Asian Pub. Corp.

www.asianpubs.org

ARTICLE

Physico-Chemical and Phytochemical Analysis of *Euphorbia prostrata*

Samra Farooq✉, Fizza Karamat and Sadia Chaman

Asian Journal of Organic & Medicinal Chemistry

Volume: 1 Year: 2016
Issue: 4 Month: October-December
pp: 126–131
DOI: https://doi.org/10.14233/ajomc.2016.AJOMC-P43

Received: 20 December 2016
Accepted: 9 January 2017
Published: 17 January 2017

ABSTRACT

The present study aimed to standardize the crude drug from *'Euphorbia prostrata'* by doing qualitative and quantitative analysis of plant pulverized parts and extracts. Physico-chemical analysis such as moisture content, total ash, water insoluble ash, sulphated ash, acid insoluble ash and water and alcohol extractives was done on powdered raw material. The moisture content and ash values were found within the recommended range (moisture content 5 % and ash value 10.5 %). The value of water soluble extract was higher as compared to acid soluble extractives. Percentage yield was found to be highest in methanolic solvent. The phytochemical analysis *i.e.* total lipids, total proteins and carbohydrates of crude powder showed that lipids and proteins contents were high.

KEYWORDS

Euphorbia prostrata, Primary and secondary metabolites, Quantitative analysis.

INTRODUCTION

The conventional use of plants in the form of crude drugs and extracts has played an important role in the maintenance and restoration of health. Pure components isolated from plants have served as pharmacologically active lead compounds that are responsible for the development of greater number of new and modern medicines [1]. The importance of natural products is apparent from a report indicating that 75 % population of world has the experience of using such products [2]. Preliminary phytochemical screening has been done in order to have an idea of the different classes of organic compounds present in different extracts of each plant [3].

The spurge family or *Euphorbiaceae* is categorized among the most diversified and largest family of flowering plants. The name 'spurge' is derived from Medieval French 'epurger', referring to the purgative properties of the seeds of genus *Euphorbia.* The plant namely '*Euphorbia prostrata'* belongs to the family *Euphorbiaceae*. The family *Euphorbiaceae* also known as spurge family is considered to be sixth largest family. There are almost 8000 species, 300 genera, 49 tribes and 5 subfamilies constituting the spurge family [4]. The family includes all types of plants comprising the large woody trees, climbing lianas and simple weeds that have a prostate habitation. The family is expanded across the tropical areas with the worldwide distribution in Indo-Malayan region and the second

Author affiliations:

Institute of Pharmaceutical Sciences, University of Veterinary and Animal Sciences, Lahore, Pakistan

✉To whom correspondence to be addressed:

Tel: +92 315 2546968
E-mail: samrach77@yahoo.com

Available online at: http://ajomc.asianpubs.org

most distribution of the species is in tropical America [5]. *Euphorbia prostrata* is analogous to *Euphorbia chamaesyce* and *Euphorbia maculata* and all these three species appear in similar type of habitat and they can be confused easily. *Euphorbia prostrata* can be easily identified among the stated species because of hairy capsule on the keels only [6].

Mishra *et al.* [7] reported physico-chemical parameters of *Euphorbia prostrata* plant powder, which showed that plant has 7.4 % moisture content, 10.72 % ash content, 4.22 % acid insoluble ash, 8.56 % water solubility and 13.76 % ethanol solubility. Another study was conducted and it reports physico-chemical parameters and qualitative chemical tests of *Euphorbia prostrata* plant extract. Fluorescence behaviour of plant powder was observed using 1 N HCl, picric acid, acetic acid, 50 % sulphuric acid, 1 N sodium hydroxide, methanol and 1 N nitric acid, under visible and short UV light (254 nm) as well as long UV light (365 nm) [8].

Pharmacological activities have been analyzed for *Euphorbia prostrata*. *Euphorbia prostrata* is anti hemorrhoidal [8]. Beneficial effects of *Euphorbia prostrata* in hemorrhoids have multiple mechanisms and these are due to active constituents like flavonoids, phenolic acids and tannins [9]. Other pharmacological studies have also been performed like antibacterial and anti-fungal, *etc.*

EXPERIMENTAL

The chemicals and solvents were of analytical grade. The fresh mature *Euphorbia prostrata* plant was collected from Botanical Garden of Institute of Pharmaceutical Sciences, Lahore and identified by Prof. Dr. Zaheer-ur-Din Khan, Department of Botany, Government College University, Lahore, Pakistan, whereby a voucher specimen was deposited vide reference no. GC/Herb/Bot/2902. The whole plant was washed with water to remove dirt and unwanted matter. It was then shade dried, after drying will pulverized to fine powder and stored in amber coloured bottles.

Proximate analysis of powdered material: Proximate analysis of material was performed according to the specifications of USP [10].

Moisture contents: Powdered plant material (2 g) was weighed in a tarred china-dish. This china-dish was kept in oven for 30 min at 105 °C for drying the plant material. After removing from oven, it was put in desiccator for cooling purpose. Then the cooled china-dish was weighed on digital balance and the weight of dried material was calculated by subtracting the empty china-dish weight from china-dish plus dried material weight. Moisture contents were calculated as follows:

Moisture contents = 100 – Weight of dried material

Total ash: 2 g Powder plant material was weighed in a tarred china-dish. Then it was incinerated in furnace at temperature 675 ± 25 °C for the duration until ash got free from carbon. After getting desired form of ash, china-dish was placed in desiccator to cool its contents. At the end the ash contents were weighed and the percentage of total ash was calculated with reference to sample weight.

Acid insoluble ash: The total ash contents obtained from 2 g of powdered plant material were boiled in 25 mL dilute HCl for 5 min. The boiled material was filtered through ash less filter paper. The soluble matter was collected as filtrate and insoluble material as residue on filter paper and it was washed with hot distilled water to ensure that all soluble material had been removed. This filter paper was then dried and ignited in tarred china-dish for the time period until ash got free from carbon. Then it was cooled in desiccator. The ash contents were weighed and the percentage of acid insoluble ash was calculated with reference to weight of total ash used in test.

Acid soluble ash: Acid soluble ash was calculated by subtracting the contents of acid insoluble ash from total ash dissolved in 25 mL of HCl.

Water insoluble ash: The total ash contents obtained from 2 g of powdered plant material were boiled in 25 mL distilled water for 5 min. The boiled material was filtered through ash less filter paper. The soluble matter was collected as filtrate and insoluble material as residue on filter paper and it was washed with hot distilled water to ensure that all soluble material had been removed. This filter paper was then dried and ignited in tarred china-dish for the time period until ash got free from carbon. Then it was cooled in desiccator. The ash contents were weighed and the percentage of water insoluble ash was calculated with reference to weight of total ash used in test.

Water soluble ash: Water soluble ash was calculated by subtracting the contents of water insoluble ash from total ash dissolved in 25 mL distilled water.

Sulphated ash: Sulphuric acid was mixed with 2 g of powdered plant material in a tarred china-dish to make a paste like material. This china-dish was ignited gently till white fumes stop originating from the surface of the material then it was cooled in desiccator. The ash contents were weighed and the percentage of sulphated ash was calculated with reference to weight of dried powdered plant material used in test.

Alcohol soluble extractives: 5 g powdered plant material was put in tarred flask. Ethanol 95 % (100 mL) was poured on it for maceration. The sample was macerated in a closed flask for 24 h with continuous stirring. The contents were filtered and 25 mL filtrate was evaporated to dryness in china dish and the residue was dried in oven at 105 °C and weighed. The percentage of alcohol soluble extractives was calculated with reference to weight of sample.

Water soluble extractives: 5 g Powdered plant material was put in tarred flask. Ethanol 95 % (100 mL) was poured on it for maceration. The sample was macerated in a closed flask for 24 h with continuous stirring. The contents were filtered and 25 mL filtrate was evaporated to dryness in china dish and the residue was dried at 105 °C in oven and weighed. The percentage of alcohol soluble extractives was calculated with reference to weight of sample [11].

Estimation of fat content: 15 g Powder material was subjected to hot extraction using petroleum ether as solvent. Solvent macerated the sample in thimble for 12 h and extraction was carried out for 24 h. Temperature was maintained within the range of 40-60 °C during the extraction procedure. After 24 h extraction, filtration was done and filtrate was dried in tared flask using rotary evaporator at 40 °C. After drying all the excess solvent, again flask was weighed and lipids content were calculated [11].

Estimation of protein content: Total protein estimation was performed by a method of Lowry *et al.* [12] with some

modification. 1 g crude powder was macerated with 10 mL of distilled water and 3-4 drops of Triton-X 100 for 10 h. The mixture was then transferred into centrifuge machine and then centrifuged for 10 min at 2700 rpm. Then, 0.1 mL of supernatant was taken in a test tube and volume was made 1 mL with distilled water and then 0.2 mL Folin-Ciocalteau reagent and 3 mL reagent C [prepared by mixing 50 mL reagent A (2 % sodium carbonate in 0.1 N sodium hydroxide) and 1 mL reagent B (0.5 % copper sulphate in 1 % potassium sodium tartarate)]. Afterwards, the tube was kept for 30 min and analyzed at 650 nm against blank containing all reagents except sample. Bovine serum albumin (1 mg/mL) was used as a standard in range of 0.01, 0.02, 0.04, 0.06, 0.1, 0.15, 0.2, 0.3, 0.6, 0.8 and 1 mg/mL. Total protein content was calculated by linear regression equation obtained from standard curve.

Estimation of total carbohydrates: Total carbohydrate was calculated by the following formula [13].

Total carbohydrate = 100 – (sum of percentage of moisture, ash, protein and fats)

Qualitative and quantitative analysis of extract

Extraction: The pulverized dried powder of *Euphorbia prostrata* (350 g) were extracted successively in petroleum ether (40-60 °C), chloroform and methanol by using 1000 mL of each solvent for soaking. Maceration was carried out in each solvent for four days with occasional shaking at room temperature (25 ± 2.5 °C) and then filtered. The solvent of each extracted material was removed under reduced pressure and temperature (35 ± 5 °C) by using rotary evaporator.

Molisch's test for carbohydrates: Few drops of Molisch's reagent were added to each of the portion of extract, dissolved in distilled water; this was then followed by addition of 1 mL of concentrated sulphuric acid by the side of the test tube. The mixture was then allowed to stand for 2 min and then diluted with 5 mL of distilled water. Formation of a red or dull violet colour at the interphase of the two layers indicated the presence of carbohydrates [14].

Test for alkaloids: The extract was evaporated to dryness and the residue was heated on a boiling water bath with 2 % hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent. The reddish brown or yellow precipitates with turbidity indicated the presence of alkaloids [15].

Test for tannins: 2 mL of extract was added to few drops of 1 % lead acetate. A yellowish precipitate indicated the presence of tannins [16].

Borntrager's test: About 0.2 g of extract was shaken with 10 mL of benzene and then filtered. Five milliliters of the 10 % ammonia solution was then added to the filtrate and thereafter the shaken. Appearance of a pink, red or violet colour in the ammoniacal (lower) phase indicated the presence of free anthraquinones [14].

Test for reducing sugars: To 0.5 mL of extract solution, 1 mL of water and 5-8 drops of Fehling's solution was added at hot. Appearance of brick red precipitate at the bottom of test tube indicated the presence of reducing sugars [15].

Test for saponins: About 2.5 g of the plant material was extracted with boiling water. After cooling, the extract was shaken vigorously to froth and was then allowed to stand for 15-20 min. Froth greater than 2 cm indicated the presence of saponins [17].

Test for flavonoids: 0.2 g extract was dissolved in diluted 10 % NaOH and 2 M HCl was added. A yellow solution that turns colourless indicated the presence of flavonoids [18].

Phlobotannins: Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1 % aqueous hydrochloric acid was taken as evidence for the phlobotannins [19].

Liebermann-Burchard test for steroids: To 0.2 g of each portion, 2 mL of acetic acid was added; the solution was cooled well in ice followed by the addition of concentrated sulphuric acid carefully. Colour development from violet to blue indicated the presence of a steroidal ring *i.e.* aglycone portion of cardiac glycoside [14].

Keller-kiliani test: About 0.5 mL of extract was taken and subjected to following test. 1 mL of glacial acetic acid containing traces of ferric chloride and 1 mL of concentrated sulphuric acid were added to the extract and observed for the formation of reddish brown colour at the junction of two layers and the upper layer turned bluish green indicated the presence of glycosides [20].

Test for terpenoids: 0.2 g of the extract was mixed with 2 mL of chloroform and concentrated 6 M sulphuric acid (3 mL) was carefully added forming a layer. A reddish brown colouration of the interface indicated the presence of terpenoids [18].

Estimation of total glycosaponins: 1 g Extract was dissolved in 50 mL methanol and refluxed for 30 min and filtered. Excessive methanol in filtrate was removed by rotary evaporator and the filtrate was concentrated to 10 mL. This concentrated extract was added drop wise to 50 mL acetone in a tared beaker. Then saponins precipitates appeared in the beaker. The precipitates were dried in oven at 100 °C to constant weight and glycosaponins were calculated by dividing the weight of precipitate with weight of extract and multiplied by 100 [21]. The test was repeated in triplicate.

$$\text{Glycosaponins} = \frac{\text{Weight of precipitate}}{\text{Weight of sample}} \times 100$$

Estimation of total proteins: 50 mg extract was mixed with 10 mL distilled water in centrifuge tube. After vortex for 2 min, tube was centrifuged at 2700 rpm for 10 min. Then 0.1 mL of supernatant was taken in a test tube and the volume was made up to 1 mL with distilled water. Then 3 mL of reagent C [Reagent C was prepared by mixing 50 mL of reagent A and 1 mL of reagent B. Reagent A composed of 2 % Na_2CO_3 in 0.1 N NaOH and reagent B was prepared by mixing 0.5 % $CuSO_4$ in 1 % potassium sodium tartarate] and 0.2 mL of Folin-Ciocalteau reagent were added to this test tube and it was then incubated for 30 min at room temperature and absorbance was recorded at 600 nm. Bovine serum albumin [BSA] (Fraction V) solution was used as standard. Blank was prepared similarly containing all the reagents except sample. Bovine serum albumin was used in different concentration range in order to plot standard curve. Total proteins content were calculated from the standard curve using linear regression equation [11]. The test was repeated in triplicate.

Estimation of total tannins: The contents of total tannins were estimated according to method of Heimler *et al.* [22] with some modification. Add 2 mL of vanillin solution (1 g vanillin in 100 mL of concentrated sulphuric acid) in 1 mL extract solution. After incubation at room temperature for 15-20 min, absorbance was measured at 500 nm against blank having all reagents except sample and standards. Gallic acid (1 mg/mL) was used as a standard in range of 0.1, 0.2, 0.3, 0.4, 0.5, 1 and 2 mg/mL to construct standard curve for estimation of tannins [11]. All samples and standards were analyzed in triplicate.

Estimation of total flavonoids: Quercetin (QTN) was used to draw the standard curve. Sample (0.2 mL) and standard (0.2 mL) were taken in test tubes and10 % aluminum nitrate solution (0.1 mL), 1 M potassium acetate (0.1 mL) and 4.6 mL distilled water were added to them. The test tubes were incubated at room temperature for 45 min. Blank was prepared similarly except analyte. Absorbance was measured at 415 nm. The flavonoid contents were determined as mg of quercetin equivalents by linear regression equation [11], obtained from the calibration curve of quercetin. Total flavonoids contents were calculated by following equation:

Total flavonoids = QTN equivalents (μg/mL) × Extract volume/Sample (g)

Estimation of total polyphenols: Singleton and Slinkard method [23] with minor changes was used for phenolic contents determination. Gallic acid was used to draw the standard curve. Sample (0.2 mL) and standard (0.2 mL) were taken in test tubes and Folin-Ciocalteu's phenol reagent (0.2 mL) was added to these test tubes and mixed them thoroughly. 1 mL of Na_2CO_3 solution (15 %) was added after 4 min. Then mixture was allowed to stand at room temperature for 2 h and at 760 nm absorbance was recorded. Blank used contained all the reagents except analyte. Gallic acid (0.1 mg/mL) was used as a standard in range of 0.004, 0.006, 0.008, 0.01 and 0.02 mg/mL. Total polyphenolics contents were determined as mg of gallic acid equivalents by linear regression equation [11], obtained from gallic acid standard curve. The total polyphenolics contents were estimated using following equation.

Total phenols = Gallic acid equivalents (μg/mL) × Extract volume/Sample (g)

RESULTS AND DISCUSSION

The results of physico-chemical properties like moisture content and ash values of crude powder of whole plant are given in Table-1.

Determination of the moisture content is important because it indicates the amount of water present in herbal material. The process of hydrolysis can be caused by the higher water content, the major process involved in the degradation of phytochemical constituents that not only result in decrease in efficacy but produced chemicals may be injurious to health [24]. In addition to this, it facilitates the microbial growth that completely destroys the medicinal value of the plants and contaminates the herb with their metabolites. Hence, it is always valuable to control the moisture contents to increase the stability of chemical constituents and to retain the medicinal importance of herbs. The observed moisture content value of *Euphorbia prostrata* plant powder is 5 %.

TABLE-1
PROXIMATE ANALYSIS OF *Euphorbia prostrata* PLANT POWDER

Physico-chemical properties	%	mg/g
Moisture content	5.0	50
Total ash	10.5	105
Acid insoluble ash	9.5	95
Acid soluble ash	1.0	10
Water insoluble ash	7.5	75
Water soluble ash	3.0	30
Sulphated ash	61.5	615
Water soluble extractive	3.0	30
Alcohol soluble extractive	2.6	26

Total ash test provides information regarding the amount of material remained after ignition that includes both physiological and non-physiological ash. Physiological ash is derived from the plant material and non-physiological ash is remaining part of the unwanted matter such as sand and oil that remain attached to surface of plant [25]. The ash values are helpful to determine the quality and purity of crude drugs. The purpose of ash test is to remove organic matter, thus on incinerating crude drugs leave an ash comprising carbonates, phosphates and silicates of potassium, sodium, magnesium and calcium. If crude drug contains high concentration of silica or calcium oxalate then acid insoluble ash is performed. Some analyst prefer mixing of sulphuric acid with powered crude drug before ashing because sulphated ash is normally less fusible than ordinary ash [26]. However, there have been a report stating that samples containing high percentage of ash content are expected to possess high level of mineral elements, which may accelerate metabolic reactions and improve the growth and development [27]. Sharma *et al*. [8] reported the values of total ash, water soluble ash, acid insoluble ash, sulphated ash and moisture content to be 10, 9.1, 0.9, 2 and 14 %, respectively. The observed total ash, acid insoluble, acid soluble, water insoluble, water soluble and sulphated ash values are in normal limits.

The results of total proteins, lipids and carbohydrates are mentioned in Table-2. The total proteins were determined by spectrophotometric method. The contents were calculated by standard curve of bovine serum albumin (1 mg/mL) solution in concentration of 0.01, 0.02, 0.04, 0.06, 0.1, 0.2, 0.5, 0.6, 0.7, 0.8 and 1 mg/mL. The linear regression equation was found to be $Y = 0.062x + 0.000$ with $R^2 = 0.992$. The contents of total lipids were determined by gravimetric method. The total carbohydrate contents were calculated by subtracting mean values of moisture content, total ash, lipids and proteins from hundred.

The estimation of proteins, lipids and carbohydrates indicated that the nutritional as well as commercial value of

TABLE-2
PRIMARY METABOLITES OF *Euphorbia prostrata* PLANT POWDER

Phytochemical contents	%	mg/g
Total proteins	9.30	93.0
Total carbohydrate	2.30	23.0
Total fat	6.69	66.9

Euphorbia prostrata plant. The contents of total protein, lipids and carbohydrates were determined which verified the result of Imaga *et al*. [28] that primary metabolites were present in *Euphorbia prostrata*.

Percentage yield of extracts: The observed extractive values showed that methanol has more extractive potential than chloroform and chloroform has more than petroleum ether (Table-3). High yield of methanol extract was obtained at the end of extraction, with green colour and hard texture. The high yield of methanolic extracts in plants may be due to stronger extraction capacity of methanol. All three extracts has same colour but different texture; methanolic and chloroform extract was hard but petroleum ether extract was gummy, which indicates that all three extracts are chemically different.

TABLE-3
PERCENTAGE YIELD OF *Euphorbia prostrata* EXTRACT

Properties	Methanol extract	Chloroform extract	Petroleum ether extract
Weight used for extraction (g)	350	350	350
Weight of extract (g)	39	29	24
Percentage yield (%)	11.1	8.2	6.8
Colour	Green	Green	Green
Texture	Hard	Hard	Gummy

Phytochemical qualitative analysis was carried out for petroleum ether, chloroform and methanol extract. The results of phytochemical screening of methanol and chloroform extract revealed the presence of alkaloids and flavonoids in both of the extracts, while reducing sugars, phlobotannins, saponins, tannins and steroids are only present in methanolic extract and absent in chloroform extract (Table-4). Petroleum ether extract does not show presence of any of these above mentioned phytochemical constituents.

TABLE-4
QUALITATIVE ASSESSMENT OF PHYTOCHEMICAL CONSTITUENTS OF *Euphorbia prostrata* PLANT EXTRACT

Tests	Methanol extract	Chloroform extract	Petroleum ether extract
Alkaloids	+	+	–
Reducing sugars	+	–	–
Saponins	+	–	–
Steroids	–	–	–
Tannins	+	–	–
Glycosides	+	–	–
Terpenoids	–	–	–
Flavonoids	+	+	–
Carbohydrates	+	–	–
Phlobotannins	+	–	–

The estimation of total glycosaponins was based on gravimetric analysis. The results indicate high percentage of glycosaponins in methanol extract. The higher contents of glycosaponins generate frothing that might be troublesome while handling, such problems can be minimized by adding anti-frothing agents or acids like HCl and tannic acid that break glycosidic linkage between glycine and aglycone.

Total tannins were investigated using linear regression equation (Y = 0.219x – 0.017), which was obtained from standard curve of gallic acid. The correlation coefficient (R^2) was found to be 0.993, which indicate good correlation between absorbance and concentration of standards.

Bitter taste of plants may be due to high content of tannins. Tannins possess astringent properties which accelerate the curative of wounds and swollen mucous membrane [29].

Total flavonoids were investigated using linear regression equation (Y = 13.20x + 0.375), which was obtained from standard curve of quercetin. The correlation coefficient (R^2) was found to be 0.995, which indicates good correlation between absorbance and concentration of standards.

Total polyphenols were investigated using linear regression equation (Y = 9.712x + 0.247), which was obtained from standard curve of gallic acid. The correlation coefficient (R^2) was found to be 0.993, which indicated good correlation between absorbance and concentration of standards.

The pharmacological activities of this plant are only due to the presence of these phytochemical constituents. Different phytochemical constituents have different therapeutic indications. It was reported that plant alkaloid and their synthetic derivatives were employed as fundamental medicinal agents, because of their analgesic, antispasmodic and antibacterial properties [30]. Similarly, saponins present in plant also have characteristic uses like as an expectorant and emulsifying agent [31]. On the other hand, their soap like characteristics makes them useful as surfactants and adjuvant for vaccines to augment macromolecule penetration [32] whereas steroids and terpenoids have shown the analgesic properties [33].

REFERENCES

1. A. Pandey and S. Tripathi, *J. Pharmacogn. Phytochem.*, **2**, 115 (2014).
2. N. Dubey, R. Kumar and P. Tripathi, *Curr. Sci. (India)*, **86**, 37 (2004).
3. A. Longanga Otshudi, A. Vercruysse and A. Foriers, *J. Ethnopharmacol.*, **71**, 411 (2000); https://doi.org/10.1016/S0378-8741(00)00167-7.
4. G.L. Webster, *Clin. Dermatol.*, **4**, 36 (1986); https://doi.org/10.1016/0738-081X(86)90062-3.
5. M.U. Saeed-ul-Hassan, *Afr. J. Pharm. Pharmacol.*, **7**, 2321 (2013); https://doi.org/10.5897/AJPP12.1027.
6. M. Milovic and M. Randic, *Natura Croat.*, **10**, 89 (2001).
7. S.M. Mishra, A. Pathak and P.K. Sharma,
8. S.K. Sharma, J. Singh and S. Singh, *Int. J. Pharm. Sci. Res.*, **3**, 1043 (2012).
9. G.D. Bakhshi, D.G. Langade and V.S. Desai, *Bombay Hosp. J.*, **50**, 577 (2008).
10. U.S. Pharmacopeial Convention, vol. 32 (2009).
11. U. Saleem, K. Hussain, M. Ahmad, N.I. Bukhari, A. Malik and B. Ahmad, *Pak. J. Pharm. Sci.*, **27**, 577 (2014).
12. O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
13. S. Al-Hooti, J.S. Sidhu and H. Qabazard, *J. Food Sci. Technol.*, **35**, 44 (1998).
14. H. Usman, F.I. Abdulrahman and A. Usman, *Afr. J. Trad. Complement. Altern. Med.*, **6**, 289 (2009).
15. H.P. Chhetri, N.S. Yogol, J. Sherchan, K. Anupa, S. Mansoor and P. Thapa, *Kathmandu Univ. J. Sci. Eng. Technol.*, **4**, 49 (2008).
16. N. Savithramma, M.L. Rao and D. Suhrulatha, *Middle East J. Sci. Res.*, **8**, 579 (2011).
17. F. Mojab, M. Kamalinejad, N. Ghaderi and H.R. Vahidipour, *Iran. J. Pharm. Res.*, **2**, 77 (2010).
18. M. Qaisar, S.N. Gilani, S. Farooq, A. Rauf, R. Naz, Shaista and S. Perveez, *Am.- Eur. J. Agric. Environ. Sci.*, **12**, 1056 (2012). https://doi.org/10.5829/idosi.aejaes.2012.12.08.1798.
19. I. Ajayi, O. Ajibade and R. Oderinde, *Res. J. Chem. Sci.*, **1**, 58 (2011).
20. A. Manjamalai, R.S.S. Singh, C. Guruvayoorappan and V.B. Grace, *Global J. Biotechnol. Biochem.*, **5**, 120 (2010).

21. K. Hussain, Z. Ismail, A. Sadikun and P. Ibrahim, *Nat. Prod. Radiance*, **7**, 402 (2008).
22. D. Heimler, P. Vignolini, M.G. Dini, F.F. Vincieri and A. Romani, *Food Chem*., **99**, 464 (2006); https://doi.org/10.1016/j.foodchem.2005.07.057.
23. K. Slinkard and V.L. Singleton, *Am. J. Enol. Vitic*., **28**, 49 (1977).
24. K.C. Waterman and R.C. Adami, *Int. J. Pharm.*, **293**, 101 (2005); https://doi.org/10.1016/j.ijpharm.2004.12.013.
25. World Health Organization, Quality Control Methods for Medicinal Plant Materials, Geneva, pp. 28-29 (1998).
26. K.R. Brain and T.D. Turner, The Practical Evaluation of Phytopharmaceuticals, Wright-Scientechnica Bristol (1975).
27. O.A. Ekpete, S.E. Onisogen and E.P. Fubara, *Br. J. Appl. Sci. Technol.*, **3**, 1447 (2013); https://doi.org/10.9734/BJAST/2014/4431.
28. N.A. Imaga, G.O. Gbenle, V.I. Okochi, S. Adenekan, T. Duro-Emmanuel, B. Oyeniyi, P.N. Dokai, M. Oyenuga, A. Otumara and F.C. Ekeh, *Sci. Res. Essays*, **5**, 2201 (2010).
29. P. Njoku and M. Akumefula, *Pak. J. Nutr.*, **6**, 613 (2007); https://doi.org/10.3923/pjn.2007.613.615.
30. J. Mbadianya, B. Echezona, K. Ugwuoke and R. Wokocha, *OSR J. Agric. Vet. Sci.*, **2**, 51 (2013).
31. R. Okigbo, C. Anuagasi and J. Amadi, *J. Med. Plants Res.*, **3**, 86 (2009).
32. D. Enyiukwu, A. Awurum, C. Ononuju and J. Nwaneri, *Int. J. Adv. Agric. Res.*, **2**, 8 (2014).
33. K. Salna, K. Sreejith, M. Uthiralingam, A.P. Mithu, M.J. Milton and T.F. Albin, *Int. J. Pharm. Pharm. Sci.*, **3**, 291 (2011).