➔DOI : 10.15740/HAS/AJBS/9.2/273-283
e ISSN-0976-8343 | ■ Visit us : *www.researchjournal.co.in*
Asian Journal of Bio Science, Volume **9** | Issue 2 | October, 2014 | 273-283
Received : 12.01.2014; **Revised :** 10.09.2014; **Accepted :** 22.09.2014

RESEARCH PAPER

In vitro antioxidant activity studies of *Artocarpus gomezianus*

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Extraction of fruits, aerial parts and roots from *Artocarpus gomezianus* were subjected for pharmacognostic analysis. Also these parts were subjected tohydro-alcohol (30:70) extraction by soxhlet extraction technique. The raw materials were dried and powdered and analyzed for various parameters. The moisture content was found to be in the range of 4.97 per cent to 11.09 per cent, extractive values - 14.92 per cent to 18.75 per cent, chloroform solubility 1.15 per cent to 6.99 per cent and water solubility ranged between 4.52 per cent to 5.77 per cent. Fluorescence analysis and ash values were also determined. Extraction yields with 30:70 solvents indicate the quantitative idea about some of the proximate components. The results are important in planning the extraction, phytochemical analysis and determination of their various beneficial biological activities. The study was undertaken to evaluate qualitatively for the contents of carbohydrates, glycosides, saponins, alkaloids, flavonoids, phenolics, tannins, Phytosterols, Triterpenoids, oils and fats present in the extracts from the Fruit, Aerial parts and Roots of *Artocarpus gomezianus*. The extracts when tested qualitatively for various phytochemicals, they found to contain carbohydrates, Glycosides, alkaloids, tannins, phytosterols and Triterpinoids. However, they do not contain saponins, oils and fats. Quantitative estimation of extracts for total phenols and total flavonoids reveals that most parts contain reasonably higher amounts. The results clearly demonstrate that the extracts can be considered for further studies which evaluate the biological activity such as antioxidant activities. Antioxidant assays such as Nitric oxide scavenging assay, Ferric ion reducing activity assay, ABTS free radical scavenging activity assay and total antioxidant assays were performed to ascertain the potency of the extracts. Fruit extract of *Artocarpus gomezianus* was found to have maximum ferric ion reducing property than other parts studied. Nitric oxide scavenging activity was found to be higher in fruit followed by aerial and root parts. ABTS radical scavenging activity of aerial part extract is found to be 5 mg/ml compared to the standard ascorbic acid with 4 mg/ml. The total antioxidant activity was found to be significantly high for the extract from fruit part than the extracts from aerial and root parts. It is clear from the studies that the extracts of aerial parts, fruits and roots possess potentially beneficial antioxidant activities. In view of their use in ancient medicine coupled with the recent understandings of these plant species, they may be considered for further exploration as they may yield very potent nutraceuticals.

Key words : *Artocarpus gomezianus*, Plant extracts, Antioxidant, Free radical scavenging

How to cite this paper : Prashanth, S.J., Suresh, D., Potty, V.H. and Maiya, P. Sadananda (2014). *In vitro* antioxidant activity studies of *Artocarpus gomezianus*. *Asian J. Bio. Sci.*, **9** (2) : 273-283.

INTRODUCTION

Consumption of plant derived medicines is wide spread andincreasing significantly in both traditional and modern medicine. According to the World Health Organization, more than 80 per cent of the world population in developing countries depends primarily on plant based medicines for basic healthcare needs (Canter *et al*., 2005). A few of these genera *viz.*, *Morus*, *Ficus* and Artocarpus are economic sources of food and widely used in traditional medicine, agriculture and industry (Jarret, 1959). These genera received a great level of scientific interest as they contain medicinally important secondary metabolites possessing useful biological activities. A number of *Artocarpus* species are used as food and for traditional folk medicines in South-East Asia, Indonesia, Western part of Java and India. Artocarpus plants offer advantages as a profitable multipurpose crop for producing fruits and timber. The exceptional medicinal value of Artocarpus has long been recognized and economically the genus is of appreciable importance as a source of edible aggregate fruit; such as *Artocarpus heterophyllus* (jackfruit), *Artocarpus altilis* (breadfruit) and *Artocarpus chempeden* (Chempedak) and yielding fairly good timber (Verheij and Coronel, 1992).

Extracts of the aerial and underground plant parts have been applied in traditional medicine for the treatment of

diarrhoea, diabetes, malarial fever, tapeworm infection and other ailments. The other uses include wound healing, antisyphilic, expectorant properties and also use to treat anaemia, asthma and dermatitis. Most of the pharmacological effects can be explained by the phenolic compounds including flavonoids, stilbenoids, arylbenzofurons (Hakim *et al*., 2006) present in all plant parts and Jacalin, a lectin (Kabir, 1998) present in seeds of certain Artocarpus species. However, future efforts should concentrate more on *in vitro* and *in vivo* studies and also on clinical trials inorder to confirm traditional knowledge in the light of a rational phytotherapy. Especially the efficacy of Jacalin, a lectin extracted from the seeds of Artocarpus in controlling viral infections, HIV and modulation of immune response to pathogens (Favero *et al*., 1993) should be further substantiated in clinical studies.

The link between conventional remedies and recent research in various areas has been well established in other plants (Das *et al*., 2007; Gutierrez *et al*., 2008; Lansky *et al*., 2008) which facilitate to determine effective mode of action of plant derived products. The present plant is known to contain several pharmacological important bio molecules as listed earlier whose efficacy is well established by several biochemical and pharmacological studies.

As a group among forest tree plants, Artocarpus species are known to occupy a variety of ecological niches across different habitats and are being diverse and numerous in various forest ecosystems. The diversity, conservation status and state of knowledge of Artocarpus (Moraceae) are not uniform worldwide. The family consists of 60 genera comprising 1400 species distributed in the tropical and sub tropical regions of Asia. The genus Artocarpus J.R. and G. Foster (Moraceae) comprises mainly of bread fruit and jack-fruit trees. It is a native of South and South-East Asia, New Guinea and the Southern Pacific having 50 species. These are restricted to evergreen forests in the humid tropical zone and usually found below an altitude of 1000 m.

Many members of the genus Artocarpus have also been used as traditional folk medicine in South-East Asia for the treatment of inflammation, malarial fever and to treat the ulcers, abscessand diarrhoea (Perry, 1980 and Heyne, 1987). The pulp and seeds of jackfruit are used as a cooling tonic and pectorial, roots in diarrhoea and fever, leaves to activate milk in women and animals, as a source to treat antisyphilic and vermifuge, leaf ash applied to ulcers wounds and the warmed leaves have healing properties if pasted on the wounds. The latex mixed with vinegar promotes healing of abscesses, snakebite and glandular swellings. The leaves and stem barks have been used to treat anaemia, asthma, dermatitis, diarrhoea, cough and as an expectorant (Balbach and Boarim, 1992). The fruits, seeds and trunk wood contained chemical compounds with aphrodisiac properties (Le Cointe, 1947 and Ferrao, 1999). The wood has as edative effect in convulsions and its pith is said to cause abortion. The root is used as a remedy against skin diseases and asthma andits extract is used to reduce fever and diarrhea (ICUC, 2003). The heartwood of *Artocarpus heterophyllus* is used by monks in rural North eastern Thailand's Forest Tradition monasteries to dye their robes. Chips of wood are boiled in water, producing a rich earth-tone dye called "gaen-kanun," which is held to have remarkable medicinal qualities. In fact, monks of this tradition never wash their robes. Once a week, the robes are re-boiled in jackfruit dye and arehung to dry in the sun. Robes treated in this manner never emitsbad odor and provides protection from fungal infections and skin disorders (Salguero, 2003).

A MeOH extract from the stem bark of Artocarpus *gomezianus* showed DPPH free radical scavenging activity. Bio-assay guided fractionation of this MeOH extract led to the isolation of four flavonoids, namely artonin E, cycloartobiloxanthone, artobiloxanthone and (+)-catechin. These compounds were shown to possess recognizable DPPH free radical scavenging activity and appreciable inhibitory effect on nitric oxide production in murine macrophage-like cells (Sritularak *et al*., 2010).

RESEARCH METHODOLOGY

Sodium nitroprusside was purchased from Merck, Germany. Mayer's reagent, methanol, HCl, H_2SO_4, NaOH, alpha naphthol, ferric chloride, naphthyl ethylene diaminedi hydrochloride, DMSO, potassium ferricyanide, trichloroacetic acid, ferric chloride, folin-ciocalteu reagent, bismuth nitrate, sodium carbonate, gallic acid and phloroglucinol from SD Fine Chemicals Company, Mumbai and sulphanilic acid (0.33 % w/v) reagent, rutin and ascorbic acid were from Hi Media Chemicals Company, Mumbai. All other solvents are of AR grade and were distilled before use.

Extraction :

The authenticated *Artocarpus gomezianus* plant materials were subjected for soxhlet extraction in which the solvent vapour generated by gently heating the reservoir, condenses and is allowed to drip back onto the porous sample cup. The liquid condensate that drips on to the sample performs the extraction which then passes through the container and back into the reservoir. The cycle is repeated continuously and can be sustained as long as needed.

The dried materials were coarsely powdered; the powder was packed in filter paper and loaded into the thimble of soxhlet extractor. The solvent hydroalcohol (70:30-ethanol:water) of 2.5 lt. was used for extraction was poured into flask. The soxhlet extraction was carried out for 10 hours. Later the extracted solvent was evaporated under reduced pressure to get waxy extract. The extractive value of the extraction was obtained by using the relation,

$$\text{Per cent of extraction} = \frac{\text{weight of dried extract}}{\text{weight of fresh material}} \times 100$$

Pharmacognosy parameters :

For pharmacognosy analysis raw materials from aerial, fruit and root part were taken from *Artocarpus gomezianus*. The raw materials were dried and powdered, the powdered materials were analysed for different parameters.

Determination of moisture content or loss on drying :

About 5 g of each raw material were accurately weighed. The air dried material was taken in a previously dried and tarred flat weighing bottle in IR moisture balance and the temperature was adjusted to 105^0C and heating was done for 5 min. The procedure was repeated for three times for different samples and the loss in weight of the formulation was calculated with respect to the original weight.

The formula used for calculating LOD is

$$Z \frac{W_1}{W_2} x100$$

W_1-weight of raw material after heating

W_2- Original weight of the raw material.

Extractive values :

Determination of chloroform soluble extractives :

5 g of air dried coarse powder of extracts macerated with 100 ml of chloroform in a glass-stoppered conical flask with frequent shaking for 6 hours and then allowed to stand for 18 hours. Thereafter it was filtered rapidly taking care against loss of solvent. About 25ml of the filtrate was evaporated in a tared flat-bottomed dish to dryness using water bath and then dried at 105^0C for 6 hours, cooled in a desiccator for 30 minutes and weighed immediately.

Determination of water soluble extractives :

5 g of air dried coarse powder of extracts macerated with 100 ml of water in a glass-stoppered conical flask with frequent shaking for 6 hours and then allowed to stand for 18 hours. Thereafter, it was filtered rapidly taking care against loss of solvent. About 25ml of the filtrate was evaporated in a tared flat-bottomed dish to dryness on water bath and then dried at 105^0C for 6 hours, cooled in a desiccator for 30 minutes and weighed immediately.

Fluorescence analysis :

The powdered extracts were treated with distilled water, Chloroform, 1N NaOH in water, 1N NaOH in methanol, 10 per cent HCl and 10 per cent H_2SO_4.

Determination of ash values (*Anonymous, 1985*) :

Ash values such as total ash, acid insoluble ash, water soluble ash, acid soluble and sulfated ash were determined. The total ash determination method is designed to measure the total amount of material remaining after ignition.

Determination of total ash :

About 3 g each of powdered parts were accurately weighed and taken separately in silica crucible, which was previously ignited and weighed. The powder was spread as a fine layer on the bottom of crucible. The powder was incinerated gradually by increasing temperature to make it dull red hot until free from carbon. The crucible was cooled and weighed. The procedure was repeated to get constant weight. The percentage of total ash was calculated with reference to the air dried powder.

Acid insoluble ash :

The ash obtained as described above was boiled with 25 ml of 2N HCl for 5 minutes. The insoluble ash was collected on an ash-less filter paper and washed with hot water. The insoluble ash was transferred into a crucible, ignited and weighed. The procedure was repeated to get a constant weight. The percentage of acid insoluble ash was calculated with reference to the air dried plant material.

Water soluble ash :

The ash obtained as described for the total ash, was boiled for 5 minutes with 25 ml of water. The insoluble matter was collected on ash less filter paper and washed with hot water. The insoluble ash was transferred into silica crucible, ignited for 15 min. and weighed. The procedure was repeated to get a constant weight. The weight of insoluble matter was subtracted from the weight of total ash. The difference of weight was considered as water soluble ash. The percentage of water soluble ash was calculated with reference to air dried parts respectively.

Acid soluble ash :

Total ash treated with dilute hydrochloric acid reacts with minerals to form soluble salts and the insoluble residue consists mainly of silica, as acid insoluble ash.

To the crucible/silica dish containing the total ash obtained by the previous test, 25ml of HCl was added, covered with a watch glass and boiled gently for 5 min on a hot plate. Watch glass was rinsed with 5ml of hot water and washings were added to the crucible. Insoluble matter was collected on an ash less filter paper by filtration and rinsed repeatedly with hot water until the filtrate was found to be neutral/free from acid. Filter paper containing the insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to a constant weight in the muffle furnace at 450°-500°C. Silica dish was allowed to cool in a desiccator for 30 min and weighed without delay. Content of acid insoluble

ash as per cent was calculated as followed.

$$\text{Acid insoluble ash per cent} = \frac{(B - C)}{A} \times 100$$

where,
A = sample weight in g
B = wt. of dish + contents after drying (g)
C = wt. in g. of empty dish.

Sulphated ash :

A silica crucible was heated to red for 10 min and was allowed to cool in a desiccator and weighed. About one gram of substance was accurately weighed and transferred to the crucible. It was ignited gently at first, until the substance was thoroughly charred. Then the residue was cooled and moistened with 1 ml of concentrated sulfuric acid, heated gently until white fumes are no longer evolved and ignited at $800^0C \pm 25^0C$ until all black particles have disappeared. The ignition was conducted in a place protected from air currents. The crucible was allowed to cool. A few drops of concentrated sulfuric acid were added and heated. Ignited as before and was allowed to cool and weighed. The operation was repeated until two successive weighing do not differ by more than 0.5 mg.

Detection of carbohydrates (*Rosenthaler,1930*) :

500 mg of extract was dissolved in 5 ml of distilled water and filtered. The filtrate was used to test the presence of carbohydrates.

Molisch's test :

10 g of alpha napthol was dissolved in 100 ml of 95 per cent methanol to prepare Molisch reagent. To the extract, two drops of Molisch reagent and few drops of concentrated H_2SO_4 are added, formation of purple-violet ring indicates the presence of carbohydrates.

Detection of glycosides (Ronsenthaler, 1930; Middeltone, 1956) :

0.5 g of the extract was hydrolyzed with 20 ml of HCl (0.1 N) and filtered. The filtrate was used to test the presence of glycosides.

Keller-Killiani test :

To the extract, few drops of glacial acetic acid and one drop of 5 per cent $FeCl_3$ and concentrated H_2SO_4 were added, formation of reddish brown colour at the junction of two liquid layers and upper layer turned bluish green indicates the presence of glycosides.

Detection of saponins (*Kokate et al., 2001*) :
Foam test :

1 ml of extract was diluted to make up to 20 ml with distilled water and slowly shaked in a graduated cyclinder for 15 minutes. 1 cm layer of foam indicates the presence of saponins.

Detection of alkaloids (*Rosenthaler, 1930; Peach and Trancey, 1955*) :

0.5 g of the extract was dissolved in 10 ml of dilute HCl (0.1N) and filtered. The filterate was used to test the presence of alkaloids by Mayer's test and Dragendroff's method.

Mayer's test :

Filtrate was treated with Meyer's reagent; formation of yellow cream coloured precipitate indicates the presence of alkaloids.

Dragendroff's test :
Dragendroff's reagent:

– Dissolve 8 g of bismuth nitrate in 20 ml of nitric acid.
– Dissolve 27.2 g of potassium iodide in 50 ml of distilled water, mix (a) and (b) and adjust the volume to 100 ml with distilled water.

Filtrate was treated with dragendroff's reagent; formation of red coloured precipitate indicates the presence of alkaloids.

Detection of flavonoids (*Shellard, 1957*) :
Alkaline reagent test :

To 100 mg of extract, few drops of NaOH solution was added in a test tube. Formation of intense yellow colour that becomes colourless on addition of few drops of dilute HCl indicates the presence of flavonoids.

Detection of phenolics and tannins (*Kokate et al., 2001*) :

100 mg of extract was boiled with 1 ml of distilled water and filtered. The filtrate was used for the following test.

Ferric chloride test :

To 2 ml of filtrate, 2 ml of 1 per cent ferric chloride solution was added in a test tube. Formation of bluish black colour indicates the presence of phenolic nucleus.

Test for tannins :

To the extract, 0.5 ml NaOH was added, formation of precipitate indicates the presence of tannins.

Detection of phytosterols and triterpenoids (*Peach and Trancey, 1955; Finar, 1959*) :

0.5 g of extract was treated with 10 ml chloroform and filtered. The filterate was used to test the presence of phytosterols and triterpenoids.

Leibermann's test :

To 2 ml of filtrate in hot alcohol, few drops of acetic

anhydride was added. Formation of brown precipitate indicate the presence of sterols.

Leiberman-Bucharat test :

To the extract, few drops of acetic acid and concentrated H_2SO_4 were added, deep red ring at the junction of two layers indicates the presence of triterpenes.

Salkowaski test :

To the extract solution few drops of Conc. sulphuric acid was added and shaken well and allowed to stand, lower layer turns red indicating the presence of sterols.

Detection of fixed oils and fats (*Ronsenthaler, 1930*) :

Oily spot test :

One drop of extract was placed on filter paper and solvent was allowed to evaporate. An oily stain on filter paper indicates the presence of fixed oil.

Scavenging of nitric oxide radical :

Sodium nitroprusside in aqueous solution at physiological pH, spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be estimated by the use of modified GriessIlosvay reaction. In the present investigation, GriessIlosvay reagent is modified by using Naphthyl ethylene diaminedihydrochloride (0.1% w/v) instead of 1-napthylamine (5%). Nitrite ions react with Griess reagent, which forms a purple azo dye. In presence of test components, likely to be scavengers, the amount of nitrites will decrease. The degree of decrease in the formation of purple azo dye will reflect the extent of scavenging. The absorbance of the chromophore formed was measured at 540 nm (Garrat, 1964; Nenadis *et al.,* 2004).

Reagents :

Sodium nitroprusside solution :

Weighed accurately 0.2998 g of Sodium nitroprusside and dissolved in distilled water to make up the volume to 100 ml in a volumetric flask (10 mM).

Naphthyl ethylene diaminedihydrochloride (*NEDD, 0.1%*) :

Weighed accurately 0.1 g of NEDD and dissolved in 60 ml of 50 per cent glacial acetic acid by heating and made up the volume to 100 ml in a volumetric flask with distilled water.

Sulphanilic acid (*0.33% w/v*) *reagent* :

Weighed accurately 0.33 g of sulphanilic acid and dissolved in 20 per cent glacial acetic acid by heating and made up the volume to 100 ml in a volumetric flask.

Preparation of sample solutions :

21 mg each of the extracts was dissolved in distilled DMSO separately to obtain solutions of 21 mg/ml concentrations. Each of these solutions was serially diluted separately to obtain lower concentrations.

Preparation of standard solutions :

Weighed accurately 10 mg of ascorbic acid and Rutin (Hi media, Mumbai) and dissolved in 1 ml of DMSO separately. From these solutions, serial dilutions were made to obtain lower concentrations using DMSO.

The reaction mixture (6 ml) containing sodium nitroprusside (10 mM, 4 ml), phosphate buffer saline (PBS, pH 7.4, 1 ml) and extract in DMSO at various concentrations or standard was incubated at 25^0C for 150 min. After incubation, 0.5 ml of the reaction mixture containing nitrite ion was removed, 1 ml of sulphanilic acid reagent was added, mixed well and allowed to stand for 5 min for completion of diazotisation. Then, 1 ml of NEDD was added, mixed and allowed to stand for 30 min in diffused light. A pink colouredchromophore was formed. The absorbance of these solutions was measured at 540 nm.

Scavenging of 2, 2'-azino-bis (*3-ethylbezothiazoline-6-sulfonic acid*) *diammonium salt*) (*ABTS*) *radical cation assay* :

ABTS assay involves a more drastic radical, chemically produced and, is often used for screening complex antioxidant mixture such as plant extracts, beverages and biological fluids. The solubility in both the organic and aqueous media and the stability in a wide pH range raised the interest in the use of ABTSradical for the estimation of the antioxidant activity (Nenadis *et al*., 2004).

13.6 mg of each of the extracts and the standards, ascorbic acid and rutin were accurately weighed and separately dissolved in 2ml of DMSO. These solutions were serially diluted with DMSO to obtain the lower dilutions. ABTS (Sigma, USA) (54.8 mg) was dissolved in 50 ml of distilled water to 2 mM concentration and potassium persulphate (17 mM, 0.3 ml) was added. The reaction mixture was left to stand at room temperature overnight in dark before use. To 0.2 ml of various concentrations of the extracts or standards, 1.0 ml of distilled DMSO and 0.16 ml of ABTS solution was added to make a final volume of 1.36 ml. Absorbance was measured spectrophotometrically, after 20 min at 734 nm.

Evaluation of total antioxidant capacity of the extract :

The total antioxidant capacity was determined by phosphomolybdenum method and is based on the reduction of Mo(VI) to Mo(V) by the antioxidant compounds and the formation of a green Mo(V) complex which has the maximal absorption at 695 nm.

Preparation of test and standard solutions :

Weighed accurately 55 mg of each extracts and the

standard, ascorbic acid and dissolved in 5 ml of DMSO. The lower dilutions were made serially with DMSO. An aliquot of 0.1 ml of the sample solution containing a reducing species in DMSO was combined in an eppendorff tube with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) (Himedia, Mumbai). The tubes were capped and incubated in water bath at 95^{0}C for 90 min. The samples were cooled to room temperature, and the absorbance of each solution was measured at 695 nm. The total antioxidant capacity was expressed as mM equivalent of ascorbic acid (Mojca *et al*., 2005).

Reducing power assay :

The principle of this assay is higher than the absorbance represents the stronger the reducing power. Weighed accurately 2 mg of each extract and the standard, ascorbic acid and dissolved in 2 ml of DMSO. Then take 0.5 of above solution then make up to 2.5 ml with phosphate buffer (0.2 M, pH 6.6). The lower dilutions were made serially with DMSO.

A method developed by Oyaizu (1986) for reducing power test was used. The above sample including MBO1 together with Ascorbic acid solutions were spiked with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1 per cent potassium ferric cyanide. The mixture was then kept in a 50^{0}C water-bath for 20 min. The resulting solution was then cooled rapidly, spiked with 2.5 mL of 10 per cent trichloroacetic acid, and centrifuged at 3000 rpm for 10 min. The supernatant (5 mL) was then mixed with 5 mL of distilled water and 1 mL of 0.1 per cent ferric chloride. The absorbance at 700 nm was then detected after reaction for 10 min. The higher the absorbance represents the stronger the reducing.

Estimation of total phenol content :

Total phenol content of the extracts was determined by using the Folin-Ciocalteu Method (Spanos and Wrolstad, 1990). This test is based on the oxidation of phenolic groups with phosphomolybdic and phosphotungstic acids. After oxidation the green– blue complex formed was measured at 750 nm.

Commercially available Folin-Ciocalteu reagent was diluted (1:10) with distilled water and used. 20.25 g of sodium carbonate was dissolved in 100 ml of distilled water and used (0.7 M).

The plant extracts (50 mg each) were dissolved separately in 50 ml of methanol. These solutions were serially diluted with methanol to obtain lower dilutions. Gallic acid monohydrate (50 mg) was dissolved in 50 ml of distilled water. It was serially diluted with water to obtain lower dilutions.

In a test tube, 200 µl of the extract (1 mg/ml) was mixed with 1 ml of Folin-Ciocalteu reagent and 800 µl of sodium carbonate. After shaking, it was kept for 2 h for reaction. The absorbance was measured at 750 nm. Using gallic acid monohydrate, standard curve was prepared and linearity was obtained in the range of 10-50 µg/ml. Using the standard curve the total phenolics content of the extract was determined and expressed as gallic acid equivalent in mg/g of the extract.

Estimation of flavonoid content (*Swain and Hillis, 1959*) :

Vanillin reagent was prepared fresh by dissolving 1 g of recrystallised vanillin dissolved in 100ml of 70 per cent Conc. H_2SO_4.

The plant extracts (50 mg each) were dissolved separately in 50 ml of methanol. These solutions were serially diluted with methanol to obtain lower dilutions. Phloroglucinol (50 mg) was dissolved in 50 ml of distilled water. It was serially diluted with water to obtain lower dilutions. 0.2 ml of the extract was taken in a test tube and the final volume was made up to 2 ml with distilled water and to this 4 ml of vanillin reagent was added rapidly. Exactly after 15 min. absorbance was recorded at 500 nm against blank. The unknown was read from a standard curve prepared using different concentration of phloroglucinol.

RESEARCH FINDINGS AND ANALYSIS

Aerial parts, fruit and root of *Artocarpus gomezianus* were collected, powdered and extracted with hydro-alcohol. The percentage yields obtained were reported. The fruits have yielded maximum extractive values of 18.75 per cent followed by root and aerial parts (Table 1). The extraction yields were expressed in terms of the solid content in the dried product per soluble solid content in plant part used on a dry basis.

Table 1: Extractive yields of plant materials

Sr. No.	Test sample	Per cent yield
1.	*Artocarpus gomezianus*- Aerial parts	14.92
2.	*Artocarpus gomezianus*- Fruit	18.75
3.	*Artocarpus gomezianus*- Root	15.44

The moisture content was found to be maximum in fruits compared to aerial parts and roots which have appreciably low levels of moisture (Table 2). The results indicate that chloroform soluble compounds were found to be higher in proportion in fruits compared to other parts. However, water soluble components were found to be more in aerial parts

Table 2 : Moisture content in the plant materials

Sr. No.	Test sample	Moisture content (%)
1.	*Artocarpus gomezianus*- Aerial parts	9.61
2.	*Artocarpus gomezianus*- Fruit	11.09
3.	*Artocarpus gomezianus*- Root	4.97

compared to root and fruit (Table 3).

Table 3 : Extractive values of plant materials

Sr. No.	Test sample	Chloroform extract (%)	Aqueous extract (%)
1.	AGA	1.15	5.77
2.	AGF	6.99	4.52
3.	AGR	1.56	5.29

In most of the cases a definite colour variations were observed under ordinary and ultraviolet light long (360 nm) and short (254 nm) wavelengths. The effect of both ordinary and ultra violet light on fluorescence properties of dried powder of various parts of three species is observed (Table 4). The total ash values and water soluble ash value were found to be higher in fruits compared to other parts except in sulfated ash value where the higher value is observed in aerial parts and in acid insoluble as value the higher value is observed in roots (Table 5).

Preliminary phyto-chemical analysis indicates that the extracts answer for most of the phyto-chemicals except saponins and oils and fats (Table 6). Phenolics, tannins, alkaloids, flavonoids, phytosterols and triterpenoids content was found to be higher in the extract from fruit and root compared to the extracts of aerial parts of the plant (Table 7, Fig 1 and 2).

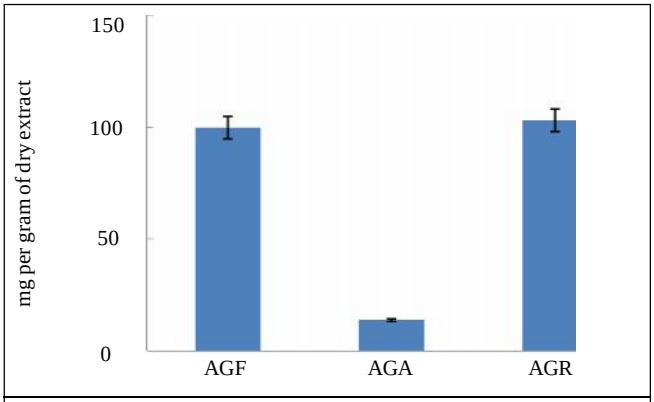

Fig. 1 : Total phenolic content of *Artocarpus gomezianus* fruit, aerial parts and root extracts

Table 4 : Fluorescence analysis of *Artocarpus gomezianus* plant materials

Sr. No.	Test sample + Solvent	*Artocarpus gomezianus* - Aerial parts		
		Ordinary	UV Short	UV Long
1.	Distilled water	Golden yellow	F green	Straw
2.	Chloroform	Green	F red	Light brown
3.	1N NaoH in water	Dark brown	Brown	Dark brown
4.	1N NaoH in methanol	Light green	F orange	Green
5.	10 (%) HCL	Straw	F green	Straw
6.	10 (%) H_2SO_4	Straw	Green	Straw
1.		*Artocarpus gomezianus*- Fruit		
2.	Distilled water	Light straw	F Straw	Straw
3.	Chloroform	Golden yellow	F golden yellow	Golden yellow
4.	1N NaoH in water	Brown	Dark Brown	Brown
5.	1N NaoH in methanol	Yellowish orange	F orange	Yellowish green
6.	10 (%) HCL	Straw	Straw	Straw
7.	10 (%) H_2SO_4	Straw	Straw	Straw
		Artocarpus gomezianus- Root		
1.	Distilled water	Red	Dark green	F green
2.	Chloroform	Orange	Light green	F orange
3.	1N NaoH in water	Dark brown	Purple	Dark green
4.	1N NaoH in methanol	Dark brown	Purple	Dark green
5.	10 (%) HCL	Straw	Light green	Light green
6.	10 (%) H_2SO_4	Straw	Light green	Light green

Table 5 : Ash values for plant materials

Sr. No.	Sample	Total ash (%)	Acid insoluble ash (%)	Water soluble ash (%)	Sulfated ash (%)
1.	AGR	10.2	6.45	2.15	2.75
2.	AGF	16.20	1.10	7.25	1.00
3.	AGA	8.29	5.75	4.00	4.00

Table 6 : *Artocarpus gomezianus* hydroalcoholic extracts

Sr. No.	Test	AGA	AGF	AGR
1.	Test for carbohydrates –	+	+	+
	Molisch's test			
2.	Test for glycosides –			
	Keller-Killiani test	+	-	-
3.	Test for saponins –			
	Foam test	-	-	-
4.	Test for alkaloids			
	Mayer's test	-	+	+
	Dragendrodroff's test	-	+	+
5.	Test for flavonoids –			
	Alkaline reagent test	+	+	+
6.	Test for phenolics and tannins –			
	Ferric chloride test	-	+	+
	Test for tannins	+	+	+
7.	Test for phytosterols and triterpenoids –			
	Leiberman-Bucharat test	-	+	+
	Salkowaski test	-	+	+
8.	Test for fixed oils and fats – Oily spot test	-	-	-

(+) Present, (-) Absent

Artocarpusgomezianus- Aerial parts	AGA
Artocarpusgomezianus- Fruit	AGF
Artocarpusgomezianus- Root	AGR

Table 7 : Total phenolic and flavonoid estimation of *Artocarpus gomezianus* fruit, aerial parts and root extracts

Samples	mg per gram of dry extract	
	Total phenols	Total flavonoids
AGF	99.59±6.1	9.86±0.26
AGA	13.72±2.9	8.30±0.42
AGR	102.92±10.6	1.67±0.17

Values are mean ± standard error of three replicates

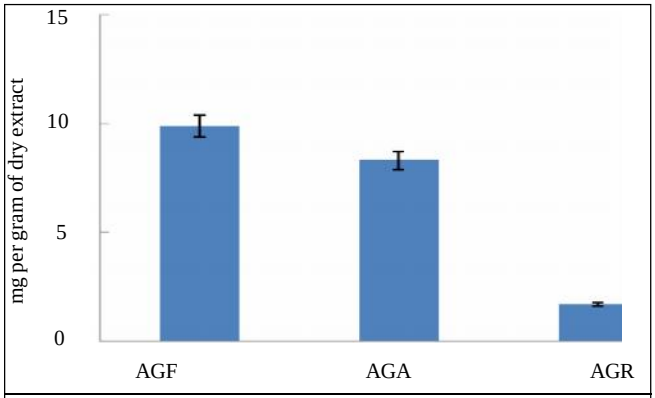

Fig. 2 : Total flavonoid content of *Artocarpus gomezianus* fruit, aerial parts and root extracts

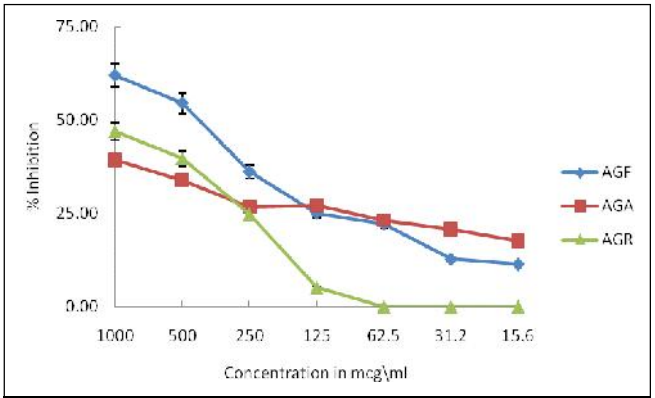

Fig. 3 : Nitric oxide radical scavenging activity of *Artocarpus gomezianus* fruit, aerial part and root extracts in terms of per cent inhibition

Ferric ion reducing property was found to be maximum for the fruit extract (Table 8, Fig. 3). The Nitric oxide scavenging activity was found to be higher in fruit followed by Aerial and root parts (Table 9 and Fig 4). ABTS radical

Table 8 : Nitric oxide scavenging activity of *Artocarpus gomezianus* fruit, aerial parts and root extracts

Samples	IC_{50} values µg/ml
AGF	430.00±56.56
AGA	>1000.00
AGR	>1000.00
Standard	Rutin 65.44

Values are mean ± standard error of three replicates

Table 9 : ABTS scavenging activity of *Artocarpus gomezianus* fruit, aerial parts and root extracts

Samples	IC_{50} values µg/ml
AGF	85.5±7.78
AGA	5.00±0.0
AGR	32.5±7.78
Standard	Ascorbic acid 4.3

Values are mean ± standard error of three replicates

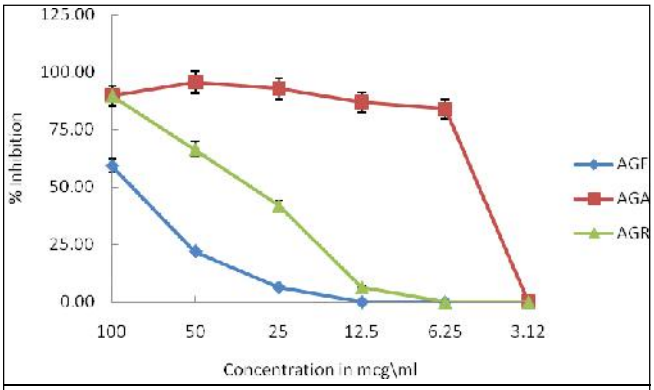

Fig. 4 : ABTS radical scavenging activity of *Artocarpus gomezianus* fruit, aerial part and root extracts in terms of per cent inhibition

scavenging activity of aerial part extract is found to be 5 mg/ml compared to the standard ascorbic acid 4 mg/ml (Table 10 and Fig. 5). However, the total antioxidant activity was found

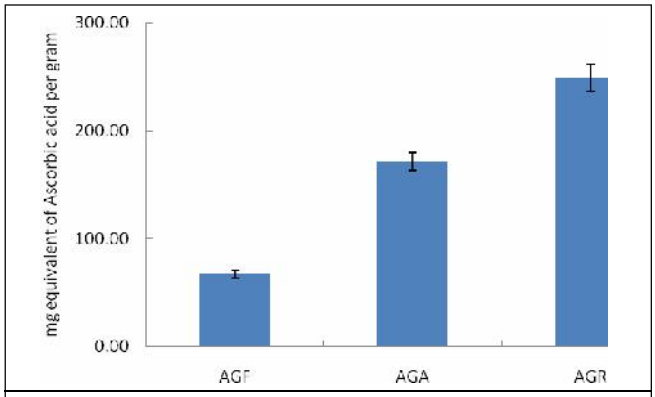

Fig. 5 : Total antioxidant capacity of *Artocarpus gomezianus* fruit, aerial part and root extracts

Table 10 : Total antioxidant activity of *Artocarpus gomezianus* fruit, aerial parts and root extracts

Samples	Total antioxidant activity*[a]
AGF	67.45±0.99
AGA	171.37±12.99
AGR	249.09±35.65
Standard	Ascorbic acid ---

*[a]The total antioxidant capacity was expressed as mg equivalent of ascorbic acid per gram of dry weight
Values are mean ± standard error of three replicates

to be significantly high for the extract from fruit part than the extracts from aerial and root parts (Table 11 and Fig. 6).

Table 11 : Ferric ion reducing power assay of *Artocarpus gomezianus* fruit, aerial parts and root extracts

Concentration In mcg/ml	Absorbance at 700 nm AGF	AGA	AGR
1000	0.081 ± 0.002	0.557 ± 0.006	0.653 ± 0.014
500	0.047 ± 0.003	0.414 ± 0.022	0.537 ± 0.018
250	0.040 ± 0.001	0.364 ± 0.006	0.347 ± 0.006
125	0.031 ± 0.001	0.227 ± 0.014	0.227 ± 0.018
61.5	0.028 ± 0.001	0.138 ± 0.001	0.133 ± 0.001
31.2	0.023 ± 0.000	0.080 ± 0.002	0.073 ± 0.008
15.6	0.021 ± 0.001	0.049 ± 0.001	0.039 ± 0.002

Values are mean ± standard error of three replicates

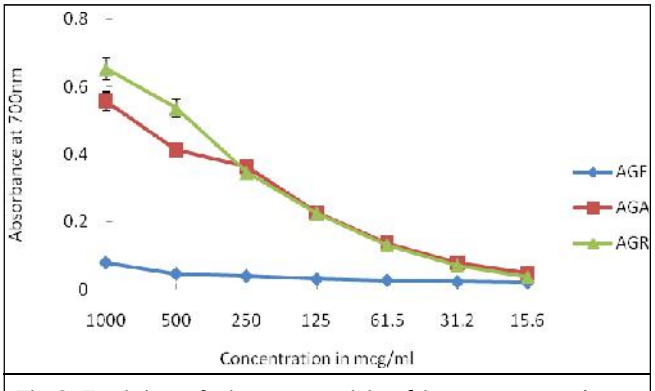

Fig. 6 : Ferric ion reducing power activity of *Artocarpus gomezianus* fruit, aerial part and root extracts

Conclusion :

The moisture content of*Artocarpusgomezianus*extracts was found to be in the range of 4.97 % to 11.09%, extractive values - 14.92 % to 18.75%, chloroform solubility 1.15 % to 6.99% and water solubility ranged between 4.52 % to 5.77%). Fluorescence analysis and ash values were also determined. Extraction yields with 30:70 solvents indicate the quantitative idea about some of the proximate components. The extracts when tested qualitatively for various phytochemicals, they found to contain carbohydrates, glycosides, alkaloids, tannins, phytosterols and triterpinoids. However, they do not contain saponins, oils and fats. Quantitative estimation of extracts for total phenols and total flavonoids reveals that most parts contain reasonably higher amounts.

Fruit extract of *Artocarpus gomezianus* was found to have maximum ferric ion reducing property than other parts studied. Nitric oxide scavenging activity was found to be higher in fruit followed by aerial and root parts. ABTS radical scavenging activity of aerial part extract is found to be 5 mg/ml compared to the standard ascorbic acid with 4 mg/ml. The total antioxidant activity was found to be significantly high for the extract from fruit part than the extracts from aerial and root parts. The results clearly demonstrate that the extracts can be considered for further studies which evaluate the biological activity such as antioxidant activities. Also, it is clear from the studies that the extracts of aerial parts, fruits and roots possess potentially beneficial antioxidant activities. In view of their use in ancient medicine coupled with the recent understandings of these plant species, they may be considered for further exploration as they may yield very potent nutraceuticals.

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