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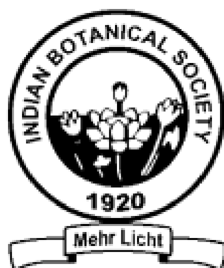

QUALITATIVE AND QUANTITATIVE ESTIMATION OF FLAVONOIDS AND PHENOLIC COMPOUNDS OF *SEMECARPUS ANACARDIUM* NUTS

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Semecarpus anacardium belongs to family Anacardiaceae is well known for its use in ayurveda, siddha and unani as a potent medicinal herb since ancient times. In ayurveda *Semecarpus anacardium* is also named as Bhallataka. It is used as remedy on different ailments viz. skin diseases, fever, analgesic, antineoplastic, also act as antitumour , antiallergic etc. These actions are attributed to a wide range of phytoconstituents present in this plant. The oil of nuts is used as mark on cloth for the purpose of identification, hence it is known as marking nut or 'Dhobi nut'. In the existing study, qualitative analysis of secondary metabolites was done by thin layer chromatographic separation method.Total flavonoids content and total phenolic content of methanolic extract of *Semecarpus anacardium* nuts was quantitatively estimated by Aluminium chloride colorimetric method (as quercetin equivalent) and Folin-Ciocalteu method (as caffeic acid equivalent) respectively. The total phenolic contents observed was 26.520±0.493 mg/gdw and total flavonoid contents was observed 6.46±0.431 mg/gdw.

Keywords: analgesic, phenolic content, flavonoids, quercetin, aluminium chloride, caffeic acid.

Semecarpus anacardium is a moderate sized deciduous tree distributed in Sub- Himalayan region It is a medium sized tree growing upto 10-15 metres in height. The bark is grey in colour and exudes an irritant secretion on incising. The leaves are 30-60 cm long and 12-30 cm broad. They are glabrous above and pubescent beneath. The flowers are greenish white, in panicles. Fruits are 2-3 cm broad. The flowering occurs in june and then onwards the plant bears fruits.It is available in hotter region up to the altitude of 3500ft. In India it is available in the forest area of West Bengal, Bihar , Orissa, Karnataka, Chattishgarh, Madhye Pradesh, Tamilnadu and Maharashtra etc.(Sharma *et al*. 2008). It is commonly known as Bhallataka, Bhilwa, Biba and Dhobi nut etc. The fruit oil is claimed to be useful in treating leprosy, neuritis, rheumatoid arthritis, piles, asthma, cough, sexually transmitted diseases like syphilis and gonorrhea, veneral disorder, nematicidal etc. (Kirthikar and Basu 1933, Nadkarni 1976). It is a potent medicinal plant as 'Ardha –Vaidya' in ayurveda meaning half Physician as it can cure almost all ailments (Nadkarni 1976). It has toxic nature and can not be consumed without detoxification. It has been used in both Ayurveda as well as Siddha system of medicine for treatment of various disease such as skin diseases, fever, analgesic, cancer ,asthma, arthritis, antineoplastic etc.

Owing to these medicinal attributes this plant holds a great value for phytochemical analysis. Plants and plant-derived products have been part of the health-care system since ancient human civilization (Kamboj 2000). Herbal remedies are popular remedies for diseases used by the vast majority of the world's population. Recent research on bioactive compounds of medicinal plants like flavonoids, alkaloids, tannin, steroids, glycosides, phenols, carotenoids, benzoic acid, cinnamic acid, folic acid, fixed oils which are stored in their specific parts of leaves, bark, flowers, seed, fruits, root etc has received much attention (Pal and Shukala 2003).

There is an increased evidence for the participation of free radicals in the etiology of various diseases like cancer, diabetes, cardiovascular diseases, autoimmune disorders, neurodegenerative diseases, aging etc. A free radical is defined as an atom or molecule possessing unpaired electrons. Antioxidants are agents which scavenge the free radicals and prevent the damage caused by

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reactive oxygen species (ROS). Antioxidants can greatly reduce the damage due to oxidants by neutralizing the free radicals before they can attack the cells and prevent damage to lipids, proteins, enzymes, carbohydrates DNA. A wide range of antioxidants from both natural and synthetic origin has been proposed for use in the treatment of various human diseases (Sharma *et al*. 2008).

Natural antioxidants have become the target of a great number of research studies in finding the sources of potentially safe, effective and cheap antioxidants. Different studies have indicated the relationship between the plant antioxidants and reduction of chronic diseases. It has been found out that plant having polyphenolic compounds such as flavonoids possess antioxidant activity(Cook and Samman 1996) and aroused considerable interest in the present time because of their potential beneficial effects on human health in fighting diseases. Some evidence suggests that the biological actions of these compounds are related to their antioxidant activity. The capacity of flavonoids to act as antioxidants depends upon their molecular structure. Quercetin, the most abundant dietary flavonol, is a potent antioxidant because it has all the right structural features for free radical scavenging activity (Pal *et al* .2009). Therefore, the objective of our present study is to determine the Qualitative separation of secondary metabolites. Quantitative determination of total phenolic content and total flavonoid content of nuts extract of *Semecarpus anacardium* using aluminium chloride colorimetric method and Folin-Ciocalteu method respectively. In the study quercetin and caffeic acid are taken as a standard for flavonoid and phenol respectively .

MATERIALS AND METHODS

Plant material: *Semecarpus anacardium* were collected from the Jhargram forest area at West Bengal in the month of November.Plant materials was identified taxonomically by the expert taxonomist cum Head of Department Dr. A.K. Mondal , Department of Botany and Forestry, Vidyasagar University,Midinapur,West Bengal. The collected sample specimen has been deposited in the institution herbarium for the future reference. I have received authentication no. RUBL211734 from herbarium of Department of Botany, University of Rajasthan, Jaipur.Plant sample were washed with distilled water and then shade dried. Dried plant material were ground into fine powder using mechanical grinder and kept in air tight container for the further analysis.

Separation of secondary metabolites by thin layer chromatography: For the thin layer chromatography studies of secondary metabolites, used thin glass plates coated with silica gel 'G' was air dried at room temperature. The dried plates were activated at 100^0C for 15 min in an oven. Extracts was applied 1cm above the edge of the chromatographic plates alongwith the reference used as marker and developed in an air tight chromatographic chamber. (Wong and Francis1968).

TLC study of flavonoids: One gram powdered nuts of *Semecarpus anacardium* were extracted with 10 ml methanol on water bath(60^0C/5min).The filtrate was condensed by evaporation, added a mixture of water and EtOAc(10:1 ml), mixed thoroughly.The EtOAc phase retained is used for further analysis.The flavonoid spots were separated using chloroform and methanol (19:1) solvent mixture.The colour and Rf values of these spots were recorded under U.V light (Wagner and Bladt 1996).

TLC study of phenols: The powered sample of nuts were lixiviated in methanol on rotary shaker for 24h. The condensed filtrate was used for chromatography. Phenols were separated using chloroform and methanol (27:03) solvent mixture. The colour and Rf values of these phenols were recorded under visible light after spraying the plates with Folin-Ciocalteu's reagent heating at

70^0C/10min (Harborne 1998).

Estimation of total flavonoid content Aluminium Chloride Colorimetric Method

Principle: Formation of acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols in addition with aluminium chloride. Aluminium chloride also forms acid labile complexes with the ortho - dihydroxyl groups in the A- or B-ring of flavonoids. For building the calibration curve, quercetin is used as a standard material. Various concentrations of standard quercetin solution were used to make a standard calibration curve (Chang *et al.*2002).

Procedure : In this method, quercetin was used to make the calibration curve. 10 mg of quercetin was dissolved in methanol and then diluted to suitable concentrations. A calibration curve was made by measuring the absorbance of the dilutions at 415 nm (λ max of quercetin) with spectrophotometer. Aluminium chloride, 1% and potassium acetate, 1M solutions were prepared(Naskar *et al.* 2011.,Kiranmai *et al.* 2011).

Preparation of sample extracts: Sample extracts of plant part (nuts) were prepared using methanol as extracting solvent. 1g of the dried powdered plant material was extracted using 100ml of methanol by soxhletion for 2 days. Crude methanolic extract was obtained by evaporating the extract to dryness and stored in refrigerator for further analysis.

Stock Solution of Extracts:100 mg of the each extract was accurately weighed and transferred to 5 ml volumetric flask and made up the volume with methanol.

Preparation of Test Solutions:0.5ml of each extract stock solution, 1.5 ml methanol, 0.1 ml aluminium chloride, 0.1 ml potassium acetate solution and 2.8 ml distilled water were added and mixed well. Sample blank was prepared in similar way by replacing aluminium chloride with distilled water. Sample and sample blank of all four extracts were prepared and their absorbance was measured at 415 nm. All prepared solutions were filtered through whatman filter paper before measuring.

Estimation of total phenolic content by Folin–Ciocalteu method

Principle: Folin–Ciocalteu's reagent, a mixture of phosphotungstic($H_3PW_{12}O_{40}$) and phospho-molybdic ($H_3PMo_{12}O_{40}$) acids, is reduced to blue oxides of tungsten (W_8O_{23}) and molybdenum (Mo_8O_{23}) during phenol oxidation. This reaction, occurring under alkaline conditions, is carried out in the presence of sodium carbonate. Blue coloration is monitored at 726 nm and reflects the quantity of phenols, usually expressed as gallic acid or caffeic acid equivalents(Singleton and Rossi 1965).

Procedure: Estimation of total phenol content in each sample was includes the preparation of a regression curve of standard phenol (Caffeic acid). A stock solution of caffeic acid was prepared by mixing 40 mg of standard phenol in 1 ml of 80% ethanol. Eight concentrations ranging from 0.1 to 0.8 ml were prepared in the test tube and volume was raised to 1 ml by addition of 80% ethanol. To each test tube, 1ml of Folin-Ciocalteu reagent and 2 ml of 20% sodium carbonate solution was added and then mixture was shaken thoroughly. The samples were placed in boiling water for 1 min and cooled under running water. These reaction mixtures were diluted to 25 ml by adding distilled water and optical density was read at 750 nm against 80% ethanol as blank. The optical density of each sample was plotted against the respective concentration of total phenols to compute regression curve.The concentrations in the test samples were calculated by referring the respective optical density of test sample against standard curve of caffeic acid.

Preparation of Test Solutions : 0.2 gm plant sample was crushed in 3 ml of 80% ethanol. Mixture was centrifuged at 1500 rpm for 20 minute at room temperature. Then 1 ml of supernatant was taken in a test tube and 1ml of Folin–Ciocalteu reagent and 2 ml of Sodium Carbonate was mixed. OD was measured by spectrophotometer at 750 nm. 80% methanol was set as blank.

RESULTS AND DISCUSSION

The data of TLC of flavonoids of *S. anacardium* is tabulated in the table1. Five spots were reported in free flavonoid(Fraction II) and three spots were reported in bound flavonoid(Fraction III) in the nut extract with different colour and Rf values.

Rf value of standards Quercetin (0.91), Lutein (0.23), Rutin(0.44), Luteolin (0.98), Caffeine (0.34), Quercitrin (0.82). It has revealed the presence of six flavonoids in the nuts .

The data of Phenols of *S.anacardium* by thin layer chromatography is tabulated in the Table 2.Five phenolic spots with different colour and Rf values (6.68,16.07,41.01,55.05,66.64) were observed.

Determination of Total flavonoid content: Flavonoids are plant secondary metabolites widely distributed in the plant kingdom. Figure 1 shows the standard calibration curve of quercetin (0.1mg/ml stock solution) for the determination of total flavonoid content in the methanolic extracts of different plant parts. Concentration values of extracts were obtained from Quercetin standard curve, by interpolating to the X- axis. Total flavonoid content was calculated by using the following formula (Mundhe *et al.*2011)

Table 1. Qualitative separation of flavonoids of *S.anacardium*.

S. no.	Colour of the spots	Rf values of Free flavonoids	Rf value of Bound flavonoids
1	Dark brown	0.23	0.34
2	Light brown	0.40	-
3	Yellow	0.82	0.82
4	Light yellow	0.91	-
5	blue	0.98	0.98

Table 2. Qualitative separation of phenols of *S.anacardium*

S.No.	Plant part (Nuts) Colour of spots	Rf values
1.	Blue	6.68
2	Dark blue	16.07
3	Intense blue	41.01
4	Blue	55.05
5	Blue	66.64

$$\text{Total Flavonoid Content} = \frac{R \times D.F. \times V \times 100}{W}$$

Where,

R – Concentration computed through standard curve of quercetin, **V** - Volume of stock Solution, **D.F.**- Dilution factor, 100- for 100 gm dried plant, **W**- Weight of the plant used in experiment (in gm)

Determination of Total phenolic content: Quantification was done on the basis of a standard curve of caffeic acid (**Figure 2**). Results were expressed percentage w/w and calculated using following formula,

$$\text{Total Phenolic Content}(\%\ w/w) = \frac{CAE \times V \times D \times 10^{-6} \times 100}{W}$$

Where,

CAE – Caffeic acid equivalent (Mg/ml), **V** - Total volume of sample (ml), **D** - Dilution factor, **W** - Sample weight

Assays were performed in triplicates. Values are expressed as means ± SD. Total flavonoids and phenolic content of plant part (nuts) extracts is shown in Table 3. Total flavonoids content is expressed as quercetin equivalent (QE; µg quercetin /100g) and total phenolic content is expressed as caffeic acid equivalent

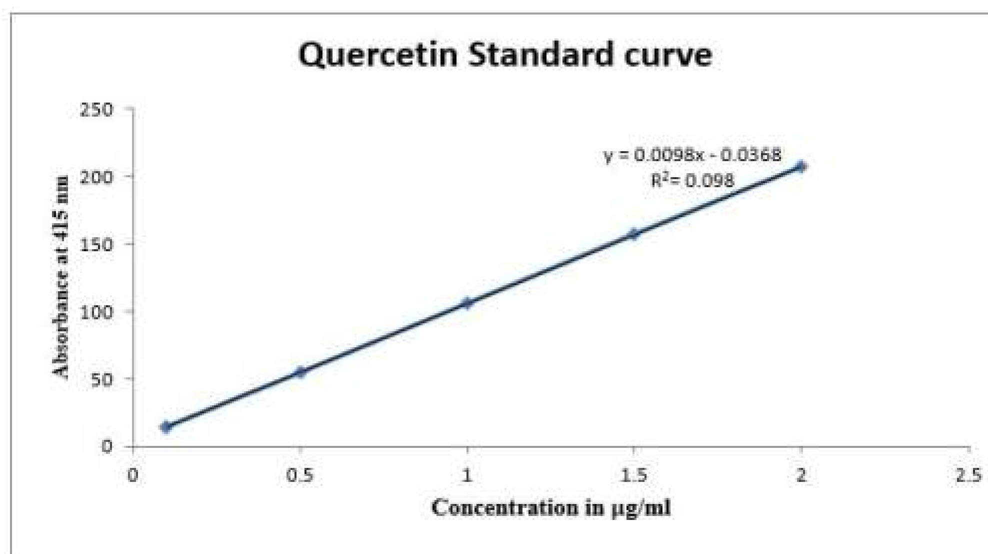

Figure 1. Standard curve of quercetin (0.2 mg/ml stock solution)

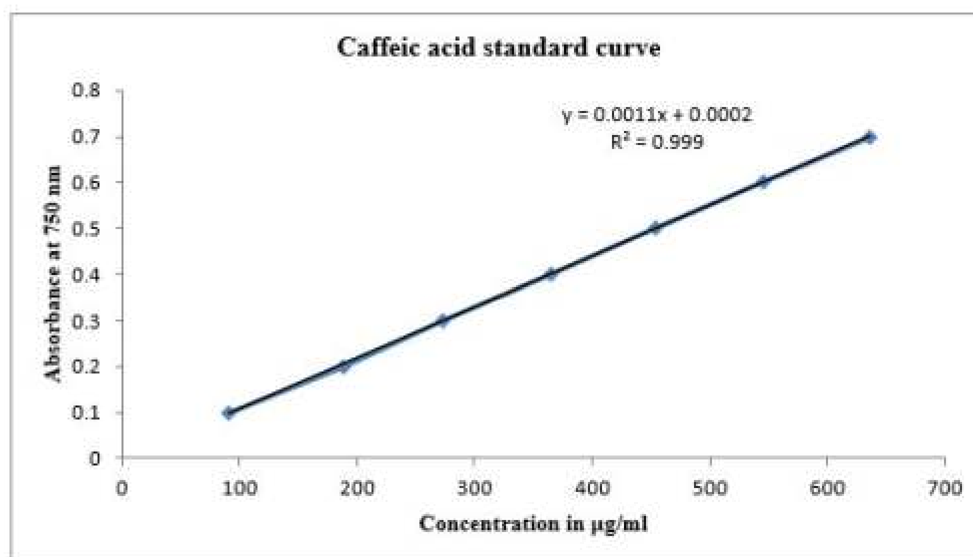

Figure 2: Standard curve of Caffeic Acid

Table 3: Results of calibration curve

Sample of *Semecarpus anacardium*	Total flavonoid content in mg/100g of dried material (in QE)	Total phenolic content in mg/100g of dried material (in CAE)
Nuts	6.46±0.431	26.520±0.493

(CAE; caffeic acid µg /100g).

DISCUSSION

Datas of Thin layer chromatography has revealed that presence of flavonoids and phenols in part of nuts. Total phenolic content recorded more than total flavonoid content in methanolic extract of *Semecarpus anacardium* nuts.The amount of phenolic content in nuts was 26.52±0.493 mg/g and total flavonoid

content was 6.46±0.431 mg/g. These data emphasize the pharmacological basis for the wide use of this plant as therapeutic agent for treating so many ailments. However, there is need to further carry out advanced spectroscopic studies in order to elucidate the structure of these compounds.

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