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RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING, QUANTITATIVE ANALYSIS OF PRIMARY AND SECONDARY METABOLITES OF MALAY APPLE LEAVES

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ABSTRACT

The study includes phytochemical screening and quantification of primary and secondary metabolites like carbohydrates, protein, lipids, phenol, tannin and flavonoids from *Syzygium malaccense* (L.)Merr. & L.M.Perry leaves. The leaves of *Syzygium malaccense* (L.) Merr. & L.M.Perry showed highest carbohydrate value than the other primary metabolites. So malay apple can act as a good remedy for nutritional disorders and pharmaceutical agent. Secondary metabolites analysis showed the potential of malay apple leaves as anticancerous, antimutagenic, anti-inflammatory, antimicrobial and astringent capacities. So malay apple leaves can act as a good therapeutic agent.

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INTRODUCTION

The plant kingdom is an important source of herbal drugs. Even in recent years, there has been an increasing awareness about the importance of medicinal plants. Generally, herbal drugs are easily available, safe, less expensive, efficient, and rarely have side effects. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs (Yadav and Agarwal, 2011). Medicinal plant drug discovery continues to provide new and important leads against various pharmacological targets, including cancer, malaria, cardiovascular diseases and neurological disorders. Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids, flavonoids and phenols. The bio-active phytocompounds are synthesized by primary or rather secondary metabolism of living organisms. Plant cells produce two types of metabolites. Metabolites are compounds synthesized by plants for both essential functions, such as growth and development (primary metabolites), and specific functions, such as pollinator attraction or defense against herbivores (secondary metabolites). Primary metabolites are involved directly in growth and metabolism. Secondary metabolites are considered products of primary metabolism and are generally not involved in metabolic activity. The genus *Syzygium*,Gaertn. comprises about 1200-1800 species belongs to the family Myrtaceae. Several species of *Syzygium*, Gaertn. bear fruit that are edible for humans, many of which are named as 'rose apple'. *Syzygium malaccense* (L.) Merr. & L.M. Perry commonly known as malay rose apple, malay apple or mountain apple. It is said to have originated in India and Malaysia, which accounts for the fruit sometimes being called Malay Apple. Its evergreen leaves are opposite, short-petioled. Though showy, the flowers are hidden by the foliage until they fall and form a lovely carpet on the ground. The fruit is oblong, obovoid, or bell-shaped (Morton, 1987). The bark of Mountain Apple is traditionally used for medicine. The leaves are also used for medicine.It is said that young leaves from saplings and the bark from mature trees are made into a warm drink for the mother of a newborn baby. This is to assist in expelling the afterbirth and to cleanse the mother's body after giving birth or even after a miscarriage. Remedies using pounded bark have been used in the mouth for lesions and also for lacerations. The leaves can be processed for a tonic, and the old fruit of this plant was considered a helpful remedy for sore throats. In the Molucca, or Spice Islands, a decoction of the bark is used to treat thrush.

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Malayans apply a powder of the dried leaves on a cracked tongue. A preparation of the root is a remedy for itching. The root acts as a diuretic and is given to alleviate edema. The root bark is useful against dysentery, also serves as an emmenagogue and abortifacient. Cambodians take a decoction of the fruit, leaves or seeds as a febrifuge. The juice of crushed leaves is applied as a skin lotion and is added to baths. In Brazil, various parts of the plant are used as remedies for constipation, diabetes, coughs, pulmonary catarrh, headache and other ailments. Seeded fruits, seeds, bark and leaves have shown antibiotic activity and have some effect on blood pressure and respiration. (Kaaiakamanu and Akina, 1973; Kepler, 1984) In Malaysia and India, the Rose Apple represents the golden fruit of immortality and is associated with the Buddha.

Previously the crude drugs/extracts prepared from plants were identified by comparison only with the standard descriptions available in the literature, but recently due to advancement in the field of pharmacognosy, various techniques have been followed for the standardization of crude drugs. Phytochemical screening is one of the techniques to identify new sources of therapeutically and industrially important compounds. Qualitative phytochemical screening will help to understand a variety of chemical compounds produced by plants and quantification of those metabolites will help to extract, purify and identify the bioactive compounds. Such phytochemical screening in various plants is reported by many workers. Many plants such as *Gloriosa superba* (Rishi and Sarin, 2009), *Ricinus communis* and *Euphorbia hitra* (Vijayavergia *et al.*, 2009), *Moringa oleifera* (Talreja, 2011) have been evaluated for their composition of primary metabolites. Likewise number of plants were screened for secondary metabolites for their medicinal values in *Eclipta alba, Morinda* citrifolia (Sharma and Sharma, 2010) and *Mangifera indica* (Gupta *et al.*, 2010). In the present investigation qualitative and quantitative phytochemical analyses were carried out using malay apple leaves grown in Cochin University of Science And Technology Campus, Ernakulam, Kerala, India.

MATERIALS AND METHODS

Collection of Plant Material

Leaves of *Syzygium malaccense* (L.) Merr. & L.M.Perry were collected from Cochin University of Science And Technology Campus,Ernakulam, Kerala,India.The plant was authenticated and the voucher specimen has been deposited in the herbarium of Kerala Forerst Research Institute, Peechi, Thrissur for future reference.

Preparation of the plant extracts

The leaves were washed under running tap water to remove the surface pollutants and the leaves were air dried under shade. The powdered leaf samples were subjected to successive extraction with petroleum ether, methanol and acetone using soxhlet apparatus. Fresh leaf material was ground using distilled water and filtered and used as an aqueous extract. The extracts obtained using solvents were concentrated using rotary vacuum evaporator and then dried. The extract thus obtained was used for various analyses.

Phytochemical Screening of extracts

Petroleum ether, methanol, aqueous and acetone extracts were used for preliminary phytochemical analyses using standard procedures (Kokate *et al.*, 1995).

Quantitative determination of primary metabolites

Determination of carbohydrate

Dubois Method was used for the determination of total carbohydrates using β D-Glucose as standard. The sample was extracted with 20ml 1M H_2SO_4 by keeping in a boiling water bath at 100°C for 1 hour. To the 1ml extract, 1ml 5% phenol and 5ml H_2SO_4 were added under cold condition. The absorbance was measured at 485nm after 30 minutes (Dubois *et al.*, 1956).

Determination of protein

The dried and powdered samples was extracted with 10 ml of 2N NaOH and kept it in boiling water bath for 2hrs at 80 ^{0} C.1.0ml of extract was pipette out and 5.0 ml of alkaline copper reagent was added and allowed it to stand for 10 min. Then 1 ml of Folin's Ciocalteau reagent was added and incubated in dark for 40 min. The intensity of the colour developed was read at 750 nm (Lowry *et al.*, 1951).

Determination of Lipid

The dried and powdered sample was extracted with 10 ml 2:1 chloroform –methanol reagent at 60^{0}C for 30 minutes. Pipette 1ml and add 1ml concentrated sulphuric acid followed by the addition of vanillin reagent and read absorbance at 520nm (Barnes and Blackstock, 1973)

Quantitative determination of secondary metabolites

Total phenolic content

The total phenolic content of the extract was determined by the Folin–Ciocalteu method (Kaur and Kapoor, 2002). Briefly, 1.0ml of crude extract was mixed thoroughly with 5.0 ml of Folin–Ciocalteu reagent for 5 min, followed by the addition of 4.0 ml of 7.5% sodium carbonate. The mixture was allowed to stand for 2 hours in the dark, and absorbance was measured at 740 nm.

Total Tannin Content

Colorimetric estimation of tannins is based on the measurement of blue colour formed by the reduction of phosphotungstomolybdic acid by tannin like compounds in alkaline solution (Ranganna, 1986). A known amount of extract was mixed with 5.0 ml of Folin- Denis reagent (FD) and Na_2CO_3 solution and made up to 100 ml, mixed well and absorbance was read at 760 nm after 30 minutes using spectrophotometer. Total tannin content as expressed as mg tannic acid equivalent /100 g of sample (Ranganna, 1986).

Determination of total flavonoid content

An 1.0 ml aliquot of sample solution was mixed with 4 ml of distilled water and subsequently with 0.3 ml of 5 % $NaNO_2$ solution. After 6 min, 0.3 ml of 10% $AlCl_3$ solution was added and allowed to stand for 6 min, and then 2 ml of 4% NaOH solution was added to the mixture. Then the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was determined at 510 nm. The analysis was performed in triplicate and the results were expressed as catechin equivalent (Chang *et al.*, 2002).

Statistical Analysis

All the analyses were performed in triplicate and the results were statistically analyzed and expressed as mean (n=3) ± standard deviation (SD).

RESULTS AND DISCUSSION

In the present investigation primary and secondary metabolites were qualitatively and quantitatively analysed using malay apple leaves and the results are shown in Table 1 & 2 and Figure 1.

Table 1. Preliminary Phytochemical screening of Malay apple leaves

Plant Constituents	Petroleum ether	Methanol	Aqueous	Acetone
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Phenolics and Tannins	+	+	+	+
Steroids and Sterols	+	+	+	+
Carbohydrates	+	+	+	+
Saponins	+	+	+	-
Glycosides	+	+	-	+
Proteins and amino acids	+	+	+	+

Table 2. Quantification of Primary metabolites of Malay apple leaves

S.No.	Primary metabolites	Weight in %
1	Carbohydrates	6.6± 0.04
2	Protein	3.2 ± 0.027
3	Lipid	3.66 ± 0.027

Values are means of 5 independent analyses ± SD(n=5)

The primary metabolites present in the sample are represented in the Table 2. Earlier reports revealed the percentage of carbohydrates and protein content in *Syzygium malaccense* (L.) Merr. & L.M.Perry fruits as 6.74 ±0.09% and 0.55 ±0.02% respectively (Oyinlade and Ogundare, 2014). In the present investigation carbohydrate and protein content in *Syzygium malaccense* (L.) Merr. & L.M.Perry leaves were found to be 6.6±0.04% and 3.2 ± 0.027% respectively. According to their findings malay apple fruits are good source of pharmaceutical agents that may be suitable for the management of hyperglycemic related conditions like obesity and diabetes mellitus. Similarly the leaves of malay apple also can be regarded as a remedy for nutritional disorders and can also act as pharmaceutical agent because of its better carbohydrate content. Secondary metabolite analysis is necessary for extraction, purification, separation, crystallization, identification of various phytocompounds. Among the secondary metabolites higher level of phenols was observed (Fig.1). The higher amount of phenol (6.11± 0.181%) is revealed the importance in regulation of plant growth, development and disease resistance. The level of flavonoid content was 1.02%. Earlier reports revealed that plant phenolic compounds including flavonoids are potent antioxidants with reported antimutageneic and anticarcinogenic effects (Middleton and Kandaswami, 1994). The presence of tannin (0.08±0.005%) in malay apple leaves was also contribute various medicinal properties such as antimicrobial, anti-inflammatory and astringent activity (Chung *et al.*, 1998) .

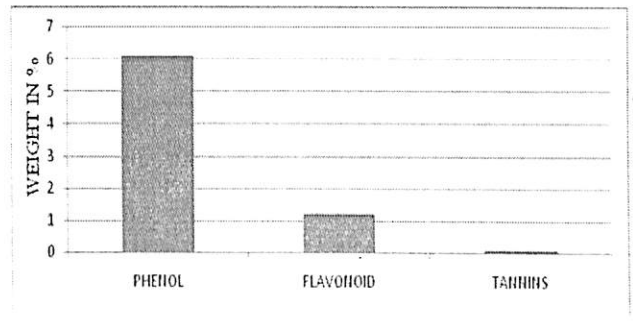

Fig. 1. Quantification of Secondary metabolites of Malay apple leaves

The same type of phytochemical metabolites was reported earlier by many workers. The present investigation showed significant variation in the contents like phenol, flavonoids, and tannin when compared to above mentioned reports (Savitha *et al.*, 2011). These variations are due to number of environmental factors such as climate, altitude, rainfall etc. as mentioned by (Kokate *et al.*, 2004).

Conclusion

Thus, the results obtained in the present study indicates malay apple leaves have the potential to act as a source of useful drugs because of presence of various phytochemical components. The results are very much encouraging but advanced work is necessary before being put into practice.

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