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Antioxidant Potency and GC-MS Composition of Leaves of *Artocarpus altilis* (Park). Fosb.

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ABSTRACT

The fruit of the plant Artocarpus altilis, widely known as bread fruit is consumed worldwide and studied for its bio-activities, yet the leaves are scarcely studied. In this study, the methanolic extract of the leaves of A. altilis was evaluated for Total Phenolic Content (TPC), total Flavonoid Content (TFC) and Total Antioxidant Content (TAC). Its antioxidant potency was evaluated using 2,2-Diphenyl Picrylhydrazyl (DPPH), Ferric Thiocyante (FTC), Ferric Reducing Antioxidant Power (FRAP), Thiobarbituric Acid (TBA) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assays. The TPC, TFC and TAC values recorded per g dry weight of the plant were 14.31 mg TAE, 33.19 µg QE and 112.55 mg TAE respectively. The EC_{50} value for scavenging DPPH radicals was determined as 35 µg/ml. The percent inhibition recorded against radicals in ABTS, FTC, TBA and FRAP assays were 97.6%, 89.2%, 61.4% and 29.4% in that order. The GC-MS studies revealed the presence of 12 different compounds, i.e. 2H-1,4-Benzodiazepin-2-one, 7-chloro-1,3- dihydro-5-phenyl-1-(trimethylsilyl)-3-[(trimethylsilyl)oxy]; Cyclodecasiloxane, eicosamethyl-; 2,15-Heptadecadiene, 9-(ethoxymethyl); Pentadecanoic acid, 14-methyl-,methyl ester; 10-Octadecenoic acid, methyl ester; Ethanol, 2-(9-Octadecenyloxy)-, (Z)-; 16-Octadecenoic acid, methyl ester; Docosanedioic acid, dimethyl ester; Stigmast-5-en-3-ol, oleate; a-Sitosterol trimethylsilyl ether; Ergosteryl acetate and 4,6,8(14)-Cholestatriene which are of either nutraceutical or pharmaceutical importance.

Keywords: *Artocarpus altilis*, Antioxidant, GC-MS, Pharmaceuticals, Nutraceuticals

INTRODUCTION

Free radicals are known to damage biomolecules such as proteins, lipids and nucleic acids, eventually leading to several diseases such as diabetes, heart diseases, cancer, hypertension, neurodegenerative disorders, etc. Antioxidants terminate these free radicals by several mechanisms and protect the cells from damage. Dietary sources of natural antioxidants are preferred, when the normal generation of antioxidants is affected and also due to the toxic nature of synthetic antioxidants [1]. In search of novel compounds of medical and economic importance, plants are studied widely. One such plant, i.e., *Artocarpus altilis* (Park). Fosb. is evaluated for its antioxidant potency and chemical composition in the present study.

A. altilis belonging to the family Moraceae, is native to the regions of New Guinea, Indonesia and Philippines. The fruit of the plant, widely known as bread fruit is consumed worldwide and studied for its bio-activities. The crop is largely used for its nutritious value [2], with emphasis on the consumption of the fruits as staple food as they are high in carbohydrates, vitamins and minerals [3,4]. The roots are used as an antiphlogistic, diuretic, expectorant and as a treatment for headache, beriberi, vomiting, parulis, and dropsy [5]. The plant also possesses antibacterial activity [6,7] and photoprotective effect against UVB-induced oxidative stress and inflammation [8]. Although the plant possesses different bio-activities, the leaves of the plant are under-utilized and are scarcely studied for their bioactivity. Traditionally, the leaves of the plant are used for the treatment of liver disorders, hypertension, diabetes [9,10] headache, enlargement of spleen and infections of the skin, eye and ear. In the present study, the methanolic extract of the leaves of *A. altilis* was evaluated for Total Phenolic Content (TPC), Total Flavonoid Content (TFC) and Total Antioxidant Content (TAC). Its antioxidant potency was evaluated using 2,2-Diphenyl picryl hydrazyl (DPPH), Ferric Thiocyante (FTC), Ferric Reducing Antioxidant Power (FRAP), Thiobarbituric Acid (TBA) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assays in this study.

MATERIALS AND METHODS

Plant source and preparation of plant extract

The leaves of the plant, *Artocarpus altilis* were collected from Thiruvananthapuram, Kerala, India. The leaves were dried under shade and pulverized. The extract was prepared through cold percolation method by adding methanol to the pulverized plant material at a ratio of 1:10 (w/v) and maintained in temperature controlled shaker (30 ± 2°C) for 48 h, following which it was filtered. The obtained crude extract was used for further analysis.

Determination of TPC

Total Phenolic content was determined using Folin-Ciocalteu method [11]. The plant extract (100 µl) was added with Folin-Ciocalteu reagent (100 µl) and distilled water (500 µl). This was incubated for 6 min at room temperature. After incubation, 1.25 ml of 7% sodium carbonate was added, the final volume made to 3 ml using distilled water and further incubated for 90 min. The absorbance was measured at 760 nm using UV-Visible spectrophotometer (CyberLab, USA). The total phenolic content was expressed as mg TAE (Tannic acid equivalents) per g of the dry weight (mg TAE/g DW) of the plant.

Determination of TFC

The total flavonoid content of the plant was determined according to the method prescribed by Moussa et al. [12]. The plant extract (200 µl) was made to a residue by allowing the solvent to evaporate. To the residue, 5 ml of 0.1 M aluminium chloride was added and allowed for incubation (40 min) at room temperature. The absorbance was measured at 415 nm. A standard plot of Quercetin was used to evaluate the total flavonoid content and expressed as mg QE/g DW of the plant material.

Determination of TAC

The total antioxidant activity was estimated by Phospho-molybdenum method [13]. To the plant extract (0.5 ml), a reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) of 4.5 m was added and maintained at 95°C in a water bath for 90 min. Upon cooling, the absorbance was measured at 695 nm. The total antioxidants in the plant was expressed as mg TAE/g DW of the plant material.

Free radical scavenging assays

The free radical scavenging ability of the methanolic extract of the leaves of *Artocarpus altilis* was evaluated through DPPH [14], FTC, TBA [15], FRAP [16] and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) [17] assays.

Gas chromatography-mass spectroscopy (GC-MS) analysis

The methanolic extract (1 µl) of the leaves of *A. altilis* was injected to GC-MS analysis in Agilent Technologies 6890N GC system coupled with JEOL Mass spectroscopy, through the Agilent J & W HP-5 capillary column (30 m × 0.2 mm × 0.25 µm) fused with silica, with the injection temperature being 220°C. The oven temperature ranged from 50-250°C, at the rate of 10°C per min. The flow rate of the carrier gas (Helium) was 1 ml/min, while the interface temperature of the GC-MS was 250°C. The compounds were identified based on Mass spectral database of NIST, USA.

RESULTS AND DISCUSSION

Determination of TPC, TFC and TAC

The methanolic extract of *Artocarpus altilis* leaves was found to contain a total phenolic content of 14.31 ± 0.534 mg TAE/g DW and flavonoid content of 33.19 ± 0.126 µg QE/g DW. The extract of the leaves has recorded 112.55 ± 0.196 mg TAE/g DW as its total antioxidant content. Phenolic compounds are acclaimed for their high free radical scavenging ability and serve as antimicrobial, antiviral and anti-inflammatory agents [18,19]. Similarly, polyphenols such as tannins and flavonoids have the ability to absorb or neutralize free radicals, quench singlet and triplet oxygen or decompose peroxides [20]. Mukesh et al. reported that the ethanolic extract of the leaves of *A. altilis* possessed total phenolic content of 26.22 mg GAE/g [21].

Free radical scavenging assays

The leaves of breadfruit tree were evaluated for their ability to scavenge free radicals through DPPH, FTC, FRAP, TBA and ABTS assays. The EC_{50} value for DPPH of the plant was found to be 35 µg/ml while that of the standard (BHA) was 25.78 µg/ml. Mukesh et al., reported the ethanolic extract of the plant leaves exhibited an EC_{50} value of 140.54 µg/ml [21]. It is evident that methanolic extract of the leaves when compared with the ethanolic extract serves as a better antioxidant. The inhibition percentage for different concentration of leaf extract and the EC_{50} value recorded in DPPH scavenging assay is presented in Table 1.

Table 1: DPPH free radical scavenging assay of the methanolic extract of *Artocarpus altilis*

Concentration (µg/ml)	% inhibition
10	33.33
20	33.33
30	44.44
40	55.56
50	100
EC_{50}	µg/ml
Artocarpus altilis	35
BHA	25.78

The methanolic extract of the leaves of *A. altilis* degraded the radicals generated during the lipid peroxidation at a percentage of 89.19 and 61.41 in FTC and TBA assays respectively. The FTC assay measures the products of the primary stage of lipid peroxidation, where the major products are hydroperoxides while the TBA assay measures the extent of lipid degradation by reacting with thiobarbituric acid and malondialdehyde [22], implying potential antioxidative of the extract against the radicals in the primary stage. Complexes containing ferric ions are converted to ferrous ions by the action of antioxidants in the FRAP assay [23]. The leaves of *A. altilis* scavenged these ferric ions to achieve 29.39%. In the ABTS assay, the methanolic extract of *A. altilis* recorded 97.58% as its percent inhibition. The percent inhibition of the methanolic extract of *A. altilis* leaves in the FTC, TBA, FRAP and ABTS assays is represented in Figure 1.

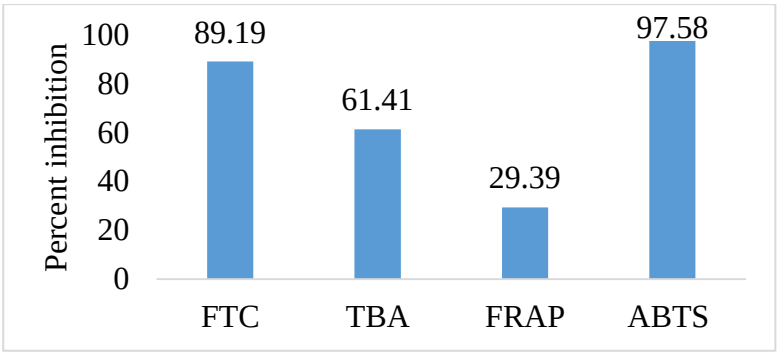

Figure 1: Percent inhibition recorded by methanolic extract of *A. altilis* leaves in FTC, TBA, FRAP and ABTS assays

Plants produce compounds which serve as exogenous antioxidants that can counteract oxidative stress by their own mechanism [24]. Although the fruits of *A. altilis* have been evaluated for their phenolic content and DPPH radical scavenging activity [25], the data on the same in relation to the leaves of *A. altilis* are not reported yet. Hence, the present study provides data on the flavonoid and antioxidant contents, in addition to their free radical scavenging activity by FTC, FRAP and ABTS assays for the first time.

GC-MS analysis

The GC-MS analysis of the methanolic extract of the leaves of *A. altilis* revealed the presence of 12 distinct peaks corresponding to the compounds: 2H-1,4-Benzodiazepin-2-one, 7-chloro-1,3- dihydro-5-phenyl-1-(trimethylsilyl)-3-[(trimethylsilyl)oxy]; Cyclodecasiloxane, eicosamethyl; 2,15-Heptadecadiene, 9-(ethoxymethyl)-; Pentadecanoic acid, 14-methyl-,methyl ester; 10-Octadecenoic acid, methyl ester; Ethanol, 2-(9-Octadecenyloxy)-, (Z); 16-Octadecenoic acid, methyl ester; Docosanedioic acid, dimethyl ester; Stigmast-5-en-3-ol, oleate; a-Sitosterol trimethylsilyl ether; Ergosteryl acetate and 4,6,8(14)-Cholestatriene. The compounds detected are tabulated in Table 2, while the chromatogram and the structure of the compounds are represented in Figures 2 and 3 respectively.

Table 2: GC-MS analysis of methanolic extract of *Artocarpus altilis*

Retention time	Name of the compound	Molecular formula	Molecular weight
9.27	2H-1,4-Benzodiazepin-2-one, 7-chloro-1,3-dihydro-5-phenyl-1-(trimethylsilyl)-3-[(trimethylsilyl)oxy]-	$C_{21}H_{27}ClN_2O_2Si_2$	431.075
12.12	Cyclodecasiloxane, eicosamethyl-	$C_{20}H_{60}O_{10}Si_{10}$	741.539
16.38	2,15-Heptadecadiene, 9-(ethoxymethyl)-	$C_{20}H_{38}O$	294.515
17.32	Pentadecanoic acid, 14-methyl-,methyl ester	$C_{17}H_{34}O_2$	270.45
19.02	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.488
19.25	Ethanol, 2-(9-Octadecenyloxy)-, (Z)-	$C_{20}H_{40}O_2$	312.53
21.05	16-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.488
23.13	Docosanedioic acid, dimethyl ester	$C_{24}H_{46}O_4$	398.62
25.58	Stigmast-5-en-3-ol, oleate	$C_{47}H_{82}O_2$	679.15
26.13	a-Sitosterol trimethylsilyl ether	$C_{32}H_{58}OSi$	486.888
28.23	Ergosteryl acetate	$C_{30}H_{46}O_2$	438.69
29.5	4,6,8(14)-Cholestatriene	$C_{27}H_{42}$	366.62

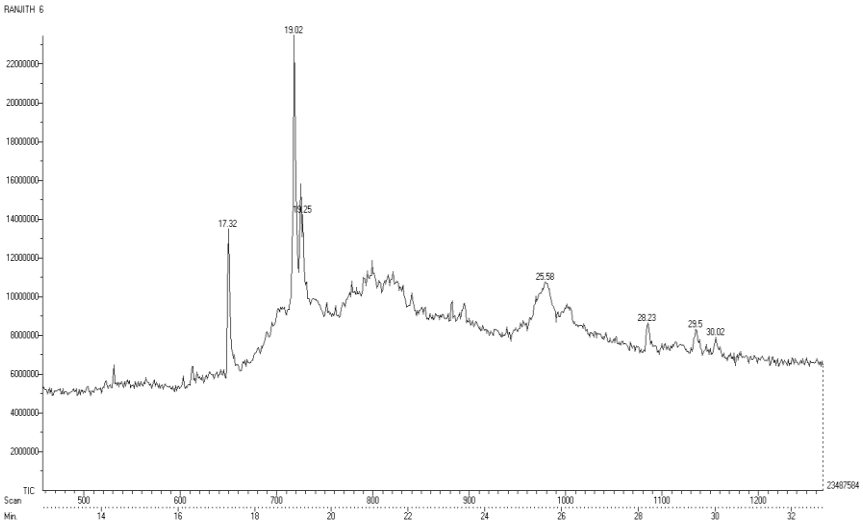

Figure 2: GC-MS Chromatogram of the methanolic extract of *Artocarpus altilis* leaves

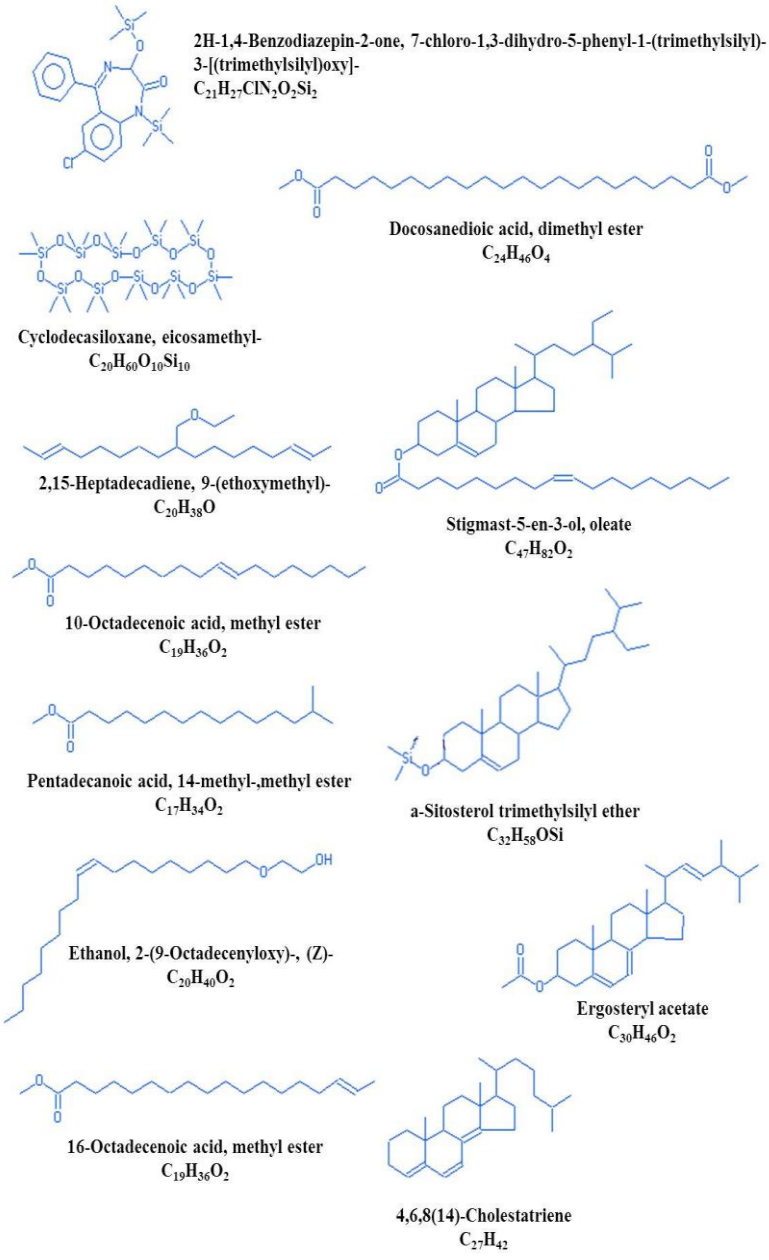

Figure 3: GC-MS analysis of the methanolic extract of leaves of *Artocarpus altilis*

Previously, compounds like Geranyl Chalcone derivatives [26], Geranyl flavonoids [27], Geranyl aurones [28] were isolated from the leaves of *A. altilis*. Prenylated flavonoids from leaves [29], root cortex [30] and heartwood [31] were also recorded.

2H-1,4-Benzodiazepin-2-one,7-chloro-1,3-dihydro-5-phenyl-1-(trimethylsilyl)-3-[(trimethylsilyl)oxy], is a derivative of Oxazepam, that belongs to a class of benzodiazepines used in relieving anxiety and insomnia. The compounds, Stigmast-5-en-3-ol, oleate and a-Sitosterol trimethylsilyl ether belongs to the group of phytosterols. Phytosterols in food, either in free or conjugated form, are of interest, owing to their nutritional aspects when consumed, such as lowering the levels of low density lipoproteins [32,33]. Thus, the compounds detected in the leaf extract of *A. altilis* can be utilized in industries.

CONCLUSION

The present study focused on the phenolic, flavanoid and antioxidant content, in addition to the antioxidant potency of the methanolic extract of the leaves of *A. altilis.* The free radical scavenging ability evaluated by the DPPH assay recorded EC_{50} of 35 µg/ml. High percent of inhibition of 89.19 and 97.58 were also recorded in the FTC and ABTS assays, respectively, which implies the antioxidant potential of the leaves of *A. altilis*. Further, 12 different compounds were detected by the GC-MS analysis, which needs to be explored for their pharmaceutical and nutraceutical benefits.

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