Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(6): 346-350
Received: 07-09-2019
Accepted: 09-10-2019

M Madhavi
Department of Botany,
Hindu College, Guntur,
Andhra Pradesh, India

M Raghu Ram
Department of Botany,
Acharya Nagarjuna University,
Guntur, Andhra Pradesh, India

Corresponding Author:
M Madhavi
Department of Botany,
Hindu College, Guntur,
Andhra Pradesh., India

Biochemical and elemental analysis of *Syzygium samarangense* Roots

M Madhavi and M Raghu Ram

Abstract
The use of plants and phytochemicals, both with known biological properties can be of great significance in treatment. The present study includes biochemical and elemental analysis of root extract of *Syzygium samarangense*. Biochemical analysis includes the estimation of amino acids, proteins, soluble sugars, starch, crude fiber, crude fat, crude protein, total nitrogen, phosphorus, also analyzed dry matter, moisture, and ash. Elements and metals present in the roots were extracted using acid digestion method and the level of calcium, magnesium, iron, manganese, zinc, copper, and cobalt were estimated by using Atomic Absorption Spectrophotometer. Sodium and Potassium were estimated flame photometrically following the standard method of flame photometer. The results, confirm the presence of aforesaid biochemical compounds and elements in the selected plant. This report may be helpful for ethnomedicine practitioners, researchers, and pharmacognosists for further validation of *S. samarangense* in depth.

Keywords: *Syzygium samarangense*, roots, biochemical analysis, moisture analysis, mineral analysis

Introduction
Since ancient times, about 80% of individuals use traditional medicine, which has chemical compounds derived from medicinal plants. Several hundred plant species and herbs in the form of whole plant, crude extract or purification, purified constituents are used in indigenous system of medicines and are of great importance to the health of individuals and communities, which have ultimately evaluated into the modern therapeutic science. Medicinal plants are an important source of life saving drugs for majority of the world population (Madhavi and Ram, 2015) [1].

Syzygium samarangense (common name - wax jambu) is a plant species in the family Myrtaceae that is widely cultivated in the tropics [2-10]. There are many traditional claims that have been reported of leaves, root, bark, fruits of the plant [11-14]. Various pharmacological activities like antidiarrhoeal activity, anticholinesterase activity, immunopharmacological activity, cytotoxic activity, anti hyperglycaemic activity, analgesic and anti-inflammatory activity are reported with various parts of the plant [15-22]. And also Investigators have found their principal constituent to be tannins, Quercetin glycosides, monoterpenes secondary metabolites those involved in pharmacological properties [23, 24]. Traditionally the root bark decoction of the *S. samarangense* is used in dysentery and amenorrhea and used as an abortifacient. Root is used as a diuretic and is given to alleviate edema. Malayans use powdered dried root preparations for itching. As plant root has significant therapeutic uses, this study is aimed to screen the phytochemicals of the root and study of its biological activities

Materials and methods
Chemicals: The solvents used for root extraction are Methanol and Ethyl Acetate. Distilled water has been used for aqueous extraction.

Apparatus: Denver electronic balance, TECHCOMP – UV 2301 Double Bean UV Visible Spectrophotometer with HITACHI 2.2 software, Tech-comp UV visible spectrophotometer, soxhlet extraction apparatus, heating mantle, incubator, autoclave.

Sample collection: Root material of *S. samarangense* plants were collected from farms in various places of East Godavari district, Andhra Pradesh, India. The roots are separated and allowed to shade dry. The root sample was ground and powdered for solvent extraction.

Solvent extraction: The phytochemicals present in the roots of the collected plants were isolated using different solvents like ethyl acetate, methanol and water in a series of extraction method from low polarity to high polarity using soxhlet extraction method.

Biochemical and elemental analysis of root

Estimation of Amino acids

Trichloroacetic acid extraction (TCA) was performed on fish samples, by following the procedure set up by Boland *et al.* 1971 [25]. 200mg of sample was added to 10mL of 7.5% (w/w) TCA and minced by means of a vertical homogenizer. The resulting product was filtered with filter paper (No. 4) from Whatman filter paper. The pH of a 1 mL aliquot was adjusted to 7.8 using 9 M KOH in an Eppendorf microfuge tube and centrifuged at 14 K rpm for 5 min in order to remove potassium trichloroacetate precipitate. The so obtained supernatant was stored at −80 °C.

A free amino acid level was estimated by the ninhydrin method as described by *Moore and Stein* [26]. To 1mLof supernatant, 2.0 mL of ninhydrin reagent was added and the contents were boiled for exactly 5min. They were cooled under tap water and the volume was made to 10mL with distilled water. The optical density of the color developed was measured using a spectrophotometer at a wavelength of 570 nm. A blank using distilled water and amino acid standards were also run similarly.

Estimation of Proteins

Total proteins were estimated following the method of Lowry *et al*. [27]. In this method protein was estimated in trichloroacetic acid precipitates, which had been treated, to remove contaminants [28]. Bovine serum albumin (Sigma) was used as standard. All determinations were carried out 3 times to permit standard evaluation.

To the sample extract, 4mL of 0.1N sodium hydroxide and to 1mL of each of these solutions, 5mL of reagent -D (mixture of 2% sodium carbonate and 0.5% copper sulphate in 50:1 ratio) was added. The samples were allowed to stand for 10 min, at the end of which 0.4mL of folin-phenol reagent(diluted with double distilled water in 1:1 ratio before use) was added. Finally volume of the solution was makeup to 10ml and then the optical density of the color developed was measured using a spectrophotometer at a wavelength of 660nm.

Estimation of Soluble Sugars

Plant root sample was extracted with 5ml of methanol. The residue was extracted again with 5 ml of methanol and the combined extract was reduced to dryness. This was resuspended in 5ml of distilled water. An aliquot 0.1 to 0.5 ml of this solution was employed for the determination of soluble sugars by Anthrone Calorimetric method [29].

The reaction was carried out under conditions similar to those used by Trevelyan & Harrison [30]. The anthrone reagent (5ml**)** was pippeted into thick walled pyrex tubes (150 x 25 mm) and chilled in ice water. The solution under test (1ml) was layered on the acid, cooled for a further 5min, and then thoroughly mixed while still immersed in ice water. The tubes were loosely fitted with corks, heated as required in vigorously boiling, constant level water bath and then cooled in water for 5min. then it was make up to 10ml with water and Absorption spectra was determined in a spectrophotometer with a 1cm cell.

Estimation of Starch

Starch was extracted from the residue of the sample after extracting the soluble sugar in methanol. This residue was boiled in water to extract the starch and estimated by I_2 KI method [29].

Dry matter and Moisture

The dry matter of the sample represents the amount of material left after the complete removal of moisture from it. The moisture of the sample was lost by volatilization caused by heat. The amount of material left after the removal of the moisture was the dry matter. Dry matter and moisture of the material were determined by following the method by AOAC (1990) [31]. Dishes were washed with detergents and then were dried at 105 ^{0}C in oven for overnight. Then dishes were removed from oven and then kept in desiccator for cooling and weights. Two gm fruit sample was taken in dishes and placed in oven at 105 ^{0}C overnight. The dry matter and moisture were calculated by using the following formulae:

$$\text{Dry matter (\%)} = \frac{(\text{Weight of dish + Weight of dried sample}) - \text{Weight of dish}}{\text{Weight of sample before drying}} \times 100$$

$$\text{Moisture content (\%)} = \frac{(\text{Weight of fresh sample} - \text{Weight of dry sample})}{\text{Weight of fresh sample}} \times 100$$

Ash

Ash value was determined by following the method of AOAC (1990) [31]. For this crucible were kept in a muffle furnace at 600 ^{0}C for 1h. Then they were transferred crucible from furnace to a desiccator and cooled to room temperature and weighed as quickly as possible to prevent moisture absorption. Two gram dry fruit powder was taken in tared silica crucible and placed in a muffle furnace at 600 ^{0}C for 6h. Then crucible was transferred to a desiccator and cooled to room temperature, crucible was transferred as quickly as possible to avoid moisture absorption. The percentage of ash was calculated by using the following formula:-

$$\text{Ash (\%)} = \frac{\text{Weight of Ash}}{\text{Weight of sample}} \times 100$$

Crude fiber

Crude fiber content was determined by following the method of *Sadasivam* and *Manikam* (1992) [32]. Two gm of dried sample of fruit was boiled with 200ml H_2SO_4 for 30 minutes with bumping chips. Then it was filtered through muslins cloth and washed with boiling water until washings were free of acid. Then the residue was boiled with 200 ml of NaOH for 30 minutes. Again it was filtered through muslin cloth and washed with 25 ml of boiling H2SO4 three 50 ml portion of water and 25 ml of alcohol. Then residue was removed and transferred to pre-weighed ashing dish (W1 g). The residue was analyzed for 2 hour at 130 ^{0}C, cooled in desiccator and weighed (W2 g). It was ignited for 30 minutes at 600 ^{0}C. After cooling in desiccator it was again reweighed (W3 g). The percentage of crude fiber was calculated by using following formula

$$\text{Crude fiber content (\%)} = \frac{\text{Loss in weight on ignition (W2 - W1)} - \text{(W3 - W1)}}{\text{Weight of sample}} \times 100$$

Where, W1= Pre-weighted ashing dish; W2= Ashing dish with dry residue; W3= Ashing dish with ash.

Crude fat

The Crude fat content was determined by following the method of *Sadasivam* and *Manikam* (1992) [302]. Two gram dried sample was taken in a thimble (prepared from Whatman No.41 filter paper) and kept it in the soxhlet apparatus. A dry pre-weighted solvent flask (‘a’ g) was connected beneath the apparatus and to it was added the required volume of solvent (petroleum ether) and then was connected to the condenser.

Then the heating was adjusted to give a condensation rate of 2-3 drops and extracted for 16 h. After the completion of extraction thimble was removed and ether was retained from the apparatus. The excess of ether was evaporate from the solvent flask on a hot water bath and dried the flask. Then it was cooled in desiccator and weighed ('b' g). Crude fat was calculated by using the following formula:

$$\text{Crude Fat content (\%)} = \frac{(b - a)}{\text{Weight of sample}} \times 100$$

Crude Protein

The sample was subjected to micro Kjeldahl method to obtain its nitrogen content. Oven dried 0.5 g root powder was taken in Kjeldahl's flask and to it added10 ml 1:1 H_2SO_4. A pinch of microsalt (200g K2SO4 +5 g dehydrated CuSO4) and a few glass beads were added to accelerate the digestion and to avoid bumping of solution in flask, respectively. Digestion was carried out till a clear solution was obtained. After cooling to room temperature, it was transferred quantitatively to 100 ml volumetric flask and the volume was made with distilled water and then stored overnight at room temperature. Next day, it was filtered through Whatman No.1 filter paper and from the filtrate; nitrogen was estimated following the method by Hawk *et al.* (1948) [33]. For this, the assay mixture contained, 1ml plant extract, a drop of 8% KHSO4 and 15 ml Nessler's reagent (Reagent A-7 g KI + 10 g HgI2 in 40 ml distilled water, Reagent B- 10 g NaOH in 50 ml distilled water. Reagents A and B were mixed in the proportion of 4:5, diluted to 50 ml with distilled water. In place of any extract, distilled water along with other assay mixture served as blank. The absorbance was recorded at 520 nm on a double beam spectrophotometer (Shimadzu UV 190). A standard curve of Ammonium sulphate (0.05 mg N.ml-1) was prepared and the nitrogen content was calculated. The total nitrogen content of the sample was multiplied by factor 6.25 to calculate the crude protein content.

Total nitrogen

Total nitrogen content in fruits was estimated according to the method given by Hawk *et al.* (1948) [33]. Oven dried five hundred milligram powder of fruit samples was taken in Kjeldahl's flask with pinch of microsalt (200g K_2SO_4 +5 g dehydrated CuSO4) and to it 5 ml $H2SO_4$ (1:1) were carefully added. A few glass beads were added to accelerate the digestion and to avoid bumping of solution in flask, respectively. Digestion was carried out till a clear solution was obtained. After cooling to room temperature, it was transferred quantitatively to 100 ml volumetric flask and the volume was made with distilled water and then stored overnight at room temperature. Next day, it was filtered through Whatman No.1 filter paper and used for the estimation of nitrogen. For this, the assay mixture contained, 1ml plant extract, a drop of 8% $KHSO_4$ and 15 ml Nessler's reagent (Reagent A-7 g KI + 10 g HgI_2 in 40 ml distilled water, Reagent B- 10 g NaOH in 50 ml distilled water. Reagent A and B was mixed in the proportion of 4:5), diluted to 50 ml with distilled water. In place of any extract, distilled water along with other assay mixture served as blank. The absorbance was recorded at 520 nm on a double beam spectrophotometer (Shimadzu UV 190). A standard curve of Ammonium sulphate (0.05 mg N.ml-1) was prepared and the nitrogen content was calculated

Estimation of Phosphorus

Phosphorus was estimated from the same acid digest by following the method described by Sekine *et al.* (1965) [34]. Two mls of acid digest were pipett out in a test tube, to which 2 ml of 2 N HNO3 were added followed by 1 ml of Molybdate-Vanadate reagent (Reagent A: 1.25 g ammonium vanadate dissolved in 1 N HNO3 and volume was made to 500 ml with 1 N HNO3. Reagent B: 25 g ammonium molybdate dissolved in distilled water and volume was made to 500 ml. Then reagent A and B were mixed in equal volumes). The volume was made to 10 ml with distilled water. The ingredients were mixed well and allowed to react for 20 minutes. After 20 minutes, yellow colour intensity was measured at 420nm by using a reaction mixture blank containing no phosphorus. The color developed by standards of known concentration of phosphorus in KH_2PO_4 solution (0.110 g KH_2PO_4 per liter = 0.025 mg P.ml-1) with Molybdate - Vanadate reagent was used for plotting the standard curve. With the help of standard curve, the concentration of phosphorus in the plant material was expressed in mg 100 g-1on dry weight basis.

Mineral Analysis

Preparation of acid digests

The acid digestion method of Toth *et al.* (1948) [35] has been followed for the analysis of inorganic constituents. Fresh fruits were washed with water. Blotted to dry and then kept in oven at 60 ^{0}C till a constant weight was obtained. The oven dried plant material was randomly mixed and powdered. Five hundred mg oven dried powder of fruits was transferred to 150 ml clean borosil beaker and to that 10 ml concentrated HNO_3 were added. It was covered with watch glass and kept for an hour till the primary reactions subsided. Then, it was then heated on hot plate till all the material was completely dissolved. It was allowed to cool to room temperature and then 10 ml of Perchloric acid (60%) was added to it and mixed thoroughly. Then, it was then heated strongly on the hot plate until the solution became colorless and reduced to about 2-3 ml. While heating, the solution was not allowed to dry. After cooling, it was transferred quantitatively to 100 ml capacity volumetric flask, diluted to 100 ml with distilled water and kept overnight. Next day the extract was filtered through Whatman No. 44 (Ash less) filter paper. The filtrate was stored properly and used for analysis of inorganic constituents.

The level of Calcium, Magnesium, Iron, Manganese, Zinc, Copper and Cobalt were estimated by using Atomic Absorption Spectrophotometer. In case needed, appropriate dilution of plant extract was made with distilled water. Sodium and Potassium were estimated flame photometrically following the standard method of flame photometer

Results & Discussion

Biochemical and Mineral analysis

The Biochemical compounds like Amino Acids, Proteins, Soluble Sugars, Crude fat and Crude Protein etc. present in the root of *S. samarangense* was studied based on the methods reported previously. Amino acids present in the root were analyzed using ninhydrin reagent method as described by *Moore and Stein* (1954). Folin-phenol reagent method as described by *Khanna et al*., 1969 was adopted for the estimation of Proteins. Anthrone Calorimetric method was used for the estimation of sugars. Traditional methods were for the determination of ash, dry matter, moisture etc. The results of the Biochemical Analysis were given in table 1.

Table 1: Biochemical analysis of root of *S. samarangense*

S. No	Parameter	Amount present* (% w/w)
1	Amino Acids	4.25±0.28
2	Proteins	2.69±0.17
3	Soluble Sugars	1.11±0.09
4	Crude fiber	0.41±0.12
5	Crude fat	3.36±0.23
6	Crude Protein	5.81±0.36
7	Total nitrogen	2.71±0.14
8	Dry matter	82.17±1.20
9	Moisture	4.25±0.31
10	Ash	6.49±0.26
11	Acid insoluble ash	0.37±0.17

* Results are expressed as Mean± Standard deviation of three replicate measurements

Elements and metals present in the roots were extracted using acid digestion method and the level of Calcium, Magnesium, Iron, Manganese, Zinc, Copper and Cobalt were estimated by using Atomic Absorption Spectrophotometer. Sodium and Potassium were estimated flame photometrically following the standard method of flame photometer. The results of the Mineral Analysis were given in table 2.

Table 2: Mineral Analysis results of root of *S. samarangense*

S. No	Element	Amount present*
1	Sodium	9.86±0.71
2	Potassium	8.14±0.53
3	Calcium	3.29±0.29
4	Magnesium	0.52±0.21
5	Iron	0.69±0.32
6	Manganese	0.11±0.03
7	Zinc	0.10±0.02
8	Copper	0.09±0.01
9	Cobalt	BDL
10	Cadmium	BDL
11	Mercury	BDL
12	Silver	BDL

BDL: Below detectable levels

* Results are expressed as Mean± Standard deviation of three replicate measurements

Conclusion

In the present study, biochemical and mineral analysis of root powder was evaluated and biochemical compounds like amino acids, proteins, soluble sugars, crude fat and crude protein etc. were present. Elements and metals present in the roots were extracted using acid digestion method and the level of calcium, magnesium, iron, manganese, zinc, copper and cobalt were estimated by using Atomic Absorption Spectrophotometer. Sodium and Potassium were estimated flame photometrically following the standard method of flame photometer. Sodium, potassium were found in high quantity.

References

1. Madhavi M, Raghu Ram M. Phytochemical screening and evaluation of biological activity of root extracts of *Syzygium samarangense*. International journal of research in pharmacy and chemistry. 2015; 5(4):753-763.
2. Whitman, William F. Five Decades with Tropical Fruit, a Personal Journey. Stuart, Florida: Quisqualis Books in cooperation with Fairchild Tropical Garden, 2001.
3. "*Syzygium samarangense*, *Syzygium javanicum*, *Eugenia javanica*". toptropicals.com. N.d. Web. 9 Dec, 2014.
4. "*Syzygium samarangense*." worldagroforestrycenter.org. Web. 1 Mar. 2015.
5. Whitman, William F. CSIR. The Wealth of India: Raw materials. Vol X Sp-W. CSIR, 1976.
6. Jensen M. Trees commonly cultivated in South-East Asia: An illustrated field guide. RAP publication: 1995/38.Bangkok, Thailand, 1995, 229pp.
7. Martin FW, Campbell CW, Ruberte RM. Perennial edible fruits of tropics: an inventory. US Department of Agriculture, Agriculture Handbook No. 642. 1987, pp 252.
8. Okuda TT, Yoshida, Hatamo T, Yazaki K, Ashida M. Ellagitannins of the Casuarinaceae, Stachyuraceae and Myrtaceae. Phytochemistry. 1982; 21(12):2871-2874.
9. Panggabean G. Syzygium aqueum (Burm.f.) Alst., *Syzygium malaccense* (L.) M. & P, and *Syzygium samarangense* (Blume) M. & P. In Coronel, R.E., et al. (Eds.): PROSEA. No. 2: Edible fruits and nuts. Prosea Foundation, Bogor, Indonesia, 1992, pp. 292-294.
10. Walter A, Sam C. Fruits of Oceania. ACIAR Monograph No. 85. Canberra, 2002, 329 pp.
11. Al-Saif AM, Sharif H, Rosna MT, Moneruzzaman KM. African Journal of Agricultural Research. 2011; 6(15):3623.
12. Ghayur MN, Gilani AH, Khan A, Amor EC, Villaseñor IM, Choudhary MI. Phytother Res. 2006; 20(1):49.
13. Reynertson KA, Basile MJ, Kennelly EJ. Ethnobotany Research & Applications. 2005, 3, 25.
14. Whistler AW, Arthur W. Species Profiles for Pacific Island., Agroforestrywww.traditionaltree.org, 2010.
15. Amor EC, Villaseñor IM, Nawaz SA, Hussain MS, Choudhar I. A dihydrochalcone from *Syzygium samarangense* with anticholinesterase activity. Philippine Journal of Science. 2005; 134(2):105.
16. Kuo YC, Yang LM, Lin LC. Isolation and immunomodulatory effect of flavonoids from *Syzygium samarangense*. Planta medica. 2004; 70(12):1237-1239.
17. Amor EC, Villaseñor IM, Antemano R, Perveen Z, Concepcion GP, Choudhary MI. Cytotoxic C-Methylated Chalcones from *Syzygium samarangense*. Pharmaceutical biology. 2007; 45(10):777-783.
18. Resurreccion-Magno MHC, Villaseñor IM, Harada N, Monde K. Antihyperglycaemic flavonoids from *Syzygium samarangense* (Blume) merr. And perry. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 2005; 19(3):246-251.
19. Ratnam KV, Raju RV. In vitro antimicrobial screening of the fruit extracts of two *Syzygium* species (Myrtaceae). Advances in Biological Research. 2008; 2(1-2):17-20.
20. Simirgiotis MJ, Adachi S, To S, Yang H, Reynertson KA, Basile MJ, Kennelly EJ. Cytotoxic chalcones and antioxidants from the fruits of *Syzygium samarangense* (Wax Jambu). Food chemistry. 2008; 107(2):813-819.
21. Soubir T. Antioxidant activities of some local bangladeshi fruits (*Artocarpus heterophyllus*, *Annona squamosa*, *Terminalia bellirica*, *Syzygium samarangense*, *Averrhoa carambola* and *Olea europa*). Chinese journal of biotechnology. 2007; 23(2):257-261.
22. Reynertson KA, Yang H, Jiang B, Basile MJ, Kennelly EJ. Quantitative analysis of antiradical phenolic constituents from fourteen edible Myrtaceae fruits. Food chemistry. 2008; 109(4):883-890.
23. Evangeline CA, Irene MV, Amsha Y, Iqbal C. Prolyl Endopeptidase Inhibitors from *Syzygium samarangense* (Blume) Merr. & L. M. Perry. Z. Naturforsch. 2004; 59c:86.

24. Nair AG, Krishnan S, Ravikrishna C, Madhusudanan KP. Fitoterapia, 1999, 70, 148.
25. Boland FE. Trimethylammine nitrogen in seadfood method colorimetric. Journal - Association of Official Analytical Chemists, 1971, 54, 9.
26. Moore S, Stein WH. A modified ninhydrin reagent for the photometric determination of amino acids and treated compounds. Journal of Biological Chemistry. 1954; 211:907-913.
27. Lowry OH, Rosebrough NJ, Farr AL, Randal RJ. Protein measurement with folin - phenol reagent, Journal of Biological Chemistry. 1951; 193:266-275.
28. Khanna SK, Mattoo RL, Viswanatha~i PN, Tewari CP, Sanwall GG. Colorimetric determination of prote~n and orthophosphates in plant tissues rich in phenolics. Journal of Biological Chemistry. 1969; 6:21-25.
29. Mc Cready RM, Guddolz J, Silviera V, Ovwens HS. Determination of starch and amylase in vegetables. Analytical Chemistry. 1950; 22:1156-1158.
30. Trevelyan WE, Forrest RS, Harrison JS. Determination of Yeast Carbohydrates with the Anthrone Reagent, Nature. 1952; 170:626-627.
31. AOAC. Official Methods of Analysis. Association of Official Analytical Chemists, Washington. DC, 1990.
32. Sathishkumar T, Baskar R, Shanmugam S, Rajasekaran P, Sadasivam S, Manikandan V. Optimization of flavonoids extraction from the leaves of *Tabernaemontana heyneana* Wall. Using L16 Orthogonal design, Nature and Science. 2008; 6(3):10-21.
33. Hawk PB, Oser BL, Summerson WH. Practical physiological chemistry (Publ.). The Blockiston Co. USA., 1948.
34. Sekine T, Sasakawa T, Morita S, Kimura T, Kuratom K. (1965). cf. labrotory manual for physiological studies of Rice (Eds.) Yoshida, S., Forno, D., Cook, J.B. and Gomez, K.A. Pub. International Rice Research institute, Manila, India, 1972.
35. Toth SJ, Prince AL, Wallace A, Mikkenlsen DS. Rapid quantitative determination of eight mineral elements in plant tissue systematic procedure involving use of a flame photometer. Soil Science. 1948; 66:459-466.