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Biological studies concerning the antioxidant and antimicrobial activities of *Pelargonium* species cultivated in Egypt (Family-Geraniaceae)

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ABSTRACT
Plant-derived pharmaceuticals have become prominent in the market place, making it a favored healthcare choice. In this study, air dried samples of aerial parts of *Pelargonium* X *fragrans* Willd. and *Pelargonium peltatum* L'Hérit. were separately extracted using successive extraction with a soxhlet apparatus. Each extract was tested for its antimicrobial activity using two Gram-negative bacterial strains (*Pseudomonas aeruginosa* and *Escherichia coli*), two Gram-positive bacterial strains (*Bacillus subtillus* and *Staphyllococcus aureus*), and clinical fungal isolates (*Aspergillus niger* and *Candida albicans)*. Also, their antioxidant activity was tested using a DPPH free radical assay. The ethyl acetate, n-Butanol and the total extracts showed moderate activity against the tested microorganisms with significant high activity against *E. coli.* The free radical scavenging property was found to be in a concentration dependent manner in all the tested fractions. The most effective antioxidant fractions in both spp. was the n-Butanol fraction (85% and 85.2%) at the concentration of 0.375µg/ml followed by the total ethanolic extracts (78.1% and 84.62%), respectively, with the same concentration compared to the standard reference ascorbic acid which showed a significant radicals scavenging potential (79.1%) in the concentration of 1µg/ml.

***Key Words*:** antimicrobial, antioxidant, DPPH, *P.* x *fragrans, P. peltatum, Geraniaceae*.

INTRODUCTION

The genus *Pelargonium* (Family: Geraniaceae) comprises over 280 species of perennial small shrubs. *Pelargonium* species are native to South Africa and commercially grown in Egypt (Mabbereley, 1997). Traditionally, *Pelargonium* was used to staunch bleeding, heal wounds, ulcers, uterine hemorrhage and skin disorders, as well as to treat diarrhea and dysentery due to its tannins content (Bown, 1995; Kokkalou and Souleles, 1988). Plants of genus *Pelargonium* were also successfully employed in modern phytotherapy for their antioxidant (Latte and Kolodziej, 2004), antimicrobial (Lis-Balchin and Deans, 1996), and immunomodulatory effects (Kayser *et al.*, 2001). The reported pharmacological studies on the genus and Clinical trials investigating its use as phytopharmaceutical preparations, indicated that *Pelargonium* species proved to be effective in treatment of many diseases depending on their antimicrobial activities as acute bronchitis (Theisen and Muller, 2012), peptic ulcer due to *Helicobacter pylori* (Wittschier *et al.*, 2007). *P.* x *fragrans* Known as Nutmeg-Scented geranium is a perennial small shrub with Spicy, pine-like fragrance, It has three lobed heart shaped grayish green leaves having trailing clusters of very small whitish flowers with pink veins (Bailey, 1949). Whereas *P. peltatum* (ivy-leaf geranium) is Perennial plant, climbing herbaceous slender-stemmed Leaves peltate, entire, bluntly lobed Flowers umbel-like inflorescences mauve, pink- mauve or almost white Although certain species of the genus *pelargonium* had attracted the attention of several authors from the Phytochemical and biological point of view, reviewing the current literature reports on the plant were scanty, so it deemed interesting to investigate the biological activities of the plant, aiming to verify the possible medicinal use. The present article reports the results of biological studies of the total extracts and their fractions thereof (petroleum ether, chloroform, ethyl acetate and n-Butanol fraction) of *P.* x *fragrans* and *P. peltatum* cultivated in Egypt.

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MATERIALS AND METHODS

Plant material

The aerial parts of *P.* x *fragrans* Willd was collected from The Experimental Station of Medicinal and Aromatic Plants, Faculty of Pharmacy, Cairo University, Giza, Egypt in January 2010. *P. peltatum* L'Hérit was collected from EL-Sharkiya governorate, Egypt in March 2011. The identity of the plants was kindly confirmed at Flora and Phytotaxonomy Department, Horticultural Researches Institute, Agricultural Research Center, Dokki-Cairo, Egypt. Voucher specimens are kept at Department of Pharmacognosy, Faculty of Pharmacy, October 6 University (# PF 7010 and # PP 8011).

Preparation of the Extracts

Air-dried powdered leaves of *P. x fragrans* (200 gm) and (100 gm) of *P. peltatum* were successively extracted using a soxhlet apparatus for continuous extraction using petroleum ether, chloroform, ethyl acetate and n-Butanol, till exhaustion. For each solvent, the extraction was continued until no residue was obtained on evaporation of a small aliquot of the colorless extract to dryness in a watch glass. Before using the next solvent, the powder was taken out of the soxhlet, carefully spread on a sheet of paper and traces of solvent-free powder was packed in the same soxhlet and extracted with the following solvent in the order mentioned before. For each extract, the solvent was removed under reduced pressure at a temperature not exceeding 40°C. The extracts were dried

Table 1: Yield of extracts of successive extraction.

Successive extracts	Yield percentage	
	P. x fragrans	*P. peltatum*
Petroleum ether	3.22	3.8
Chloroform	2.4	0.3
Ethyl acetate	4.2	5.3
n-Butanol	8.5	11.7

to constant weight in vacuum desiccators over calcium chloride and kept for further biological studies (table 1).

Chemicals

Organic solvents: petroleum ether, chloroform, ethyl acetate, n-Butanol and Dimethyl formamaide; purchased from (Adwic, Nasr Pharma, Egypt), DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical, purchased from Sigma Co., USA., Standardized extract of Vitamin.C. , purchased from Memphis Co., Egypt.

Antimicrobial Activity

Plant extracts were tested against two standard Gram-positive bacteria: *Staphylococcus aureus* ATCC 25923 and *Bacillus subtillus* NCIB-3610; two standard Gram negative bacteria: *Escherichia coli* ATCC 25992 and *Pseudomonas aeruginosa* NCIB9016; and the fungal strains were (*Aspergillus nige*r Ferm- BAM C- 21 and *Candida albicans* ATCC 48274. The culture media used for bacteria was nutrient agar medium, malt extract agar medium used for cultivation of *Candida albicans* and Czapeks Dox agar medium was used for *Aspergillus niger*.

Antimicrobial activity test: The strains were first checked for purity on the basis of standard microbiological tests and then used for their sensitivity on the test samples. The bacteria and fungi were maintained on nutrient agar medium and Czapeks Dox agar medium, respectively. The agar media were inoculated with different test microorganisms. The potential antimicrobial activities of the different plants extracts were studied through determination of the zones of inhibition by agar well method (Cooper, 1972). 100µl of each extract diluted with 100µl of DMF and only 50 microliter of solubilized extract was placed in the well. After 24 hours of incubation at 30°C for bacteria and 48 hours of incubation at 28°C for fungi. Inhibition zones were measured to the nearest mm using vernier calipers.

Standards with the concentration of 1 mg/ml were used as positive controls miphincol (Misr Co, Egypt) for Gram positive, Keflix (Nile Co, Egypt) for Gram negative bacteria, flucoral (Sedico Co, Egypt) for fungi.

Antioxidant activity

DPPH radical scavenging activity: The dried extracts were dissolved in ethanol 90% to a final concentration of 100 µg ml-1 (sample stock solution), then the different concentration of each sample (0.05, 0.1, 0.15, 0.2 and 0.25 µg/ ml) were prepared. Several trials had to run to determine the solution concentration that provides an appropriate range of date points. The standard extract of ascorbic acid was prepared in 90% ethanol to a final concentration 1mg/ml and then diluted to serial dilutions of (0.2, 0.4, 0.6, 0.8 , 1 µg/ ml) to be used as positive controls.

The reaction mixture for each of the fractions consisted of 1ml of 0.125 Mm DPPH/ethanol solution, 0.5 ml of 0.05M Tris-HCL buffer (pH7.4) and 1.5 ml of the tested fraction. Decrease in DPPH free radical was measured by reading the absorbance at 517 nm at room temperature exactly 30 seconds after adding each dilution to the reaction mixture for the tested dilution, that dilution was added to absolute ethanol and buffer to be used as blank

Table 2: Antimicrobial activity studies showing mean diameter of inhibition zones of various fractions of test plants.

Plant	Microorganism / Tested fraction	Mean diameter of inhibition zone (mm)					
		G +ve bacteria		G -ve bacteria		Fungi	
		B. subtillus	*E. coli*	*S. aureus*	*P. aeruginosa*	*C. albicans*	*A. niger*
P. peltatum	Petroleum ether	11.5	12	15	14	11	21
	Chloroform	11	15	16	11	15	-
	Ethyl acetate	14	16	19	18	14	-
	n-Butanol	14	20	20	20.5	16	-
P. x fragrans	Petroleum ether	18.5	16	17	19	13	-
	Chloroform	13.5	14	14.5	14	15	-
	Ethyl acetate	16	17	19	16.5	15	-
	n-Butanol	15.5	20	18	18	14	-
	Standards	31.5(a)	30(a)	32(b)	37.5(b)	25(c)	23(c)

a: standard for G +ve bacteria (Miphinicol); b: standard for G -ve bacteria (Keflix); c: standard for fungi (Flucoral)

Table 3: Free radical scavenging activity of different extracts of *P. x fragrans* and *P. peltatum* using DPPH photometric method.

Plant	Fraction	% Scavenging of DPPH radical				
		0.075µg/ml	0.15µg/ml	0.25µg/ml	0.3µg/ml	0.375µg/ml
P. x fragrans	Petroleum ether	N.D	N.D	N.D	N.D	N.D
	Chloroform	2.63±0.002	10.95±0.003	20.88±0.001	35±0.001	40±0.003
	Ethyl acetate	28±0.004	57.77±0.003	74.88±0.003	76.97±0.001	79.25±0.003
	n-Butanol	37.47±0.002	69.15±0.001	80.23±0.001	82.91±0.001	85±0.002
	Total extract	34.2±0.004	59.3±0.004	71.2±0.002	75±0.002	78.1±0.002
P. peltatum	Petroleum ether	N.D	N.D	N.D	N.D	N.D
	Chloroform	58.65±0.004	70.1±0.003	71.71±0.002	74±0.004	77±0.003
	Ethyl acetate	21.67±0.003	53.93±0.003	60.91±0.004	75.91±0.001	79.72±0.001
	n-Butanol	69.24±0.001	79.58±0.001	84.37±0.002	84.43±0.002	85.2±0.001
	Total extract	58.17±0.002	69.62±0.003	79.57±0.003	83.5±0.003	84.62±0.002
	Vit-C	0.2µg/ml	0.4µg/ml	0.6µg/ml	0.8µg/ml	1µg/ml
		15.54%±0.001	31.51%±0.001	48.18%±0.003	64.15%±0.001	79.1%±0.001

N.D= not detected; (Mean ±SEM) of triplicates

while DPPH solution was added to absolute ethanol and buffer to be used as negative control.

The % of antioxidant activity (%AA) was deduced from the following equation (Munir, 2003):

$$\%AA = 100 - \left\{\frac{(Abs\ sample - Abs\ blank)}{Abs\ control} \times 100\right\}$$

RESULTS AND DISCUSSION

Total extracts of air-dried samples of *P. peltatum* L'Hérit, *P.* x *fragrans* Willd and their fractions were subjected to biological investigation. Table 2 summarizes the antimicrobial activity of the investigated extracts; the petroleum ether fraction of *P. peltatum* showed a powerful antifungal activity against *A. niger*, it has almost the same activity of the standard flucoral. Ethyl acetate and n-Butanol fractions of both species have a significant activity against all the tested bacteria especially against *E. coli*, *S. aureus*. Chloroform fractions of both *P. peltatum* and *P.* x *fragrans* had a moderate activity for all tested microorganism. The significant antibacterial activity of the ethyl acetate and n-Butanol fractions of both species may be attributed to their high phenolic content (Fauconneau, 1997).

Where the antioxidant activity revealed at table 3 that free radical scavenging property was found to be in a concentration dependent manner in all the tested fractions. The most effective antioxidant fractions of *P.* x *fragrans* and *P. peltatum* was the n-Butanol fraction (85% and 85.2%) at the concentration of 0.375µg/ml followed by the total ethanolic extracts (78.1% and 84.62%) respectively, with the same concentration compared to the standard reference ascorbic acid which showed a significant radicals scavenging potential (79.1%) in the concentration of 1µg/ml. The high activity of the n-Butanol fraction is probably due to the presence of phenolic substances, where DPPH free radical abstracts the phenolic hydrogen of the electron donating molecule (Fauconneau, 1997).

CONCLUSION

This study presents the free radical scavenging and antimicrobial activities of *P. peltatum* and *P.* x *fragrans*, the preliminary microbiological investigation encourages establishing further studies on broader range of microbial strains and, the above study was effective for selection of the most effective antioxidant fraction (n-butanol), furthermore detailed studies on the chemical composition of that fraction is essential to isolate antioxidant agents. It could be concluded that the plants is of phytopharmaceutical importance and studies with these plants may yield nature friendly strong antioxidant, anti-fungal and antibacterial agents of medicinal importance.

REFERENCES

Bailey, L.H. (1949). Manual of cultivated plants most commonly grown in the continental United States and Canada. Macmillan, New York, U.S.A". P.597.

Bown,D. (1995). "Encyclopedia of herbs and their uses", The Herb Society of America, (1st Ed., P.172) Dorling Kindersley Ltd.

Cooper, K.E. (1972). "Analytical Microbiology F.W. Kavanageh, (E.D). Vol. I &II, Academic press, New York and London.

Fauconneau, B., Waffo,T.P., Huguet, F., Barrier, L., Decendit, A., Merillon, J.M.(1997). Comparative study of radical scavenger and antioxidant properties of phenolic compounds from *Vitis vinifera* cell cultures using *in vitro* tests. Life Sci. 61 (21). [DOI]

Kayser, O., Kolodziej, H., Kiderlen, A.F. (2001); "Immunomodulatory Principles of *Pelargonium sidoides*.", Phytother. Res., 15(2), 122-126. [DOI] PMid:11268110

Kokkalou, E. and Souleles, C. (1988). " Flavonoid Constituents of *Pelargonium x asperum* Enrh. ex Willd. Geraniaceae." Plant Med. Phytother., 22(4), 247-253.

Latte, K.P. and Kolodziej, H. (2004) "Antioxidant properties of phenolic compounds from *Pelargonium reniforme*.", J. Agric. Food Chem., 52(15), 4899-4902. [DOI] PMid:15264932

Lis-Balchin, M. and Deans, S.G. (1996). "Antimicrobial effects of hydrophilic extracts of *Pelargonium* species (Geraniaceae). Letters in Applied Microbiology. 23 (4): 205–207. [DOI] PMid:8987691

Mabbereley, D.J. (1997). "The plant Book", (2nd Ed., P.536). Publisher: Cambridge University Press.

Munir, O., Ihami, G., Rafn, O. and Kufrevio, L. (2003) "Determination of In vitro antioxidant activity of Fennel (*Foeniculum vulgare*) Seed Extracts." Lebensmittel-Wissenschaft und Technologi, 36(2), 263-271. [DOI]

Theisen, L.L. and Muller, C.P. (2012). "EPs® 7630 (Umckaloabo®), an extract from *Pelargonium sidoides* roots, exerts anti-influenza virus activity *in vitro* and *in vivo*." Antiviral Research. 94(2): 147-56. [DOI] PMid:22475498

Wittschier, N., Faller, G. and Hensel, A. (2007). An extract of *Pelargonium sidoides* (EPs® 7630) inhibits in situ adhesion of *Helicobacter pylori* to human stomach. Phytomedicine. 14(4):285-8. [DOI] PMid:17350240