

Standardization of Polyphenol Content for certain Species Belonging to Genus *Pelargonium* cultivated in Egypt

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ABSTRACT

Traditionally, *Pelargonium* species due to its tannin contents; was used to staunch bleeding, heal wounds, ulcers, uterine hemorrhage, a comparative study was carried out on *Pelargonium peltatum* L'Hérit and *Pelargonium x fragrans* In order to incorporate these two active extracts into pharmaceutical preparations, they need to be standardized by quantitative means. The total polyphenol contents of the aqueous extract of both plants were determined by Folin-Ciocalteu assay for estimation the total polyphenol (TP) contents of the aqueous extract. TP were expressed as mg of gallic acid equivalents (GAE)/g of the dry plant material, revealed that it was relatively high in both species representing 12.46 and 12.67 mg GAE/g dry weight respectively.

Keywords: total phenolics, Folin-Ciocalteu, *Pelargonium*, *Geraniaceae*

INTRODUCTION

There has been an increased movement towards a more "green" ideology in recent years, due to renewed interest in herbal and homeopathic medicines, making it a favored healthcare choice.

The genus *Pelargonium* (Family: *Geraniaceae*) contains 280 species; most of them are native to South Africa and few in Tropical Africa (Mabberley, 1997). Phytochemical studies concerning *Pelargonium* species had attracted the attention of many authors. It has been reported that species are rich in essential oils (Lalli et al. 2006), phenolic constituents including flavonoids (Kokkalou and Souleles, 1988), tannins (Kolodziej et al. 1995), phenolic acids (Contour and Louquet. 1985). Traditionally, due to its tannin contents; was used to staunch bleeding, heal wounds, ulcers, uterine hemorrhage and skin disorders, as well as to treat diarrhea, dysentery (Bown, 1995). *Pelargonium* spp. were also successfully employed in modern phytotherapy for their antioxidant (Latte and Kolodziej, 2004), antimicrobial (Lis-Balchin et al. 1998) and immunomodulatory effects (Kayser et al. 2001), haemostatic effects (Páez and Hernández, 2003). *Pelargonium x fragrans* Willd. known as Nutmeg-Scented, it is a perennial small flowering shrub growing to about one foot high (Bailey, 1949). The fresh leaf juice is considered to be antiseptic and astringent and of use in the treatment of sore throat and ulceration of oral mucosa in form of gargle preparation (Lis-Balchin and Deans, 1996). *Pelargonium peltatum* L'Hérit is a climbing herbaceous slender-stemmed perennial plant, containing tannins such as pelargonin A (Latté, 1999). The leaf sap has been taken for sore throats and the leaves have been used as an antiseptic (Miller, 1996). Several methods were used for quantitative estimation of tannin content in plants including the gravimetric method for determination of the total tannin content adopting the hide powder method (Harinder, 1989). In order to incorporate these two active extracts into pharmaceutical preparations, they need to be standardized by quantitative means. The total polyphenol contents of the aqueous extract of both plants were determined by Folin-Ciocalteu assay.

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. *P. peltatum* L'Hérit was collected from EL-Sharkiya governorate, Egypt in September 2014. 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 6 October University (# PF 7010 and # PP 8011).

Preparation of the plant extracts

Crude polyphenol (CPP) fraction was prepared by the following procedure (Fig.1). The powdered dried plant material (50gm) were extracted with cold water and allowed to stand for 12 hr at 3 °C. The extract was filtered

to remove sugars present in the plant. This extraction procedure was performed twice. Then the residue of each plant was extracted with water at room temperature and boiled for 10 min with stirring. The sample was allowed to stand for 12 hr at 25 °C. After filtration, the filtrate was concentrated and dried with spraying to give CPP.

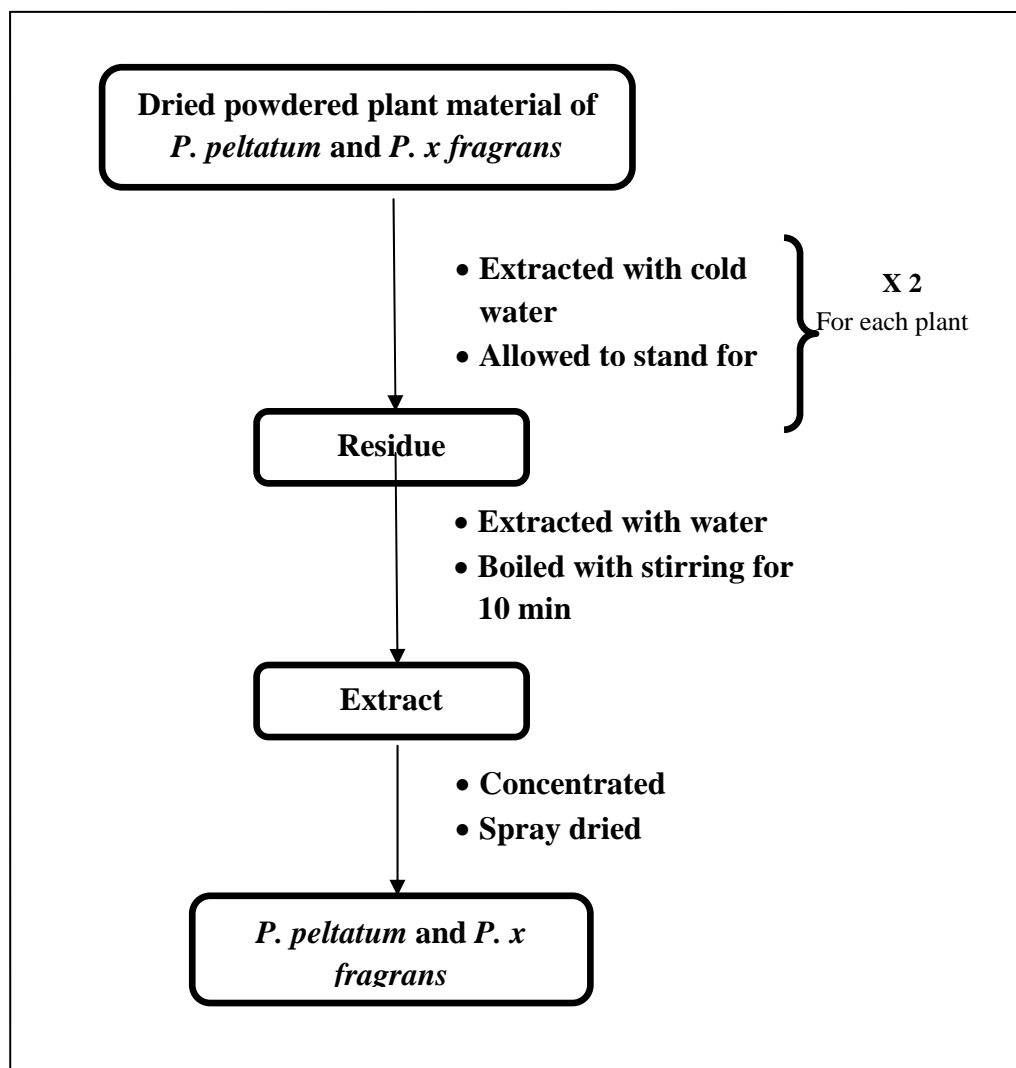


Fig (1): Scheme for preparation of the crude polyphenol of *P. peltatum* and *P. x fragrans*

Colorimetric determination and antioxidant activity of total polyphenol content of the aqueous extracts

Total polyphenol (TP) content of both plants was determined according to the procedure adopted by (Kumazawa *et al.*), and the procedure in the European Pharmacopoeia 4th edition (2002) using Folin-Ciocalteu colorimetric method. TP were expressed as mg of gallic acid equivalents (GAE)/g of the dry plant material.

Standard preparation

The standard solution was prepared by dissolving 50 mg of gallic acid in 100 ml distilled water (0.05% solution). A calibration curve was constructed over the range of 2-7 µg/ml by diluting the stock solution with distilled water.

Preparation of the standard calibration curve

Each concentration was prepared from the stock solution of the standard (0.5 ml) was mixed with 0.25 ml of Folin-Ciocalteu reagent, 10 ml distilled water were added and the mixture was diluted to 25 ml using 290g/L solution of sodium carbonate. After 30 minutes the absorbance was measured at $\lambda_{\max} = 730$ nm against blank prepared at the same time, using 0.5 ml distilled water as a compensating liquid.

Estimation of the total polyphenol content of the plants

The mean absorbance of the prepared aqueous extracts of *P. peltatum* and *P. x fragrans* was determined using the test extract instead of the standard solution. The results were compiled in Table (1). The gallic acid content of the tested extract could be calculated from the linear regression analysis.

The total polyphenol content calculated as gallic acid could be calculated from the following equation:

$$TP = C.V.m/M \text{ Where,}$$

TP = total polyphenol content in mg GAE/g dry weight.

C = concentration of gallic acid in the tested extract (established from the calibration curve in $\mu\text{g/L}$).

V = dilution factor.

m = total weight of CPP in grams.

M = weight of the dried plant material in grams.

Results and Discussion

Phytochemical studies concerning *Pelargonium* species had attracted the attention of many authors. It has been reported that species are rich in essential oils, phenolic constituents. Traditionally, due to its tannin contents; was used to staunch bleeding, heal wounds, ulcers, uterine hemorrhage and skin disorders, as well as to treat diarrhea, dysentery. In order to incorporate these two active extracts into pharmaceutical preparations, they need to be standardized by quantitative means. The total polyphenol contents of the aqueous extract of both plants were determined by Folin-Ciocalteu assay. TP (total polyphenol content), calculated as gallic acid, of the aerial parts of *P. peltatum* and *P.x fragrans* estimated in its respective aqueous extract by Folin-Ciocalteu method revealed that it was relatively high in both species representing 12.46 and 12.67 mg GAE/g dry weight respectively.

Table (1): Results of quantitative estimation of the total polyphenol content of the aqueous extract of *P. peltatum* and *P. x fragrans*

Item	Value	
	<i>P.peltatum</i>	<i>P. x fragrans</i>
M	50 g	50 g
m	1.978	1.98
V	50	50
Mean absorbance	0.596	0.603
C	6.3 $\mu\text{g/ml}$	6.4 $\mu\text{g/ml}$
TP	12.46 mg GAE/g dry weight	12.67 mg GAE/g dry weight

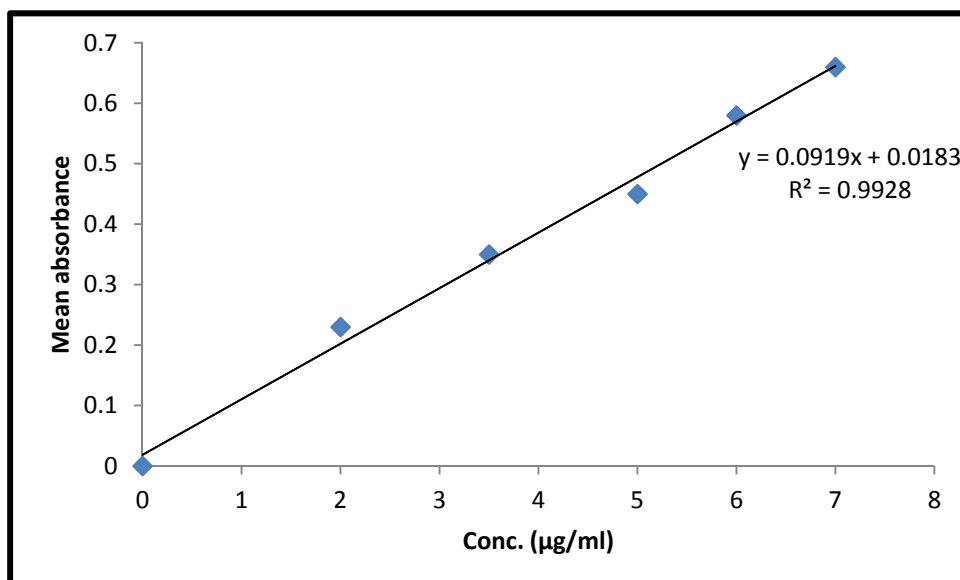


Figure (2): Calibration curve of standard gallic acid

REFERENCES

- [1] Mabberley, D.J. (1997). "The plant Book", (2nd Ed., P.536). Publisher: Cambridge University Press.
- [2] Lalli, J.Y.Y., Viljoen, A.M., Baser, K.H.C., Demirci, B. and Ozek, T. (2006): "The essential oil composition and chemotaxonomical appraisal of South African Pelargoniums (Geraniaceae)" J.Essent.Oil Res., 18,89-105
- [3] Kokkalou, E. and Souleles, C. (1988). "Flavonoid Constituents of Pelargonium x asperum Enrh.ex Willd. Geraniaceae." Plant Med. Phytother., 22(4), 247-253.
- [4] Kolodziej, H., Kayser, O. and Gutmann, M. (1995) "From The Zulu Medicine to the European Phytomedicine Umckalabo®", Dtsch.Apoth.Ztg., 135(10), 23-26,29-30,33-34.
- [5] Contour, A.D. and Louguet, P. (1985). Short-term effect of light on phenolic compounds in isolated leaf epidermis of Pelargonium hortorum. J. Plant Physiol., 120(3), 223-231.
- [6] Bown, D. (1995). "Encyclopedia of herbs and their uses", The Herb Society of America, (1st Ed., P.172) Dorling Kindersley Ltd.
- [7] Latte, K.P. and Kolodziej, H. (2004) "Antioxidant properties of phenolic compounds from Pelargonium reniforme.", J. Agric. Food Chem., 52(15), 4899-4902.
- [8] Lis-Balchin, M., Buchbauer, G., Ribisch, K., and Wenger, M.T. (1998). "Comparative antibacterial effects of novel Pelargonium essential oils and solvent extracts. Letters in Applied Microbiology. 27 (3): 135-41.
- [9] Kayser, O., Kolodziej, H., Kiderlen, A.F. (2001); "Immunomodulatory Principles of Pelargonium sidoides.", Phytother. Res., 15(2), 122-126.
- [10] Páez, X. and Hernández, L. (2003). "Topical hemostatic effect of a common ornamental plant, the Geraniaceae Pelargonium zonale. Journal of Clinical Pharmacology .43(3):291-295.
- [11] 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.
- [12] 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
- [13] Latté, K.P. (1999). "Phytochemische und pharmacologische Untersuchungen an Pelargonium reniforme Curt." PhD thesis, University of Berlin.
- [14] Miller, D. (1996). Pelargoniums: a gardener's guide to the species and their cultivars and hybrids. Portland, Timber Press.
- [15] Harinder Paul S. Makkar, (2004). "Protein precipitation methods for quantitation of tannins." J. Agric. Food Chem., 1989, 37 (4), pp 1197-1202
- [16] Kumazawa, S., Taniguchi, M, Suzuki, Y., Shimura, M., Kwon, M., and Nakayama, T. "Antioxidant activity of polyphenols in Carob pods" J. Agric. Food Chem. 2002, 50, 3733-377