

Original Research Article

Preliminary Phytochemical and Physicochemical Characterization of *Gynura segetum* (Lour) Merr (Compositae) Leaf

Lay Jing Seow*, Hooi Kheng Beh, Amirin Sadikun and Mohd Zaini Asmawi

School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang, Malaysia

*For correspondence: Email: layjing80@yahoo.com; Tel: +604-6533888 ext 4711; Fax: +604-6570017

Received: 12 August 2012

Revised accepted: 12 July 2013

Abstract

Purpose: To carry out phytochemical and physicochemical studies on the leaves of *Gynura segetum*.

Methods: Preliminary phytochemical and physicochemical studies using chromatographic techniques, were carried out. Parameters evaluated include ash value, loss on drying and extractive value, amongst others.

Results: Phytochemical results revealed that the leaves of *Gynura segetum* contain alkaloid, terpene, flavonoid, tannin and saponin. Mean ash value (%) was 13.5 (total), 2.49 (acid-insoluble ash) and 6.14 (water-soluble ash). Loss on drying was 10.70 % while extractive value was 20.60 % in water and 14.13 % in ethanol. Thin layer chromatography (TLC) analysis indicates the presence of rutin in the methanol extract of *G. segetum* and this was supported by high performance liquid chromatography (HPLC) studies.

Conclusion: Useful quantitative and descriptive data essential for identifying and characterizing the plant for the purpose of quality control are presented.

Keywords: *Gynura segetum*, Quality control, Extractive, Ash value

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Herbs are the earliest source of medicine. The use of medicinal plants for healing dates as far back as prehistoric times and has since been woven into the culture and civilization of various peoples [1]. Plants contain phytochemicals that can be used for therapeutic purpose or as precursors for pharmaceutical synthesis [2]. As a result, global market demand for herbs and their products has increased tremendously in recent years. Therefore, quality control of herbal products for the purpose of efficacy and safety is essential.

World Health Organization (WHO) has prescribed a number of quality control tests that medicinal plant materials should undergo. Quality

control is determined on the basis of identity, purity, content, and other chemical, physical and/or biological properties, as well as by manufacturing process [3].

Gynura segetum, family Compositae (Asteraceae) is a cultivated species and can be found growing in the tropical regions of Indonesia and Malaysia. The leaves are shortly petiole, fleshy with serrated margin and both surfaces are pubescent. The upper surface is green and lower surface is purplish green. The stem branches profusely, purplish green in color and slightly hairy. The yellowish orange flowers have a strong and foetid odour. The plants produce numerous roots which are attached to a tuber.

G. segetum has drawn a lot of attention due to its uses in traditional medicine. The plant is known for its use for the treatment of cancer, inflammation, diabetes, hypertension and skin afflictions [4,5].

A literature survey indicates lack of research on *G. segetum* plant. Therefore, investigation of *G. segetum* would be desirable. The aim of the present study was to carry out preliminary phytochemical screening and physicochemical of the plant to assure the quality, safety, and efficacy of this formulation.

EXPERIMENTAL

Plant material

The leaves of *G. segetum* were collected from Penang State and identified by Mr. Adenan Jaafar from School of Biological Sciences, University Sains Malaysia. A voucher sample of the plant, with reference no. 11013 was deposited at the Herbarium of School of Biological Sciences, University Sains Malaysia.

Phytochemical screening

Phytochemical screening of the leaves was carried out to identify the major chemical constituents. The standard methods or some modifications have been used to screen for the presence of alkaloids, terpenes flavonoids, anthraquinones, tannins, and saponins (6,7).

Alkaloid test

Five ml of ammonia solution (5%) was added to 1 g of dried powdered leaves to moisten the material. Twenty ml of chloroform was then added and shaken for 15 min to effect extraction. The mixture was then filtered and the filtrate (extract) was washed further with 10 ml chloroform and evaporated to dryness over a water-bath. The residue was transferred to a test tube, and 1 ml of 5 % HCl was added, stir well with spatula. Dragendorff's reagent was added slowly to test tube and the presence of orange-coloured precipitate indicated the presence of alkaloids.

Liebermann-Burchard test for terpenes

Two gram plant material was mixed with 10 ml of methanol and then heated for 30 min. The mixture was filtered while hot and a further 5 ml of the solvent was added, heated and soluble materials were rinsed away. The solvent was evaporated off by rotary evaporator and triturate with ether in a test tube and a few drops of

Liebermann-Burchard reagent was added carefully. Blue-green ring between layers indicated the presence of steroids while pink-purple ring indicated the presence of terpenes.

Test for flavonoids

One gram of plant material was added to 100 ml of water and heated for 5 min. The mixture was then filtered and the filtrate evaporated to dryness over a hot water-bath. The extract was treated with NaOH, followed by addition of dilute HCl (5%); the solubility and colour changes were noted. A yellow solution on addition of NaOH, which turns colourless after addition of dilute HCl indicated the presence of flavonoids.

Borntrager's test for anthraquinone derivatives

One gram of plant material was added to 15 ml of chloroform and then filtered. Two ml of the filtrate was transferred to a test tube and 1 ml of dilute ammonia (10 %) was added. The mixture was shaken and a pink red colour in the lower ammoniacal layer indicated the presence of anthracene derivatives.

Ferric chloride solution test for tannins

One gram of plant material was added to 20ml of water and boiled for 15 min. The mixture was filtered and the filtrate treated with 15 % ferric chloride test solution. A blue-green colour indicated the presence of tannins.

Frothing test for saponins

Water extract was obtained by boiling over a water bath. The extract was transferred into a test tube, shaken vigorously, left to stand for 10 min and the result noted. A thick persistent froth indicated the presence of saponins.

Physicochemical characterization of *Gynura segetum* leaf

Ash value, loss on drying and extractive value were determined as per World Health Organization (WHO) guidelines [8].

Total ash value

About 2 g of dried powder of *G. segetum* leaves accurately weighed in a previously ignited and tared crucible. The material was ignited by gradually increasing the temperature to 600 °C until it became white. The crucible was cooled in a desiccator and weighed, and then again ignited to constant weight. The content of total ash was

calculated as mg/g of air-dried material and expressed as a percentage. The experiment was carried out in triplicate.

Acid-insoluble ash value

Twenty-five milliliters of HCl was added to the crucible containing the total ash, covered with a watch-glass and boiled for 5 min. The insoluble matter was collected with an ashless filter paper and washed with hot water until the filtrate was neutral. The filter was then transferred to the original crucible and ignited to constant weight. The crucible was cooled in a desiccator and weighed. The content of acid-insoluble ash was calculated in mg/g of air-dried material and expressed as percentage. The experiment was carried out in triplicate.

Water soluble ash value

Twenty-five milliliters of water was added to the crucible containing total ash and boiled for 5 min. Insoluble matter was filtered using an ashless filter paper. The filter paper was washed with hot water and ignited in a crucible for 15 min at a temperature not exceeding 450 °C. The crucible was then weighed to constant weight. Water-soluble ash was calculated by subtracting the residue weight in mg from the weight of total ash and expressed in percentage. The experiment was carried in triplicate.

Loss on drying

Water content of the crude plant extract was determined based on the test of loss on drying. About 2g of the sample weighed in a previously dried and tarred petri dish. The sample was dried in an oven at 105°C to constant weight, cooled and weighed. The experiment was repeated three times and the result was calculated as loss of weight in percent.

Extractive values

About 4 g of powdered *G. segetum* leaf was accurately weighed in a glass-stopped conical flask, and 100 ml water was added to the flask and weighed. The flask was shaken and allowed to stand for 1 h. A reflux condenser was attached to the flask and boiled gently for 1 h. The mixture was then filtered, 25 ml of the filtrate transferred to a tarred Petri dish and evaporated to dryness over a water-bath. The Petri dish was then dried in an oven at 105 °C for 6 h, cooled in a desiccator (silica gel) for 30 min and weighed. The experiment was done in triplicate and the content of extractable matter was then calculated in mg per g of air-dried material and expressed

as percentage. The same procedure was repeated but with water replaced with 95 % ethanol, and the results obtained compared.

Chromatographic analysis of the leaves of *Gynura segetum*

TLC analysis

Freshly collected leaves were dried in an oven at about 45 °C and ground to a fine powder. The dried powder was macerated overnight with methanol, filtered and evaporated in a rotary evaporator at a maximum temperature of 45 °C. The concentrated extract was stored in a refrigerator until used.

The methanol extract of the leaves of *G. segetum* was spotted on TLC plates (Merck TLC aluminium sheets, silica gel-60 ART-5554, measuring 20 x 20 cm² and 10 x 10 cm, layer thickness of 0.2 mm) using micropipette tubes (20 µl). The TLC plates were then developed using butanol: acetic acid: water (5: 1: 4) solvent system. The developed plate was dried in open air and then viewed under day light and ultraviolet light (UV) at 254 nm and 365 nm. Detection of compounds was made by spraying with diphenylboric acid-2-aminoethyl ester) solution (NP-reagent, Sigma, USA), under daylight and UV light (254 and 365 nm). Thin layer chromatography (TLC) photographs (viewed under UV light and sprayed with reagent) were taken with a digital camera (Sony Cyber-shot DSC-T33).

HPLC analysis

HPLC analysis was performed on Agilent Technologies series 1100 system equipped with Agilent G1314 UV detector and a Multi Technique HP ChemStation (Agilent Technologies; Waldbronn, Germany). A LiChrosorb RP-18 column (4.6 i.d x. 250 mm, 5 µm particle size, Merck) was used with the following analytical conditions: a mobile phase of acetonitrile:water (20 : 80), a flow rate of 1.0 ml/min and the signals detected at 280 nm. The temperature was 24 °C, and the injection volume 20 µl.

An accurately weighed methanol extract (5 mg) was dissolved in 1 ml of HPLC grade methanol. The resultant solution was sonicated and filtered through a 0.45 µm syringe filter (Sigma, USA) prior to HPLC analysis. Rutin, used as reference standard, was prepared by weighing an accurate amount of rutin (5 mg, Sigma, USA) and dissolving it in 1 ml of HPLC grade methanol. The methanol extract and rutin (5000 ppm each)

were injected into the HPLC equipment under the same conditions. The separation and retention times were compared.

A quantity of rutin (500 µl from the stock sample) was added to 500 µl methanol extract (5000 ppm) and mixed to ensure dissolution. The resultant solution was then injected into HPLC system to ensure the presence of rutin in the methanol extract.

RESULTS

Phytochemical profile

Preliminary phytochemical screening results for *G. segetum* leaf are recorded in Table 1.

Gravimetry

The results of physicochemical characterization of the leaves of *G. segetum* are given in Table 2.

TLC

The TLC chromatograms of the extract on the developed plates are shown in Figure 1. Rutin was used as reference standard ($R_f = 0.49$) and showed yellow color band in daylight which changed to orange yellow after chemical

treatment (sprayed). The R_f value (0.48) of the extract and the color of the spot observed in daylight and after UV treatment (365 nm) suggest the presence of rutin in the extract.

HPLC

The TLC results provided indication of the presence of rutin which was then used as a marker in HPLC analysis. HPLC analysis of methanol extract and reference standard gave good separation under the same conditions (Figures 2 and 3). The authenticity of the rutin in the methanol extract was checked by running a standard injection of a mixture of methanol extract and rutin (Figure 4). The result showed the presence of rutin in methanol extract. In this study, the TLC chromatograms indicate the presence of rutin in methanol extract of *G. segetum*. This was confirmed by HPLC chromatogram.

DISCUSSION

Medicinal plants have played a key role in global health. The correct identity of the crude herbal material as well as botanical quality are of prime importance in establishing the quality of herbal drugs [9]. Voucher specimens are reliable reference sources.

Table 1: Preliminary Phytochemical tests of leaves of *Gynura segetum*

Test	Observation	Inference
Dragendorff's test	Orange precipitate	Alkaloids present
Liebermann-Burchard test	Bright red coloration	Terpenes present
Flavonoids test	Yellow solution with NaOH; turns colourless with HCl.	Flavonoids present
Borntreger's test	No pink color in ammoniacal layer	Absence of anthraquinone derivatives
Ferric chloride solution test	Blue-green precipitate	Tannins present
Frothing test	Formation of thick stable foam	Saponins present

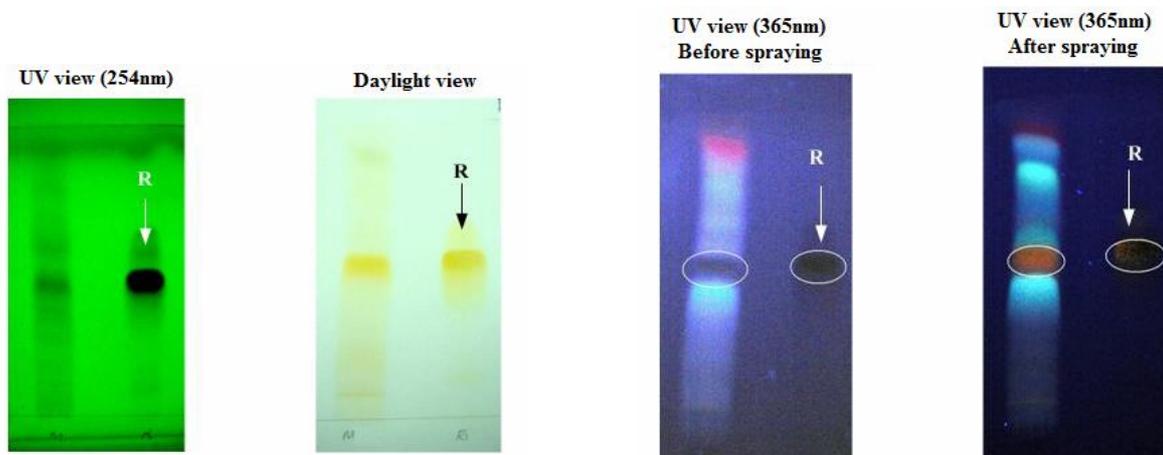
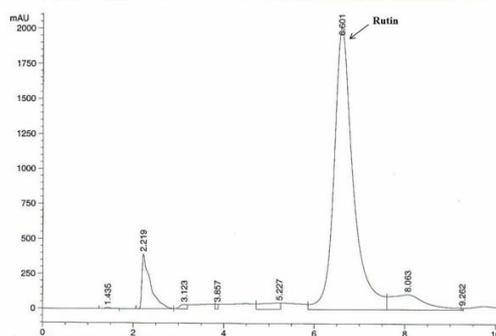
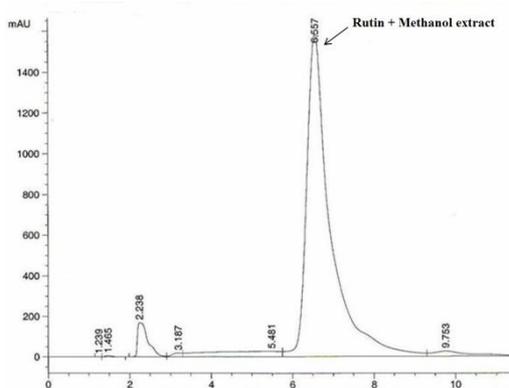


Figure 1: TLC profiles of the crude methanol leaf extract of *G. segetum* and reference standard rutin (R).

Table 2: Physicochemical characteristics of leaves of *Gynura segetum*

Parameter	Result (%)
Total ash	13.5
Acid-insoluble ash	2.49
Water-soluble ash	6.14
Loss of weight on drying	10.7
Water extractive values	20.60
Ethanol extractive values	14.13

**Figure 3:** HPLC chromatogram of standard rutin**Figure 4:** HPLC chromatogram of mixture of methanol extract and rutin

Phytochemical screening of the plant is preliminary and important aspect of the process of establishing herbal medicine quality. Preliminary phytochemical analysis is helpful in determining the chemical constituents of plant materials. They are also useful in locating the source of pharmacologically active chemical compounds.

Preliminary phytochemical revealed the presence of alkaloids, terpenes, flavonoids, tannins and saponins in leaves of *G.segetum*. The presence of phenolic compounds in the plants indicates that this plant may possess anti-microbial properties. This agrees with the findings of Seow *et al.* [10]. Aguinaldo *et al* [11] reported that tannins may possess potential value such as cytotoxic and antineoplastic agents. Flavonoids are potent water-soluble antioxidants and free radical scavengers, which can prevent oxidative

cell damage and exhibit anti-cancer effect [12]. Saponins are used in hyperglycemia, antioxidant, anticancer and anti-inflammatory, etc [13]. Recent studies by Seow *et al.* [14] show that the leaf extracts of *G.segetum* reduced the growth of blood vessels and exhibited potent anti-angiogenic activity. The inhibition of angiogenesis may lead to control of tumor growth.

Assurance of safety, quality and efficacy of medicinal plants is of utmost importance. To establish identity and purity, criteria such as physical constants, contaminants, moisture, ash content and solvent residues have to be checked. Ash value represents the level of 'cleanness', and high values may be due to inappropriate handling procedures during the sample collection process.

Extraction with water gave higher yield than extraction with ethanol. The test for loss on drying is a measure of both water and volatile matter. An excess of water in plant materials encourages microbial growth and deterioration following hydrolysis. Therefore, this method is important for determining the water content of plant material.

Thin layer chromatography (TLC) is used widely for qualitative determination of small amounts of substances and has proved valuable in chemistry. The marker compound (rutin) detected by HPLC indicates that the TLC method has good sensitivity and utility in the identification of *G. segetum*. However, the plant leaf needs to be further studied to identify and isolate its chemical constituents.

CONCLUSION

Suitable parameters for the phytochemical screening and physicochemical characterization have been for *G. segetum* leaf. These parameters can serve as quality characters and criteria for the evaluation of the identity and authenticity of the plant. Further studies are recommended to isolate and characterize the chemical constituents which may be responsible for the pharmacological activities of the plant.

ACKNOWLEDGMENT

The authors acknowledge the financial support given by the Universiti Sains Malaysia (USM). The authors also thank the School of Pharmaceutical Sciences, USM for providing the required facilities and excellent technical assistance. The first-named author was supported by MyBrain 15 (MyPhD) scholarship

provided by the Ministry of Higher Education (MOHE), Malaysia.

REFERENCES

1. Indu BJ, Ng LT. *Herbs: The green pharmacy of Malaysia*. Malaysia: Vinpress Sdn Bhd; 2000.
2. Makut MD, Gyar SD, Pennap GRI, Anthony P. Phytochemical screening and antimicrobial activity of the ethanolic and methanolic extracts of the leaf and bark of *Khaya senegalensis*. *Afr. J. Biotechnol* 2008; 7(9): 1216-1219.
3. Ameh SJ, Obodozie OO, Inyang US, Abubakar MS, Garba M. Quality Control Tests on *Andrographis paniculata* Nees (Family: Acanthaceae) – an Indian 'Wonder' Plant Grown in Nigeria. *Trop J Pharm Res* 2010; 9(4): 387-394.
4. Suharmiati, Maryani H. *Khasiat dan manfaat daun dewa & sambung nyawa*. Jakarta: Agromedia Pustaka; 2003.
5. Yuan SQ, Gu GM, Wei TT. Studies on the alkaloids of *Gynura segetum* (Lour.) Merr.. *Acta Pharmaceutica Sinica* 1990; 25: 191-197.
6. Goh SH, Svepadmo E, Chuah CH. *Phytochemical Guide to Malaysian Flora* (2nd ed.). Kuala Lumpur, Malaysia: Institute of Advanced studies. University of Malaya; 1993.
7. Onwukaeme DN, Ikuegbvweha TB, Asonye CC. Evaluation of Phytochemical Constituents, Antibacterial Activities and Effect of Exudate of *Pycnanthus Angolensis* Wedl Warb (*Myristicaceae*) on Corneal Ulcers in Rabbits. *Trop J Pharm Res* 2007; 6(2): 725-730.
8. *Quality Control Methods for Medicinal Plants Materials*. Geneva: World Health Organization; 1998; pp 1–115.
9. Bandaranayake WM. Quality control, screening, toxicity and regulations of herbal drugs. In Ahmad I, Aquil F., Owais M. *Modern Phytomedicine: Turning medicinal plants into drugs*. Germany: WILEY-VCH Verlag GmbH & Co; 2006; pp 25-57.
10. Seow LJ, Beh HK, Ibrahim P, Sadikun A, Asmawi MZ. Antimicrobial activity of *Gynura segetum*'s leaf extracts and its active fractions. *International Journal of Genuine Traditional medicine* 2012; 2(2): 1-20.5.
11. Aguinaldo AM, El-Espeso, Guevara BQ, Nanoto MG. Phytochemistry. In: Guevara BQ (ed). *A Guide book to plant screening and biological*. University of Santo Tomas, Manila. Philippines; 2005.
12. Doss A, Phil M. Preliminary phytochemical screening of some Indian medicinal plants. *Ancient Science of Life* 2009; 29(2): 12-16.
13. Rathore SK, Bhatt S, Dhyan S, Jain A. Preliminary phytochemical screening of medicinal plant *Ziziphus mauritiana* Lam. Fruits. *Int J Curr Pharm Res* 2012; 4(3): 160-162.
14. Seow LJ, Beh HK, Majid AMSA, Murugaiyah V, Ismail N, Asmawi MZ. Anti-angiogenic activity of *Gynura segetum* leaf extracts and its fractions. *J Ethnopharmacol* 2011; 134: 221-227.