Quantitative Evaluation of Some Phenolic Compounds in the leaf of *Syzigium cumini* L.

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ABSTRACT

A rapid method has been developed for doing the quantitative estimation of phenolic compounds present in the leaves of Syzigium cumini L. (Family - Myrtaceae). The alcoholic extraction of stem was subjected for the quantitative estimation of these compounds. The alcoholic extract of S. cumini was prepared by the maceration using ethanol followed by quantitative estimation of phenolics compounds by visible spectroscopy. The results reveal the presence of 5900 µg/g of total phenols, 940 µg/g of ortho-dihydric phenols, 680 µg/g of quinones, 740 µg/g of flavonols and 650 µg/g of tannins respectively in the stem of C. quadrangularis. Thus, the therapeutic potential of C. quadrangularis may be due to these phytochemicals.

Keywords: *Syzigium cumini, Phenolic compounds, Spectroscopy, Phenol.*

INTRODUCTION:

Plant phenolics are a chemically heterogenous group of nearly 10,000 individual compounds. Some are soluble only in organic solvents while some are water soluble carboxylic acids and glycosides whereas others are large insoluble polymers. Keeping their chemical diversity, phenolics play a variety of roles in plants. Many of the phenolics compounds serve against herbivores and pathogens whereas, others function in mechanical support, in attracting pollinators and fruit dispersers, absorbing harmful Ultraviolet radiation and also in reducing the growth of nearly competing plants (Taiz and Zeiger, 1998). Depending on the property of the absorbing the light at particular wavelength in the visible region of the electromagnetic spectrum different phenolics compounds can be quantitatively estimated with the help of studying their absorption spectra at their respective wavelength of light where they show maximum absorption respectively. In the present work the phytochemical quantitative analysis was done with the help of UV-Visible absorption spectra of the various phenolics compounds in the aerial parts of *Syzigium cumini* L.

The major antioxidant capacities of plants in fruits and vegetables are Vitamins C and E and phenolic compounds, chiefly flavonoids. Phenolic compounds possess different biological activities, but most important of all, they show antioxidant activities. The phenolics are able to scavenge reactive oxygen species due to the electron donating properties (Re, *et al*., 1999). The antioxidant effectiveness in food depends not only on the number and location of hydroxyl groups but also on factors such as physical location and interaction with other food components and also environmental conditions (e.g., pH). In many studies, phenolic compounds has demonstrated higher antioxidant activity than antioxidant vitamins and carotenoids (Velioglu, *et al*., 1998). Therefore the objectives of this work is to investigate the amount of phenolic compounds present in plants.

Phenolic Compounds are mainly synthetized from cinnamic acid, which is formed from phenylalanine by the action of L-phenyloalanine ammonia-lyase (PAL), the branch point enzyme between primary (shikimate pathway) and secondary (phenylopropanoid) metabolism (Dixon and Paiva, 1995). The significance of this route can be supported by the fact that, in normal growth conditions, 20% of the total carbon fixed by the plants flows through this pathway (Diaz, *et al*., 2001).

Syzigium cumini belonging to Family- Myrataceae is a large evergreen tree. It has been valued in Ayurveda and Unani systems of medication for possessing variety of therapeutic properties. Most of the plant parts are used in traditional system of medicine in India. According to Ayurveda, its bark is acrid, sweet, digestive and astringent to the bowels, antihelminthic and good for sore throat, bronchitis, asthma, theist, biliousness, dysentery, blood impurities and to cure ulcers (Kirtikar and Basu, 1975). In Unani medicine system the leaves are used for strengthening the teeth and the gums, the seeds are astringent, diuretic, stops urinary discharge and remedy for diabetes and the bark showed good wound healing properties (Nadkarni, 1954). *Syzigium cumini* is a medicinal plant whose parts were pharmacognosically proved to possess hypoglycaemic (Kannabiran kavitha, *et al*., 2011), antibacterial, anti diarrhoea effects, anti-inflammatory activity of leaf and barks (Muruganandan, *et.al*. 2001).

Syzigium cumini is a tree reaching 20Meter in height; bark ash white; branches terete, glabrous. Leaves opposite, oblong-elliptic, 8-16 x 4-6 cm, rounded or acute at base, acuminate, glabrous on both surfaces. Flowers in paniculate cymes arising from old leaf scars; peduncles short. Calyx 4-5 mm long with cup-shaped limb, tuncate or obscurely 4-toothed. Petals white, calyptrate, ovate-orbicular, 2-3mm long. Stamens exerted. Fruits dark violet, globose or ellipsoid, smooth, variable in size, 1-3 cm long, crowned with truncate calyx limb. Seed solitary, globose or oblong, greyish brown. Flowering and fruiting period is March to July. (Naik, 1998)

MATERIALS AND METHODS:

The aerial parts i.e. stem of *Syzigium cumini* was collected during the month of February and March at reddish clay loam soil from the Botanical garden of the Department of Botany, Shri Shivaji College of Arts, Commerce and Science, Akola. 1gm of the plant material was weighed and taken each time and crushed with the help mortar and pestle in the suitable solvent (80% Alcohol, 90% Alcohol, Absolute Alcohol, Chloroform, Distilled Water, Phosphate buffer, Methanol. Ether, etc.) each time for the test. UV- Visible spectrophotometer was used to carry out the photometric assays (Thimmaiah, 2009).

Quantitative photometric assays: Photometry was used to determine the concentration of a light absorbing compound present in a solution. The principles of quantitative photometric assays involved direct photometric quantitative measurements of light absorbing compounds. The assays included reaction of the compound to be assayed with a limited excess of one or more reagents that from specific colours with this compound, under defined reaction conditions and within a given concentration range. The intensity of colour formed is proportional to the quantity of original colourless compound.

Colourless compound + colour forming reagents → Colour proportional to be assayed amount of colourless compound

The big advantage of the technique is that complete isolation of the compound is not necessary and the constituents of a complex mixture like blood can be determined after little treatment. Then working with photometric assays in which colours form as a result of a chemical reaction, one has to prepare a series of reaction tubes that leads to a standard curve.

Standard curve:

A standard curve is the plot obtained by plotting concentration of a given standard along X-axis and the corresponding absorbance values along Y- axis on a graph sheet resulting a straight line which passes through the origin. It is used to quantify the amount of a given compound present in an unknown sample whose absorbance value is to be matched against to that of standard along Y-axis and a corresponding concentration could be read off along X-axis.

It is important to note that the standard curve should be linear. For measurement of a compound from an unknown sample, its absorbance value should be within the linear or valid quantitative photometry range. If not, the unknown sample may be appropriately diluted in order to obtain the absorbance values within the range (Thimmaiah, 2009).

Quantitative photometric assays were taken for various phenolic compounds for total phenols, ortho-dihydric phenols, quinones, flavonols, and tannins at specific wavelength of light where the respective compound showed absorbance of light. The various steps followed for each of the respective phenolic compound groups are stated as under.

Estimation of total phenols:

1gm of sample was grinded with the help of mortar and pestle in 10 time volume of 80% ethanol. The homogenate was centrifuged at 10,000 rpm for 20 minutes. Supernatant was kept and the residue was re-extracted with 5 times volume of 80% ethanol, centrifuged and pooled. Then the supernatant was evaporated to dryness in a water bath. Then the residue was dissolved in the known volume (5 ml) of distilled water.1 ml of the aliquot was pipette out in a test tube, and volume make up to 3 ml with distilled water. To it 0.5 ml of Folin - Ciocalteu reagent was added. After 3 minutes, 2 ml of 20% Na_2CO_3 solution was added, then mixed thoroughly and the test tube was kept in boiling water bath for exactly one minute, then allowed to cool and absorbance was measured at 650nm against a reagent blank.

Standard curve was prepared using different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1ml) of catechol (Thimmaiah, 2009).

Estimation of Ortho-dihydric phenols:

1gm of sample was grinded with the help of mortar and pestle in 10 time volume of 80% ethanol. The homogenate was centrifuged at 10,000 rpm for 20 minutes. Supernatant was kept and the residue was re-extracted with 5 times volume of 80% ethanol, centrifuged and pooled. Then the supernatant was evaporated to dryness in a water bath. Then the residue was dissolved in the known volume (5 ml) of distilled water.1 ml of the aliquot was pipette out in a test tube. To it 1 ml of 0.05N HCl, 1 ml of Arnow's reagent, 10 ml of water and 2ml of 1N NaOH was added. Absorbance was measured at 515 nm against a reagent blank lacking only extract.

Standard curve was prepared using different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1ml) of catechol (Thimmaiah, 2009).

Estimation of Quinones:
1gm of sample was grinded with the help of mortar and pestle in with chilled phosphate buffer (5 ml for each gm of tissue). The supernatant was collected by centrifugation at 2000rpm for 30 minutes at 4^0 C, this was used as enzyme extract. 3 ml of buffer, 3 ml of standard catechol or caffeic acid and 1.5 ml of enzyme extract was pipette out in a test tube. It was shaken gently and incubated in water bath. 4 ml of TCA (Trichloro acetic acid) reagent (without ascorbic acid) to one and 4 ml of TCA reagent (with ascorbic acid) was added. Precipitate was filtered. Absorbance was measured at 400 nm against a reagent blank lacking only extract.
Standard curve was prepared using different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1ml) of working standard of catechol (Thimmaiah, 2009).

Estimation of Flavonols:
1gm of sample was grinded with the help of mortar and pestle in ethanol and the supernatant was collected by centrifugation at 10,000 rpm for 20 minutes. The supernatant was evaporated to dryness; then the residue was dissolved in a known volume of distilled water (5 ml). 1 ml of extract was pipette out into 25 ml cap, conical flask and 1 ml of distilled water was added. Then 4 ml of vanillin reagent was added from a burette rapidly within 10-15 sec to flask A and 4ml of 70% H_2SO_4 to flask B. A blank flask C was prepared containing 4 ml vanillin reagent and 2ml water. Both the flasks A and B were shaken in the water bath at temperature below 35^0C. Flasks were kept at room temperature for exactly 15 minutes. Absorbance was measured at 500 nm of flask A, B, and C against 47% H_2SO_4 (Flask D). The absorbance's of the flasks B and C from that of A. The flavonol content was calculated using a standard curve prepared from phloroglucinol or kaempferol (100 µg/ml) (Thimmaiah, 2009).

Estimation of Tannins (Vanillin hydrochloride method):
1g of sample was extracted in 50 ml methanol, after 20-28 hours it was centrifuged and supernatant was collected. 1 ml of supernatant was pipette out into a test tube and quickly 5ml of vanillin hydrochloride reagent was added and mixed. Then the readings were taken on spectrophotometer at 500nm after 20 minutes. A reagent blank was prepared with vanillin hydrochloride reagent alone. A catechin standard graph was prepared from working standard (100 µg/ml) of catechin and amount of tannins was calculated (Thimmaiah, 2009).

RESULTS AND DISCUSSIONS:
On the basis of the spectrometric analysis following readings for the different phenolics compounds were done at the respective wavelengths, as mentioned in the tables below:

Table 1: Spectrometric analysis for the estimation of Total Phenols–Standard and Sample

Standard Graph for Total Phenols		
T. T. No.	Concentration of Catechol (ml) per 10 ml	Absorbance (at 650 nm)
1	0.1	0.03
2	0.2	0.04
3	0.3	0.05
4	0.4	0.05
5	0.5	0.06
6	0.6	0.06
7	0.7	0.07
8	0.8	0.09
9	0.9	0.08
10	1	0.09

Sample	Vol. of sample taken for analysis	Absorbance (at 650 nm)
Syziygium cumini	0.2 ml	0.146 ± 0.002

Table 2: Spectrometric analysis for the estimation of Ortho-dihydric Phenols – Standard and Sample

Standard Graph for Ortho-dihydric Phenols		
Test Tube Number	Concentration of Catechol (ml) per 10ml	Absorbance (at 515 nm)
1	0.1	0.115
2	0.2	0.123
3	0.3	0.131
4	0.4	0.136
5	0.5	0.105
6	0.6	0.099
7	0.7	0.110
8	0.8	0.128
9	0.9	0.147
10	1	0.165
Sample	Vol. of sample taken for analysis	Absorbance (at 515 nm)
Syziygium cumini	1 ml	0.156 ± 0.001

Table 3: Spectrometric analysis for the estimation of Quinones – Standard and Sample

Standard Graph for Quinones		
Test Tube Number	Concentration of Catechol (ml) per 10ml	Absorbance (at 400 nm)
1	0.1	0.125
2	0.2	0.128
3	0.3	0.130
4	0.4	0.134
5	0.5	0.143
6	0.6	0.140
7	0.7	0.145
8	0.8	0.150
9	0.9	0.147
10	1	0.162
Sample	Vol. of sample taken for analysis	Absorbance (at 400 nm)
Syziygium cumini	1 ml	0.158 ± 0.002

Table 4: Spectrometric analysis for the estimation of Flavonols – Standard and Sample

Standard Graph for Flavonols				
Test Tube Number	Concentration of phlorogucinol or kaempferol (ml)per 10ml			Absorbance (at 500 nm)
1	0.1			0.113
2	0.2			0.097
3	0.3			0.108
4	0.4			0.124
5	0.5			0.129
6	0.6			0.132
7	0.7			0.134
8	0.8			0.139
9	0.9			0.141
10	1			0.141
Sample	Absorbance at 500 nm			A - (B+C)
	Flask A	Flask B	Flask C	
Syziygium cumini	0.553	0.292	0.120	0.141 ± 0.003

Table 5: Spectrometric analysis for the estimation of Tannins – Standard and Sample

Standard Graph for Tannins		
Test Tube Number	Concentration of catechin (ml) per 10ml	Absorbance (at 500 nm)
1	0.1	0.081
2	0.2	0.089
3	0.3	0.093
4	0.4	0.098
5	0.5	0.102
6	0.6	0.139
7	0.7	0.145
8	0.8	0.138
9	0.9	0.126
10	1	0.143

Sample	Vol. of sample taken for analysis	Absorbance (at 500 nm)
Syziygium cumini	1 ml	0.135 ± 0.002

Aerial parts of *Syziygium cumini* L. was taken for the phytochemical evaluation for phenolics compounds. The extracts of the plant parts were taken and their extracts were subjected to chemical reactions and then were analyzed on the UV-Visible spectrophotometer. The optical densities for the *Syziygium cumini* were measured at the respective wavelengths for total phenols, ortho-dihydric phenols, quinines, flavonols and tannins. The absorbance was plotted on the standard graph for respective phenolics compounds to be analyzed and the amount of compounds per gram of the plant material was calculated and the following results were obtained.

Syziygium cumini contained 5900 µg/g of total phenols; 940 µg/g of ortho-dihydric phenols; 680 µg/g of quinines; 740 µg/g of flavonols; 650 µg /g of tannins.

CONCLUSIONS:

By seeing the values it is said that the plant *Syziygium cumini* contain considerable amount of phenolics compounds and thus justify their traditional medicinal use and importance. Phenolics also function in the defence mechanism of the plant protecting the plant from microbes, pathogens and herbivores. Phenolic compounds contain active groups in their molecular structure which are capable of forming weak interactions, like, hydrophobic interactions, hydrophilic interactions, hydrogen bonding and van-der-Waal's forces, with water and other cell organelles. Weak interactions are important for life and life processes, as permanent bond formation is a permanent and also its breakage consumes lot of amount of energy. A large amount of energy is conserved due to weak interactions and are very much important to sustain life. Phenolics compounds are present in all parts of the plant. They are capable of absorbing the harmful UV- radiations due to the chemical bonding present in the molecule of phenolic compounds. Pollens dispersed in atmosphere absorb the UV-radiations and help to lessen up harmful effects of these radiations by absorbing certain amount of UV-rays.

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