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Syzygiumaqueum (Burm. f.) – Watery Rose Apple: Its Morphological, Ethnobotanical and Phytochemical Appraisal

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ABSTRACT

Syzygiumaqueum (Burm. f.) Alston is a medicinally valued Myrtacean member. The fruit is edible and has been used in several parts of the world, especially the South-East Asian countries including India. The fruit is of immense benefit to human health and is used in cure of several ailments. However, phytochemical studies pertaining to its phenolic and flavonoid content of the fruit has been performed in the on-going study. Besides antioxidant activity of the 80% aqueous ethanolic extract is also performed.

Keywords: *Syzygiumaqueum*, ethnobotany,medicinal, phenolic, flavonoid, antioxidant.

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INTRODUCTION

Syzygiumaqueum (Burm. f.) Alston (*Eugenia aquea*Burm.f.) of Myrtaceae family is a priced medicinal plant. Its native distribution includes Southern India to Eastern Malaysia. This is a small evergreen tree with a dense, spreading and much branched crown. Commonly called watery rose apple, is a rare species indeed. It grows and thrives best in climates that have a substantial dry season, but cannot tolerate water stress and prefers growing in locations where they have ready access to water.

Etymology:

The etymology of the name: Generic epithet "Syzygium" is derived from the Greek word "suzugos" which means joined. Syzygium used to be the name of a Jamaican plant species with paired leaves and branches (Brambach et al., 2017). The specific epithet "aqueum" implies to the juicy fruits of this species. It comes from the Latin word "aqueus" which means watery.

Morphology:

Bisexual flowers are borne in small terminal or axillary clusters. The fruit is fleshy red berry which are pear-shaped, apex has a shallow cavity that is partially covered by 4 fleshy sepals. The epicarp is shiny, thin and waxy;mesocarp is white, juicy and crisp. Fruits are often seedless, but sometimes have 1 - 4 small seeds.The fruits have slightly sweet taste with slight astringency and can be preserved for months in household refrigerator.

Ethnobotanical uses:

This medicinal species has been extensively used for cure of body ailments (Rahmatullah et al., 2012; Palaisamy et al., 2011; Palanisamy and Manaharan, 2015). It protects against diabetes, improves immune system, lowers cholesterol, protects against certain types of cancers, eliminates fungal and bacterial infections, reduces fever and cures epileptic seizures. Some other important uses include:

- The fruit is a stimulant for the liver and brain. In

India, it is used to boost the liver and brain function.

- The sweet prepared from its flowers is used to treat fever.
- The leaves decoction is used to treat sore eyes and rheumatism.

  Leaves extract has revealed anti-oxidant and anti-diabetic potential.
- The infusion of roasted seeds is used to treat diabetes.
- It prevents wrinkles and premature aging.
- It is used to brighten the skin. It makes the skin glow.
- The fruit infusion acts as a diuretic.

Apart from the above mentioned uses, this species is also highly priced amongst the aboriginals for various uses. The fruits are eaten fresh or preserved. Sometimes these are boiled and sweetened with sugar. They are also added to soups and fruit salads. The fruit skin is rich in Vitamin A. In Hawaii, bark decoction is used to treat thrush. Malaysian women post child delivery, are given a ceremonial salad containing this fruit to eat. In Indonesia, young foliage is used to wrap snacks sold by street vendors. The reddish wood is hard and used to make ornamental objects.In Cuba, epilepsy is treated with its roots.In parts of Colombia, fruit is used as pain killer.The dried leaves are eaten with vegetables or the fresh leaves are eaten raw, as a treatment for malaria and pneumonia. An infusion of the leaves is used in the treatment of dysentery and stomachache.

Despite wealth of information on its uses by all and sundry, very few studies have been conducted to know the phytochemical properties of the fruits. In the foregoing study, antioxidant activity of the 80% aqueous ethanolic extract of the *Syzygiumaqueum*fruit and its quantification of phenolic acids and flavonoids using HPLC studies have been performed and assayed.

Phytochemical evaluation:

1. Preparation of methanolextractof plant sample

Extraction of plant material

The air-dried and coarse powdered plant sample, here fruit (one gm) of was extracted with (25) ml 80% aqueousethanol with stirring in a magnetic shaker at room temperature. The extract was filtered and residue was again extracted with same solvent for another five days and filtered. The filtered extracts were combined and concentrated using a rotary evaporator, under reduced pressure at approximately 40°C and lyophilized to obtain the 80% aq.ethanol extract of the plant. The total phenolic, flavonoid and flavonol content, reducing power and their free radical scavenging capacity of the chloroform extract of the plants were also investigated and quantification of phenolic acids (gallic acid, methyl gallate, caffeic acid, syringic acid, ferulic acid, *p*-coumaric acid, sinapic acid) and flavonoids (catechin, rutin, quercetin, naringin, myrecetin , apigenin, kaempferol) with the help of a reversed-phase high-performance liquid chromatograph using photodiode array detector with gradient elution. The extracts were filtered through a 0.45 µm polyvinyl difluoride (PVDF) membrane for HPLC analysis.

2. Total phenolic content

The amount of total phenolic content of crude extracts of each plant was determined according to Folin-Ciocalteu procedure . The tested extracts (100 l) were introduced into test tubes. 1.0 ml of Folin-Ciocalteu reagent and 0.8 ml of sodium carbonate (7.5%) were added. The tubes were mixed and allowed to stand for 30 min. Absorption at 765 nm was measured (UV-visible spectrophotometer Shimadzu UV 1800).

Table 1 : Total phenolic content in the Plant materials (mg/gm dry ext)

Sl No.	Name of the plant	Parts used	Total phenolic content GAE equivalent Mean ±SEM
1.	*S. aqueum*	Fruit	14.75 ±0.44

Each value in the table was obtained by calculating the average of three experiments and

data are presented as Mean ± SEM.

3. Estimation of total flavonoids

Total flavonoids were estimated using the method of Ordonez et al., 2006. To 0.5 ml of extracts, 0.5 ml of 2% $AlCl_3$ ethanol solution was added. After one hour, at room temperature, a yellow color developed, indicated the presence of flavonoids and the absorbance was measured at 420 nm (UV-visible spectrophotometer Shimadzu UV 1800).

Table 2

Sl No.	Name of the plant	Parts used	Total flavonoid content Rutin equivalent (mg/gm dry ext) Mean ±SEM
1.	*S. aqueum*	Fruit	6.60 ±0.02

Each value in the table was obtained by calculating the average of three experiments and

data are presented as Mean ± SEM.

4. Estimation of total flavonols

Total flavonols in the plant extracts were estimated using the method of Kumaran and Karunakaran, 2006. To 2.0 ml of sample (standard), 2.0 ml of 2% $AlCl_3$ ethanol and 3.0 ml (50 g/L) sodium acetate solutions were added. The absorption at 440 nm (UV-visible spectrophotometer Shimadzu UV 1800) was read after 2.5 h at 20°C. Totalflavonol content was calculated as quercetin equivalent (mg/g) using the following equation based on the calibration curve: $y = 0.0049x + 0.0047$, $R^2 = 0.9935$, where y was the absorbance and x was the quercetin equivalent (mg/g).

Table 3

Sl No.	Name of the plant	Parts used	Total flavonoid content Quercetin equivalent (mg/gm dry ext) Mean ±SEM
1.	*S. aqueum*	Fruit	5.35 ±0.06

Each value in the table was obtained by calculating the average of three experiments and

data are presented as Mean ± SEM.

5. Measurement of reducing power

The reducing power of the extracts was determined according to the method of Oyaizu, 1986. Extracts (100 µl) of plant extracts were mixed with phosphate buffer (2.5 mL, 0.2 M, *p*H 6.6) and 1% potassium ferricyanide (2.5 mL). The mixture was incubated at 50°C for 20 min. Aliquots of 10% trichloroacetic acid (2.5 mL) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and a freshly prepared ferric chloride solution (0.5 mL, 0.1%). The absorbance was measured at 700 nm. Reducing power is given in ascorbic acid equivalent (AAE) in milligram per gram (mg/g) of dry material using the following equation based on the calibration curve: $y = 0.0023x - 0.0063$, $R^2 = 0.9955$ where y was the absorbance and x was the ascorbic acid equivalent (mg/g).

Table 4

Sl No.	Name of the plant	Parts used	Total flavonoid content Quercetin equivalent (mg/gm dry ext) Mean ±SEM
1.	*S. aqueum*	Fruit	10.51 ±0.23

Each value in the table was obtained by calculating the average of three experiments and

data are presented as Mean ± SEM.

6. Determination of DPPH free radical scavenging activity

The free radical scavenging activity of the plant samples and butylated hydroxyl toluene (BHT) as positive control was determined using the stable radical DPPH (1,1-diphenyl-2-picrylhydrazyl). Aliquots (20 - 100 l) of the tested sample were placed in test tubes and 3.9 mL of freshly prepared DPPH solution (25 mg L^{-1}) in methanol was added in each test tube and mixed. The absorbance was measured at 517 nm (UV-visible spectrophotometer Shimadzu UV 1800) after 30 min. The capability to scavenge the

DPPH radical was calculated, using the following equation:

DPPH scavenged (%) = {(Ac – At)/Ac} x 100

Where Ac is the absorbance of the control reaction and At is the absorbance in presence of the sample of the extracts. The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration in mg of dry material per ml (mg / ml) that inhibits the formation of DPPH radicals by 50%. Each value was determined from regression equation.

Table 5

Sl No.	Name of the plant	Parts used	DPPH radical scavenging activity (% of Inhibition) Mean ±SEM
1.	*S. aqueum*	Fruit	23.93±0.24

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM.

1. Scavenging activity of ABTS radical cation

The 2,2´-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation ($ABTS^{\cdot+}$)-scavenging activity was measured according to the method described by Re *et al*.. ABTS was dissolved in water to a 7 mM concentration. The ABTS radicals were produced by adding 2.45 mM potassium per sulphate (final concentration). The completion of radical generation was obtained in the dark at room temperature for 12–16 h. This solution was then diluted with ethanol to adjust its absorbance at 734 nm to 0.70 0.02. To determine the scavenging activity, 1 mL of diluted $ABTS^{\cdot+}$ solution was added to 10 l of plant extract (or water for the control), and the absorbance at 734 nm was measured 6 min after the initial mixing, using ethanol as the blank. The percentage of inhibition was calculated by the equation:

ABTS scavenged (%) = $(A_{cont} - A_{test}) / A_{cont}$ 100

Where, A_c and A_s are the absorbencies of the control and of the test sample, respectively. From a plot of concentration against % inhibition, a linear regression analysis was performed to determine the IC_{50} value of the sample.

Table 6

Sl No.	Name of the plant	Parts used	ABTS radical scavenging activity (% of Inhibition) Mean ±SEM
1.	*S. aqueum*	Fruit	43.461.07

Each value in the table was obtained by calculating the average of three experiments and

data are presented as Mean ± SEM.

Quantification of phenolic acids and flavonoids in the methanol extract of plant by HPLC

HPLC analyses for the quantification of phenolic acids and flavonoids in the plant extracts were performed using Dionex Ultimate 3000 liquid chromatograph including a diode array detector (DAD) with 5 cm flow cell and with Chromeleon system manager as data processor. Separation was achieved by a reversed-phase Acclaim C18 column (5 micron particle size, 250 x 4.6 mm). 20 L of sample was introduced into the HPLC. The method was validated according to the USP and ICH guidelines.

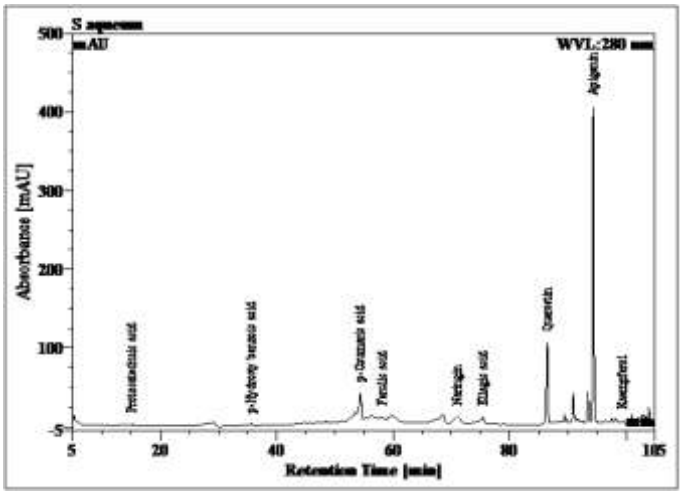

Fig.1. HPLC chromatogram of the 80%aq.ethanol extract of *S.aqueum*.

Table 7. Phenolic acids and flavonoid content in *S. aqueum*

Sl No.	Phenolic acids/ Flavonoids	ug/gm plant material *S.aqueum*
1.	Gallic acid	ND
2.	Protocatechuic acid	2.71
3.	Gentisic acid	ND
4.	p-Hydroxy benzoic acid	5.92
5	Catechin	ND
6	Chlorogenic acid	ND
7	Vanillic acid	ND
8	Caffeic acid	ND
9	Syringic acid	ND
10	p-Coumaric acid	15.09
11	Ferulic acid	0.84
12	Sinapic acid	ND
13	Salicylic acid	ND
14	Naringin	22.09
15	Rutin	ND
16	Ellagic acid	7.93
17	Myricetin	ND
18	Quercetin	56.34
19	Naringenin	ND
20	Apigenin	157.51
21	Kaempferol	1.39

CONCLUSION

Studies on phenol and flavonoid content of *Syzygiumaqueum*(Burm. f.) Alston fruits have been performed for the first time. For this methanolic extract has been used and the method has been validated according to the USP and ICH guidelines.Photodiode array detector revealed the presence of an array of phenolic acids such as; gallic acid, methyl gallate, caffeic acid, syringic acid, ferulic acid, *p*-coumaric acid, sinapic acid. Flavonoids is represented by catechin, rutin, quercetin, naringin, myrecetin ,apigenin, kaempferol.Free radical scavenging activity is noticed in the fruit extract. Therefore this can act as a significant source of natural antioxidant compounds that may have tremendous beneficial health effects.

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