard methods

Phytoconstituents	Flavonoids	Phenols and Ary-lamines	Reducing Sugars	Glycosides	Alkaloids	Amines
P. graveolens Leaves)	+++	+++	++	++	+++	+

Table 2: The Qualitative results of phytoconstituents by using standard methods.

Key: +++ = Rich, ++ = Moderate, + = Low-level

Table 1 which showed the results obtained by TLC tests for the qualitative screening of phytoconstituents were revealed that each of Tannins Saponins, Terpenoids and Volatile Oils were present in the *P. graveolens* leaves. Ultimately, Table 2 exhibited the presence of the phytoconstituents, hence, each of Flavonoids, Phenols, Aryl amines and Alkaloids present in abundance, while, each of the Reducing Sugars and Glycosides hers presence was average, whilst the Amines present in low levels. Many products of secondary metabolisms, such as phenols, tannins, saponins, alkaloids, glycosides, steroids, etc., belong to different families and are distributed in different parts of the plant. As its distribution may be concentrated in more parts than other parts, and perhaps this is what makes this part of the plant specialize in use, such as treatment for a disease, more than the other parts of the same plant. These components also have an effective and influential role in the human body, thus, their role is in the prevention of diseases such as strengthening the immune system or in eliminating free radicals such as phenolic compounds, or eliminating bacteria and limiting their growth and multiplication and many other benefits.

Quantitative phytoconstituents

Phytoconstituents	*P. graveolens* leaves (%)
Flavoring's	63.4
Saponins	60.3
Alkaloid's	53.2
Polysaccharides	48

Table 3: The results of quantitative determination of some phytoconstituents.

From table 3, the quantitative analysis results for flavonoids, saponins, alkaloids and polysaccharides were 63.4, 60.3, 53.2 and 48 %, respectively. The highest percentage of productivity was for flavonoids, followed by saponins, and after that came alkaloids, polysaccharides, and these secondary metabolic products in the leaves of *P. graveolens* are given nutritional, preventive and curative importance in terms of the existence of such important compounds.

Antibacterial activities

As revealed in table 4 the results of the crude extract of the *P. graveolens* was showed the diameter of the inhibitory zone (mm) against bacterial strains which included *Escherichia coli,*

Bacterial strains	*P. graveolens* extract Diameter of inhibitory zone (mm)	Antibiotic (mm)
Escherichia coli	17	21
Pseudomonas aeruginosa	14	19
Bacillus subtilis	12	17
Staphylococcus aureus	15	20

Table 4: The results of antibacterial activity as the diameter of zone of inhibition (mm) around the crude extract and standard discs.

Pseudomonas aeruginosa, Bacillus subtilis and *Staphylococcus aureus* were 17, 14, 12 and 15 mm. Somewhat, we note that the result of the highest inhibition was against *Escherichia coli*, followed by *Staphylococcus aureus,* and after that came both *Pseudomonas aeruginosa* and *Bacillus subtilis*. As a result, the secondary metabolic products exert an effect on pathogenic microbes such as many types of bacteria. Particularly on the cell membrane of bacteria so that these products act on disturbance, cytolytic leakage and swelling that would hinder the functions of cells [20]. Constituents like phenolic, oxygenated and terpenes compounds, are accountable with regard to an assortment of biological activities [21]. Ascertain by many previous studies, which showed that this type of plant possesses effective antimicrobial effects, particularly some pathogenic species that were used in this research [22].

Conclusion

It is attainable to conclude from this study that the leaves of the plant *P. graveolens* enjoy the presence of many active biological compounds, whether they as additives food, as an aromatic flavour or as used for prevention and treatment of some diseases. Thus, conducting other in-depth studies and using advanced methods of separation and analysis, will have an effective impact to introduce more about this plant for food and pharmaceutical industries. Consequently, because the functioning extract demonstrated an effective antibacterial vitality, against both Gram-positive and Gram-negative bacteria, also through the results obtained, it is achievable to use this plant as an aromatic or as a therapeutic plant for prevention and in the treatment of the diseases.

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Bibliography

1. Pelargonium graveolens. "Germplasm Resources Information Network (GRIN)". Agricultural Research Service (ARS), United States Department of Agriculture (USDA). (2007).

2. Pandey AK., *et al*. "Essential Oils: Sources of Antimicrobials and Food Preservatives". *Frontiers in Microbiology* 7 (2017): 2161.

3. Akhtar BD and Azam T. "Antimicrobial activity of essential oils extracted from medicinal plants against the pathogenic microorganisms: A review". *Biological Sciences and Pharmaceutical Research* 2 (2014): 1-7.

4. Roeschenbleck J., *et al*. "Phylogenetics, character evolution and a subgeneric revision of the genus Pelargonium (Geraniaceae)". *Phytotaxa* 159.2 (2014): 31-76.

5. Saraswathi J., *et al*. "Phytopharmacological importance of Pelargonium species". *Journal of Medicinal Plant Research* 5.13 (2011): 2587-2598.

6. TLC Reagents and Detection Methods - Physical and Chemical Detection Methods: Fundamentals, Reagents, I, Vol 1a, H. Jork, W. Funk, W. Fishcer, and H. Wimmer, Wiley, NY, 1990. Practice of Thin Layer Chromatography, 3rd edition, J.C. Touchstone, Wiley-Interscience, NY (1992).

7. TLC Reagents and Detection Methods - Physical and Chemical Detection Methods: Activation Reactions, Reagent Sequences, Reagents, II, Vol 1b, H. Jork, W. Funk, W. Fishcer, and H. Wimmer, Wiley, NY (1994).

8. E Stahl. Springer-Verlag, NY. Thin Layer Chromatography, 2nd edition (1969).

9. Sofowra. Medicinal Plants And traditional Medicine In Africa, (Ibadan Nigeria: Spectrum Books, (1993): 191-289.

10. G Trease and W Evans. "Pharmacognosy". (11th ed), (London: Bailliere) (1989): 45-50.

11. J Harborne. "Phytochemical methods". (London: Chapman and Hall, (1973): 49-88.

12. Talukdar A and Chaudhary B. "Phytochemical Screening of ethanolic extracts of Rubia Cordiofolia". *Pharmaceutical and Biological Sciences* 1.4 (2010): 530-536.

13. Boham BA., *et al*. "Flavonoids and condensed tannins from leaves of Hawaiian vaccinium vaticulatum and V. calycinium". Pacific Sciences 48 (1974): 458-463.

14. Obdoni Bo and Ochuko PO. "Phytochemical studies and comparative efficacy of the crude extracts of some Homostatic plants in Edo and Delta States of Nigeria". *Global Journal of Pure and Applied Sciences* 8 b (2001): 203-208.

15. Harborne JB. "Phytochemical methods, London". Chapman and Hall, Ltd. (1973): 49-188.

16. Ahmed HM and Al-Zubaidy AMA. "Exploring Natural Essential Oil Components and Antibacterial Activity of Solvent Extracts from Twelve Perilla Frutescens L. Genotypes". *Arabian Journal of Chemistry* 13 (2020): 7390-7402.

17. Elegbede JA., *et al*. "Silver-Gold Alloy Nanoparticles Biofabricated by Fungal Xylanases Exhibited Potent Biomedical and Catalytic Activities". *Biotechnology Progress* 35 (2019): e2829.

18. Balouiri M., *et al*. "Antifungal Activity of Bacillus Spp. Isolated from Calotropis Procera AIT. Rhizosphere against Candida Albicans". *Asian Journal of Pharmaceutical and Clinical Research* 8 (2015): 213-217.

19. El Barnossi A., *et al*. "Antifungal Activity of Bacillussp. Gn-A11-18isolated from Decomposing Solid Green Household Waste in Water and Soil against Candida Albicans and Aspergillus Niger". E3S Web Conf. 150 (2020): 02003.

20. Yang L., *et al*. "Durable antibacterial cotton fabrics based on natural borneol-derived Anti-MRSA agents". *Advanced Healthcare Materials* 9 (2009): e2000186.

21. Pinto, R. "Estudo da atividade antibacteriana da Carvona e seus derivados". Dissertacão de Mestrado. Universidade da Beira Interior, Covilha, Portugal (2014).

22. Mativandlela SPN., *et al*. "Antibacterial, antifungal and antitubercular activity of (the roots of) Pelargonium reniforme (CURT) and Pelargonium sidoides (DC) (Geraniaceae) root extracts". *South African Journal of Botany* 72 (2006): 232-237.