Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 12 [4] March 2023 : 49-52

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Phytochemical and Antibacterial studies of *Pelargonium graveolens* cultivated in Iraq

Zaineb Aziz Ali[1], Seror Ali Abdul Hussein Shalash[1],Rafif Raad[1], Sevan A. Dushan[1], Maiss S. Baqer[1],Sumia S Tayeh[1]
[1]Department of Pharmacy, AL-Esraa University College, Baghdad, Iraq.

ABSTRACT
Pelargonium graveolens (P. graveolens), commonly known as rose geranium it is native to the southern parts of Africa. Phytochemical studies show that this plant is rich in flavonoids, terpenoids, volatile oil, tannins, and glycosides. It has important pharmacological activities including antibacterial, antifungal, and antidiabetic and antioxidant activity. This is a novel study of Pelargonium graveolens cultivated in Iraq. In this study, the preliminary tests indicate the presence of active constituents in the leaves including terpenoids, flavonoids, alkaloids and tannin. This study also involved studying the antibacterial activity of Pelargonium graveolens hexane and ethanol leaves extracts and was performed on gram negative bacteria species (Escherichia coli) and one gram positive bacteria (Staphylococcus aureus). The antibacterial study was performed by ager well diffusion method. The ethanol extract shows good results against examined bacteria while hexane extract shows no antibacterial activity against examined bacteria.
***Keywords**: Pelargonium graveolens, rose geranium, antibacterial activity, agar well diffusion method.*

Received 14.11.2022 Revised 30.01.2023 Accepted 10.03.2023

INTRODUCTION

Pelargonium graveolens (P. graveolens), commonly known as rose geranium is one of more than 250 species within the *Pelargonium* genus, belonging to the Geraniaceae family and that are native to the southern parts of *Africa* [1]. However, not all species of the genus reside there. Some have branched out and found growing naturally in *Australia*, eastern Africa, New Zealand, and Madagascar. They are multi-branched plants and have succulent stems. The leaves appear deeply incised, soft to the touch (hairy), velvet-like, and can vary in shapes and scents. In general, *Pelargonium graveolens* plants are seasonal bloomers and produce flowers from late summer through mid-winter (*August* to *January*) [2]. Their blossoms exhibit different shades of white, pink, red, lavender, and also beautiful mixes of these tints. Phytochemical studies show that this plant is rich in flavonoids, terpenoids, volatile oil, tannins, and glycosides. These active ingredients have very important antibacterial activity. A plant of medicinal use since prehistoric times. It has an important pharmacological activities including antibacterial [3, 4], antifungal [5], and antidiabetic [6] and antioxidant [7] activity. Despite of availability of antibacterial agents now a day, but there uses are limited by number of factors like side effects, drug toxicity, and the most important factor is the emergence of resistant strains of bacteria. This phenomenon results in many clinical problems including the disease treatment caused by these microorganisms. Therefore, it is important to discover new, safer, and more effective antibacterial agents from natural sources like plants. The aim of this study is phytochemical screening of the most important active constituent in the plant and to evaluate the antibacterial activity of Pelargonium *graveolens* ethanol and hexane leaves extracts against one gram negative bacteria species (*Escherichia coli*) and one gram positive bacteria (*Staphylococcus aureus*).

MATERIAL AND METHODS

Collection of plant: The leaves of Pelargonium graveolens were collected from AL-Washash nursery. The plant material was collected in February and dried at room temperature in the shade. Then, grinded as powder and weighed.

Preliminary Phytochemical study of *Pelargonium graveolens leaves*:

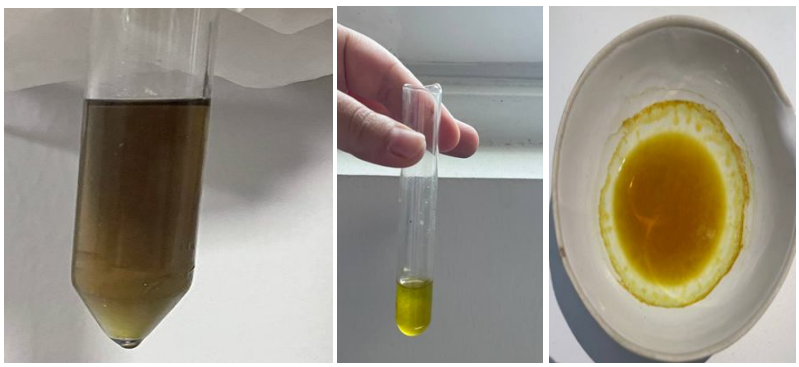
The plant extract was phytochemically screened for the qualitative investigation of major classes of secondary metabolites:

Test for saponins (Foam Test):
Ethanolic extract of the leaves (1 mL) was mixed with distilled water (5mL) in a test tube and then shaken until persistent foam appear [8].
Test for terpenoids (Salkowski Test):
Ethanolic extract of the leaves (5 mL) was mixed with chloroform (2 mL) and concentrated sulphuric acid (3 mL) was carefully added. A reddish–brown color of interface will form to indicate the presence of terpenoids [9].
Test for flavonoids:
Ethanolic KOH (2 mL) was added to (1 mL) of ethanolic extract of the leaves. A formation of yellow color will indicates the presence of flavonoids.
Test for tannins:
Powdered sample of the plant (0.5 g) was boiled with distilled water (20 ml) in a beaker and then filtered after that 1% of ferric chloride was added to the filtered sample. The brownish - green or a blue - black color will indicates that tannins are present [8].
Test for Alkaloid
1- Mayer test: few drops of Mayer reagent were added to ethanolic extract of the plant(2 mL).(Positive result is cream precipitate).
2- Wagner test: few drops of Wagner reagent were added to ethanolic extract of the plant (2 mL).(Positive result is brown reddish precipitate).
Preparation of extracts: 50 g of shade-dried pulverized plant materials, from plant leaves were packed in the thimble of soxhlet apparatus and extracted with 500 mL of hexane for 18 hours. The extract was filtered, and the solvent had been evaporated using a rotary evaporator to get a dry brown extract. The residue was packed again in the thimble of soxhlet apparatus and extracted with 500 mL of ethanol for 16 hours. The extract was filtered, and the solvent was evaporated using a rotary evaporator to get a dry dark brown extract and were left at 4 0C until assessment for their antimicrobial activities. The stock solution of hexane (2 gram/ 2 ml) was prepared by dissolving 2 grams of hexane extract in 1 ml of hexane. The dilution serials of 1 gram/ml, 500 mg/ml were prepared for antibacterial assay by agar well diffusion method. Stock solution of ethanol (2gram/ 1 ml) was prepared by dissolving 2 gram of ethanol extract in 1 ml of ethanol. The dilution serials of 1 gram/ml, 500 mg/ml were prepared for antibacterial assay by agar well diffusion method.
Tested microorganisms: one gram negative bacteria species (*Escherichia coli*) and one gram positive bacteria (*Staphylococcus aureus*) were tested. The isolates were obtained from microbiological laboratory /Department of Pharmacy/ Al-Esraa University College.
Agar well diffusion bioassay of ethanol extract: The antibacterial activity of ethanol extract was determined by agar well diffusion method and carried out by using pure culture for all species of bacteria. Stock cultures of the test bacteria were grown in Muller Hinton Broth (MHB, Merck, Germany) medium at 37˚C for 22 hours. The final cell concentrations were adjusted to 1.5 × 108 CFU/ML with reference to the McFarland turbid meter [10, 11]. The media were allowed to solidify and wells were prepared in the seeded agar plates with the help of a cup borer (6 mm) into agar. Subsequently, in each agar plate of tested bacteria four wells were made and (100µl) of dilutions of the extracts (2 gram/ml, 1 gram/ml and 500 mg/ml) introduced into wells on the plate. The ethanol was used as the negative control. The plates were kept at 37 °C for 24 hours and the antimicrobial action was estimated by determining the diameter of the inhibition zone. Evaluation of antibacterial action was based on extent of the diameter of inhibition zone formed all over the place of the well.

RESULTS AND DISCUSSION

The preliminary tests indicate the presence of active constituents in the leaves including terpenoids, flavonoids, alkaloids and tannin as shown in table (1), Fig (1).

Extract	Terpenoids	Alkaloids	Flavonoids	Saponins
Leaves	+++	+++	+++	-



A. (+) (terpenoids) test B. (+) flavonoid test C. (+) alkaloid test

Fig (1): Tests for (A) terpenoids, (B) flavonoid (C) alkaloid.

Anti-bacterial assay of Pelargonium graveolens leaves ethanolic extract by agar well diffusion method:*Pelargonium graveolens* leaves ethanolic extract was screened for its antibacterial activity by agar well diffusion method and ethanol used as a negative control against one gram negative bacteria (*E.coli*) and one gram positive bacteria (*S. aureus*) at concentration of 2000mg/ ml, 1000mg /ml and 500 mg/ml of ethanol extract as shown in table (2) and figure (2). The ethanol extract shows variable results at different concentration towards bacteria. The most effective concentration was 2000 mg/ml followed by 1000mg/ml and the weakest concentration was the lowest one 500mg /ml shows moderate activity against all bacteria understudy. *E. coli* was more resistant bacteria to the ethanol extract and the most sensitive one was *S. aureus*. This study show that *Pelargonium graveolens* leaves ethanolic extract has good antibacterial study and this result is confirmed by other studies in the world, one of these studies show that *Pelargonium graveolens* has an excellent antibacterial activity against *S. aureus* and *E. coli*[7,8]. The flavonoid and alkaloid content of *Pelargonium graveolens* leaves ethanolic extract may be responsible for the antibacterial activity. The presence of flavonoid and alkaloid content of *Pelargonium graveolens* leaves ethanolic extract confirmed by the preliminary testsas shown in table (1) and Figure (1). They are responsible for the antibacterial activity and this activity was confirmed by other studies in the word, one of these studies show that flavonoids have antibacterial activity byproposed antibacterial mechanisms of flavonoids are as follows: inhibition of nucleic acid synthesis, inhibition of cytoplasmic membrane function, inhibition of energy metabolism, inhibition of the attachment and biofilm formation, inhibition of the porin on the cell membrane, alteration of the membrane permeability, and attenuation of the pathogenicity [12].The alkaloids antibacterial activity was confirmed by other studies one of these studies show that most alkaloids act through, the down regulation and inhibition of efflux pump ATPase cause disruption of the ABC transporters in the bacterium and reduce oxygen consumption. This causes disruption of bacterial homeostasis and further compromises the outer membrane and cytoplasmic membrane integrity of the bacterium. At last, the series of inhibitions and disruptions causes leakage of cytoplasmic contents leading to anti-microbial activity [13].

Table (2): Antibacterial activity of *Pelargonium graveolens* leaves ethanol extract with different bacterial species measured in millimeter by agar well diffusion method.

Bacterial species	**Antibiotic code/concentration in microgram (mcg) inhibition zone in millimeters**		
	2g/ml	**1g/ml**	**500 mg/ml**
Staphylococcus aureus	**25 S**	**18 S**	**16 S**
Escherichia coli	**15 S**	**14 S**	**12 S**

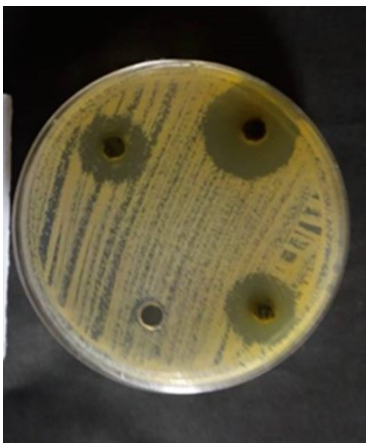


Fig (2): Antibacterial activity of *Pelargonium graveolens* leaves ethanol extract with different bacterial species measured in millimeter by agar well diffusion method.

CONCLUSION

1. Phytochemical study of *Pelargonium graveolens* cultivated in *Iraq* revealed the presence of important bioactive compounds which are flavonoid, alkaloids and terpenoids.
2. A good antibacterial activity of ethanol extract against Gram negative (*Escherichia coli*) and Gram positive bacteria (*Staphylococcus aureus*).
3. This study looked into the ethanol extract preference of Gram-positive bacteria over Gram-negative bacteria. It concluded that the Gram-positive bacteria were preferred because they lacked the hydrophilic polysaccharide chain that acts asa barrier for Gram-negative bacteria. This difference is what allows Gram-negative bacteria to be less susceptible to the *Pelargonium graveolens* extract.

REFERENCES

1. Mativandlela SP, Lall N, Meyer JJ (2005) Antibacterial, antifungal and antitubercular activity of (the roots of) *Pelargonium reniforeme* (CURT) and *Pelargonium sidoides* (DC) (Geraniaceae) root. South African Journal of Botany, 72: 232-237.
2. Cavar S, Maksimovic M (2011) Antioxidant activity of essential oil and aqueous extract of Pelargonium graveolens. Food Control, 23: 263-267.
3. Boukhatem MN, Kameli A, Saidi F (2013) Essential oil of Algerian rose-scented geranium (Pelargonium graveolens): Chemical composition and antimicrobial activity against food spoilage pathogens. Food Control 34: 208-213.
4. Seidel V, Taylor P (2004). In vitro activity of extracts and constituents of *Pelargnoium* against rapidly growing mycobacteria. International Journal of Antimicrobial Agents 23: 613-61.
5. Dzamic AM, Sokovic MD, Ristic MS, Grujic SM, Mileski KS, et al. (2014) Chemical composition, antifungal and antioxidant activity of *Pelargonium graveolens* essential oil. Journal of Applied Pharmaceutical Science 4: 1-5.
6. Boukhris M, Bouaziz M, Feki I (2012) Hypoglycemic and antioxidant effects of leaf essential oil of *Pelargonium graveolens* in alloxan induced diabetic rats. Lipids in Health and Disease.
7. D.VijisaralElezabeth and Arumugam Subramanian. (2013). Identification of phytochemical constituents and antimicrobial activity of *Indigofera suffruticosa* leaves. International Journal of Current Biotechnology. 1.100-109
8. Singh P, Shivhare Y, Singhai AK, Sharma A.(2010). Pharmacological and phytochemical profile of Chenopodium album linn. Research Journal of Pharmacy and Technology. 3(4), 960963.
9. Burt SA, Reinders RD. Antibacterial activity of selected plant essential oils against Escherichia coli O157:H7. Lett ApplMicrobiol. 2003;36(3):162-7.
10. Thamer Mouhi Jasiem, Suhad Faisal Hatem Al-mugdadi, Ibrahim S. Aljubory, Qabas Nather Latef, (2016). Phytochemical Study and Antibacterial 15 Activity of Crude Alkaloids and Mucilage of Cordiamyxa in Iraq, Inter. J. Pharma. Scien. Review and Research, 39(1), Pages: 232-236
11. Xie Y, Yang W, Tang F, Chen X, Ren L. (2015). Antibacterial activities of flavonoids: structure-activity relationship and mechanism. Curr Med Chem. 22(1):132-49. doi: 10.2174/0929867321666140916113443. PMID: 25245513.
12. Majumder, Avishek (2019). "Antibacterial activities of plant-based alkaloids and terpenoids." Knowledge Tank, Project Guru, https://www.projectguru.in/antibacterial-activities.

CITATION OF THIS ARTICLE

Z A Ali, S A A Hussein Shalash,R Raad, S A. Dushan, M S. Baqer, S S Tayeh. Phytochemical and Antibacterial studies of *Pelargonium graveolens* cultivated in Iraq. Bull. Env.Pharmacol. Life Sci., Vol 12 [4] March 2023: 49-52