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VOLATILE COMPOUND ANALYSIS USING GC-MS, PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF *FICUS AMPLOCARPA* GOVIND AND MASIL FRUIT

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ABSTRACT
The present study was designed to investigate qualitative, quantitative phytochemical, *in vitro* antioxidant properties and GC MS analysis of various extracts from edible fruit of *Ficus amplocarpa* (Moraceae). Qualitative phytochemical analysis for carbohydrates, proteins, amino acids, alkaloids, saponins, phenolic compounds, flavonoids, flavanol glycosides, cardiac glycosides and phytosterols. quantitative phytochemical analysis for total phenolics, tannin and total flavonoid content, *in vitro* antioxidant activity 2,2-diphenyle 1-picrylhydrazyl, 2, 2 azinobis (3-ethyl-benzothiozoline)-6-sulfonicacid disodium salt, phosphomolybdenum, ferric reducing antioxidant power, super oxide anion radical scavenging reducing power assay and GC MS analysis. Among the various solvents, ethyl acetate extract displayed maximum total phenolics (227.07 mg GAE/g extract), tannin (220.31 mg RE/g extract) and flavonoid content (229.75 mg RE/g extract). Result of antioxidant studies revealed that the ethyl acetate of fruit possessed an efficient IC_{50} for DPPH assay (21.59µg/mL), $ABTS^{+\cdot}$ scavenging activity (64.11 mg TEAC/g extract), phosphomolybdenum reduction (32.93 mg AAE/g extract), ferric reducing antioxidant power (263.76 mM Fe(II)/mg extract), superoxide (36.02%) radical scavenging, reducing power possesses higher activity. GC-MS revealed that 25 different types of low and high molecular weight compounds present. In this study, various extracts from edible fruit of *F. amplocarpa* possess major biologically active compounds that were identified, characterized and analysed for antioxidant activity spectroscopically thus, the identified compounds reflect its pharmaceutical potentialities.

KEY WORDS: Antioxidant activity, *F. amplocarpa*, GC-MS.

INTRODUCTION

Antioxidants or free radical scavengers are nutrients and enzymes that are said to play important role in preventing and development of chronic diseases such as cancer, heart diseases, diabetics *etc*. Antioxidants neutralises the effectiveness of free radicals, which are safely interact with the free radicals and form chain reaction before vital molecules get damaged. Phenolics, flavanoids, vitamins (E and C), numerous minerals (Cu, Mn, Zn, Se and Fe) glutamine are one of the most widely recognised antioxidants (Abhishek *et al.,* 2013). Recent reports have indicated that there is an adverse effect in an inverse relationship between the dietary intake of antioxidant rich foods and human diseases (Sies, 1993). The usage of synthetic antioxidants like Butylated hydroxytoluene (BHT) and Butylated hydroxyanisole (BHA) are widely used in food industries and these compounds are responsible for many diseases namely liver damage and Carcinogenic to human body.

The genus *Ficus* is one of the medicinally important plants which have many of the biologically active compounds which are helpful in treatment of hypotentive, anti-inflammatory, anti-diabetic, laxative and anti-rheumatic (Amgad *et al.,* 2015). Among *Ficus* species *F. amplocarpa* Govind & Masil. Is also a most prominent species belongs to the family Moraceae. It is a scan dent root climber distributed chiefly in Western Ghats of Kerala, Tamil Nadu rarely in Karnataka at above 800 m elevation. There is no report related to the antioxidant and chemical composition of *F. amplocarpa*, hence the present study was under taken to investigate the qualitative and quantitative phytochemical analysis, antioxidant activities and GC MS analysis.

MATERIALS AND METHODS

Collection of Plant material

Fresh fruits of *F. amplocarpa* were collected in the month of July 2017 from the Shola forest of Coonoor, Nilgiri district, Tamil Nadu, India. The mature fruits were collected and washed in running tap water to remove dirt and were shade dried.

Chemicals

Folin ciocalteu, polyvinyl polypyrrolidone, 2, 2-diphenyle 2-picrylhydrazyl (DPPH), 2,2-azinobis (3-ethylbenzothio zoline)-6-sulfonicacid, dimethyl sulphoxide, 6-hydroxy-2,5,7,8-tetramethylchroman-2carboxylic acid (Trolox), ammonium molybdate, ethylenediamine tetracetic acid disodium salt, nitro blue tetrazolium, All other chemicals and solvents used were of analytical grade.

Preparation of Extracts

The dried, powdered fruit samples were successively extracted with five different solvents such as Petroleum ether, Chloroform, Ethyl acetate, Methanol and Water using Soxhlet apparatus. Each time before the extraction with the next sample the thimbles were air dried. The different solvent extracts were concentrated by a rotary

vacuum evaporator (Yarnato, BO410, Japan) and then air dried. The dried extracts obtained with each solvent were weighed and the percentage of yield was calculated. The extracts thus obtained were used directly to find out the phytochemicals and assess the antioxidant potential.

Qualitative Phytochemical Screening

The fruit extracts of *F. amplocarpa* were analyzed for the presence of major phytochemicals such as carbohydrates, proteins, amino acids, alkaloids, saponins, phenolic compounds, tannins, flavonoids, glycosides, flavanol glycosides, cardiac glycosides, phytosterols, fixed oils, fats, gums and mucilages according to standard methods such as Hager's test, Frothing test, Borntrager's test, Keller Killiani test, Libermann and Burchard's test, saponification test *etc*. (Raaman, 2006).

Determination of total Phenolics and Tannins

The total phenolics of different extracts of *F. amplocarpa* fruits were determined according to the method described by (Makkar, 2003). In this method 100µL of plant extracts were taken into a series of test tubes and made up to 1 mL with distilled water. A test tube with 1 mL of distilled water served as the blank. Then, 500µL of Folin–Ciocalteau Phenol reagent (1 N) was added to all the test tubes including the blank. After 5 minutes, 2.5 mL of sodium carbonate solution (10%) was added to all the test tubes. The test tubes were vortexed well to mix the contents and incubated in dark for 40 minutes. The formation of blue colour in the incubated test tubes indicated the presence of phenolics. Soon after incubation the absorbance was read at 725 nm against the reagent blank. Gallic acid standard was also prepared and the results were expressed as Gallic acid equivalents (mg GAE/g extract). The analyses were performed in triplicates.

The amount of total tannins was calculated by subtracting the total non- tannin phenolics from total phenolics. For the determination of total non- tannin phenolics (Makkar, 2003). 500 µL of each plant samples were incubated with 100 mg of polyvinyl polypyrrolidone (PVPP) and 500 µL of distilled water taken in a 2mL eppendorf tube for 4 hours at 4º C. After incubation the eppendorf tubes were centrifuged at 4000 rpm for 10 minutes at 4ºC. The supernatant contains only the non- tannin phenolics since the tannins would have been precipitated along with PVPP. The supernatant was collected and the non- tannin phenolics were determined by the same method described for the quantification of total phenolics. The analyses were also performed in triplicates and the results were expressed in Gallic acid equivalents (mg GAE/g extract). From these two results, the tannin content of the plant samples were calculated as follows,

Tannins = Total phenolics – Non- tannin phenolics

Determination of total flavonoids

The flavonoid contents of all the extracts were quantified according to the method described by (Zhishen, *et al*.**.** 1999). About 500 µL of *F. amplocarpa* fruit extracts were taken in different test tubes and 2 mL of distilled water was added to each test tube. A test tube containing 2.5 mL of distilled water served as blank. Then, 150 µL of 5% $NaNO_2$ was added to all the test tubes followed by incubation at room temperature for 6 minutes. After incubation, 150 µL of 10% $AlCl_3$ was added to all the test tubes including the blank. All the test tubes were incubated for 6 minutes at room temperature. Then 2 mL of 4% NaOH was added to all the test tubes which were then made up to 5mL using distilled water. The contents in all the test tubes were vortexed well and they were allowed to stand for 15 minutes at room temperature. The pink colour developed due to the presence of flavonoids was read spectrophotometrically at 510 nm. All the experiments were done in triplicates and the results were expressed as mg/g Rutin equivalents.

***IN VITRO* ANTIOXIDANT STUDIES**

DPPH˙ radical scavenging activity

The antioxidant activity of different extracts of *F. amplocarpa* fruit was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH**,** according to the method of (Braca *et al*., 1958). Sample extracts at various concentrations (20-100 µg/ml) were taken and the volume was adjusted to 100 µL with methanol. About 3 mL of a 0.004 % methanolic solution of DPPH was added to the aliquots of samples and standards (BHT and Rutin) and shaken vigorously. Negative control was prepared by adding 100 µL of methanol in 3mL of methanolic DPPH solution. The tubes were allowed to stand for 30 minutes at 27°C. The absorbance of the samples and control were measured at 517 nm against the methanol blank. Radical scavenging activity of the samples was expressed as IC_{50} which is the concentration of the sample required to inhibit 50% of DPPH˙ concentration.

$ABTS^{\bullet+}$ radical scavenging activity

The total antioxidant activity of the plant samples was measured by ABTS radical cation decolourization assay according to the method of (Re *et al*., 1999). $ABTS^{\bullet+}$ was produced by reacting 7 mM ABTS aqueous solution with 2.4 mM potassium persulfate in the dark for 12–16 hours at room temperature. Prior to assay, this solution was diluted in ethanol (about 1:89 v/v) and equilibrated at 25°C to give an absorbance of 0.700 ± 0.02 at 734 nm. About 1 mL of diluted ABTS solution was added to about 30µL sample solution and 10µL of Trolox (final concentration 0–15 µM) in ethanol. A test tube containing 1 mL of diluted ABTS solution and 30 µL of ethanol served as the negative control. All the test tubes were vortexed well and incubated exactly for 30 minutes at room temperature. After incubation the absorbance of samples and standards (BHT and Rutin) were measured at 734 nm against the ethanol blank. The results were expressed as the concentration of Trolox having equivalent antioxidant activity expressed as µg/ g sample extracts.

Phosphomolybdenum assay

The antioxidant activity of the plant samples was evaluated by the green phosphomolybdenum complex formation according to the method of (Prieto *et al*., 1999). An aliquot of 100 µL of samples and standards (BHT and rutin) were taken into a series of test tubes and were made up to 300µL with methanol. About 300 µL methanol taken in a test tube was considered as the blank. All the test tubes were added with 3mL of reagent solution (0.6 M

sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and vortexed well to mix the contents. The mouth of the test tubes were covered with foil and incubated in a water bath at 95°C for 90 minutes. After the samples were cooled to room temperature, the absorbance of the mixture was measured at 695nm against the reagent blank. Ascorbic acid was used as the reference standard and the results were expressed as milligrams of ascorbic acid equivalents per gram extract.

Super Oxide Radical scavenging activity

The assay was based on the capacity of various extracts to inhibit formazan formation by scavenging the superoxide radicals generated in riboflavin–light–NBT system (Beauchamp and Fridovich, 1971). About 3mL of reaction mixture containing 50 mM sodium phosphate buffer (pH-7.6), 20µg riboflavin, 12 mM EDTA and 0.1 mg NBT was added to 100µL sample solution, BHT and Rutin. Reaction was started by illuminating the reaction mixture with samples for 90 seconds. The illuminated reaction mixture without sample was used as the negative control. Immediately after illumination, the absorbance was measured at 590nm against the blank (unilluminated reaction mixture without plant sample). The scavenging activity on superoxide anion generation was calculated as:

Scavenging activity (%) = [(Control OD – Sample OD)/Control OD] X 100

Ferric reducing antioxidant power (FRAP) assay

The antioxidant capacities of different extracts of samples were estimated according to the procedure described by (Pulido *et al.* 2000). FRAP reagent (900 µL), prepared freshly and incubated at 37°C, was mixed with 90µL of distilled water and 30 µL of test sample or methanol (blank). BHT and rutin were used as the standards. All the test tubes were incubated at 37°C for 30 minutes in a water bath. The final dilution of the test sample in the reaction mixture was 1/34. The FRAP reagent was prepared by mixing 2.5 mL of 20 mM TPTZ in 40 mM HCl, 2.5 mL of 20 mM $FeCl_3.6H_2O$ and 25 mL of 0.3 M acetate buffer (pH-3.6). At the end of incubation, the absorbance of the blue colour developed was read immediately at 593 nm against the reagent blank. Methanolic solutions of known $FeSO_4.7H_2O$ concentration ranging from 500 to 4000 µM were used for the preparation of the calibration curve. The parameter Equivalent Concentration was expressed as the concentration of antioxidant having a ferric-TPTZ reducing ability equivalent to that of 1 mM $FeSO_4{\cdot}7H_2O$.

Gas Chromatography- Mass Spectroscopy

Preparation of extract

An amount of 1μL of ethyl acetate extract of *Ficus amplocarpa* fruit was studied for GC-MS analysis.

Instruments and Chromatographic conditions

The GC-MS analysis of the *F. amplocarpa* ethyl acetate fruit extract was carried out at the South India Textile Research Association (SITRA), Coimbatore. The GC was analysed by using Thermo-GC Trace Ultra Version 5.0 equipment with running time of 38.94 min and the MS was carried out by using Thermo MS-DSQ II equipment (Thermo Fisher Scientific Inc., Waltham, MA, USA). Auto sampler and GC interfaced to a MS instrument employing the following conditions: column TR 5-MS capillary standard non-polar column (30 M, i.d.: 0.25 mm, FILM: 0.25µm) composed of 5% phenyl polysilphenyle-siloxane; helium (99.999%) was used as carrier gas at a constant flow of 1 mL/min; and an injection volume of 0.5 EI was employed (split ratio of 10:1) injector temperature 250^{o}C; ion-source temperature 280^{o}C. The MS of the unknown component was compared with the spectrum of the known components stored in the National Institute of Standard Technology (NIST) library. The name, molecular weight and structure of the components of the test samples were ascertained.

Statistical Analysis

All the experiments were done in triplicates and the results were expressed as mean±standard deviation (SD).

RESULTS

Extract Recovery Percent

The percentage yield of *F. amplocarpa* fruit extracts from different solvents are presented in Table 1. The maximum yield for fruit was obtained from methanol extract (13.67%). The second highest yield was revealed by hot water extract (9.77%). The yield percentages of chloroform, ethyl acetate and petroleum ether of fruit were found to be low. These results conclude that the high polar solvents could dissolve more constituents in fruit parts of the plant *F. amplocarpa.*

TABLE 1: Percentage yield of *F. amplocarpa* extracts

S. No.	Solvents	Yield Percentage
1	Petroleum ether	4.47
2	Chloroform	1.97
3	Ethyl acetate	5.64
4	Methanol	13.67
5	Hot water	9.77

Qualitative Phytochemical Screening

The qualitative phytochemical screening was carried out in various fruit extracts of *F. amplocarpa* for major primary and secondary phytochemicals are shown in Table 2. The results revealed that the primary metabolites such as carbohydrates, proteins and amino acids are present in appreciable amount in the different extracts of fruit. The secondary metabolites such as alkaloids, saponins, phenolic compounds, alkaloids, flavonoid-glycosides, cardiac- glycosides, phytosterols, fixed oils, fats, gums and mucilages were found to be variously distributed in different extracts of fruits. Among the extracts examined ethyl acetate extract of *F. amplocarpa* fruit exhibit the maximum number of secondary metabolites which were

found to play key role for pharmacological properties of studied plant.

TABLE 2: Phytochemical screening of *F. amplocarpa*

Phytochemicals	*F. amplocarpa*				
	Fruit				
Extracts	Petroleum ether	Chloroform	Ethyl acetate	Methanol	Water
Carbohydrate	+	+	++	+	+
Protein	+	+	+++	+	+
Amino acid	-	-	++	++	++
Alkaloid	+	+	+++	++	++
Flavonol glycosides	+	+	++	+	+
Phytosterol	+	+	++	++	++
Flavonoids	+	+	+++	++	++
Cardiac glycosides	+	++	++	+++	+
Phenolic compound	+	+	+++	+	++
Saponins	-	++	+	+	++

(+): Presence of chemical compound, (-): Absence of chemical compound
(+) < (++) < (+++): Based on the intensity of characteristic colour

Total Phenolics, flavonoids and tannin

The non-enzymatic antioxidants like total phenols, flavonoids and Tannins content of different extracts *F. amplocarpa* fruit were determined with Gallic acid equivalents and Rutin equivalents (Table 3). The highest amount of phenolic (227.07 mg GAE/g extract) and tannin (220.31 mg RE/g extract) were noted in ethyl acetate extract. However, the total flavonoid content was high in ethyl acetate 229.75 mg RE/g. The studied chemical compounds widely dispersed in plants have highest consideration due to their anti-mutagenic, anti-tumour, anti-diabetic, antioxidants which have beneficial effect in human health (Davis *et al.*, 2009).

TABLE 3: Total phenolic and tannins contents of *F. amplocarpa* fruit extracts

Samples	Extracts	Total Phenolics (mg GAE/g extract)	Tannins (mg GAE/g extract)	Flavonoids (mg RE/g extract)
Fruit	Petroleum ether	28.00±4.08	30.45±3.21	24.20±3.85
	Chloroform	83.97±2.10	166.94±3.79	74.23±2.35
	Ethyl acetate	230.10±7.62	229.75±7.29	220.86±8.43
	Methanol	218.31±6.08	71.15±1.05	200.59±4.39
	Water	47.94±4.08	28.00±3.79	43.66±4.04

GAE – Gallic Acid Equivalents; RE– Rutin equivalents.
Values are mean of triplicate determination (n=3) ± standard deviation.

***IN VITRO* ANTIOXIDANT ASSAY**

DPPH assay

In the DPPH assay, the antioxidants were able to reduce the stable DPPH radical to the yellow coloured diphenyl-picrylhydrazine. The free radical-scavenging activities of different fruit extracts (petroleum ether, chloroform, ethyl acetate, methanol and water) of *F. amplocarpa* along with natural and synthetic antioxidant standards such as rutin, and BHT respectively were determined by the DPPH radical scavenging assay. Among the different extracts the highest free radical scavenging activity was noted in ethyl acetate (21.59µg/mL) extract of fruit (Fig-1).

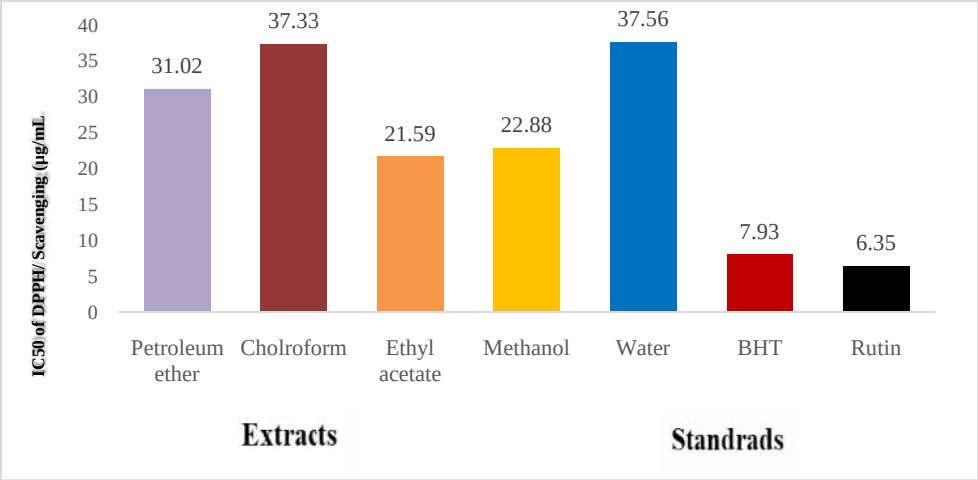


FIGURE 1: DPPH radical scavenging activity of *F. amplocarpa*

$ABTS^{\bullet+}$ radical scavenging activity

$ABTS^{\bullet+}$ radical decolourisation assay, is one of the most commonly employed method to measure antioxidant capacity, which measures the ability of a compound to scavenge $ABTS^{\bullet+}$. The results were measured in µM TE/g dry weight of plant material. The result of $ABTS^{\bullet+}$ Cation radical scavenging activity of different extracts of *F. amplocarpa* fruit were shown in Table 4. The highest scavenging activity of fruit was observed in ethyl acetate extract (64111.11 µg TE/g extract).

Phosphomolybdenum Assay

The Phosphomolybdenum assay evaluated based on the capacity to reduce Mo (VI) to Mo (V), a green phosphate by the antioxidant compound present in the sample with the maximum absorption at 695 nm. Estimation of Mo reduction capacity of different extracts of fruits of *F. amplocarpa* was studied. The results were calculated in Ascorbic acid equivalents. The results depicted that the highest activity showed in ethyl acetate extract of fruit (32.93 mg AAE/g extract).

Ferric reducing antioxidant power (FRAP) assay

The antioxidant potential of various extracts of *Ficus amplocarpa* fruit was estimated for their ability of reacting with Fe (III)-TPTZ complex and producing a coloured Fe (II)-TPTZ) complex by a reductant at low pH, was adopted and the results depicted in Table 4. Among various solvent extracts ethyl acetate extracts of fruit 263.76 mM Fe(II)/mg extract registered higher antioxidant activity even than standards used (BHT-412.59 mM Fe(II)/mg extract and Rutin: 246.41 mM Fe(II)/mg extract).

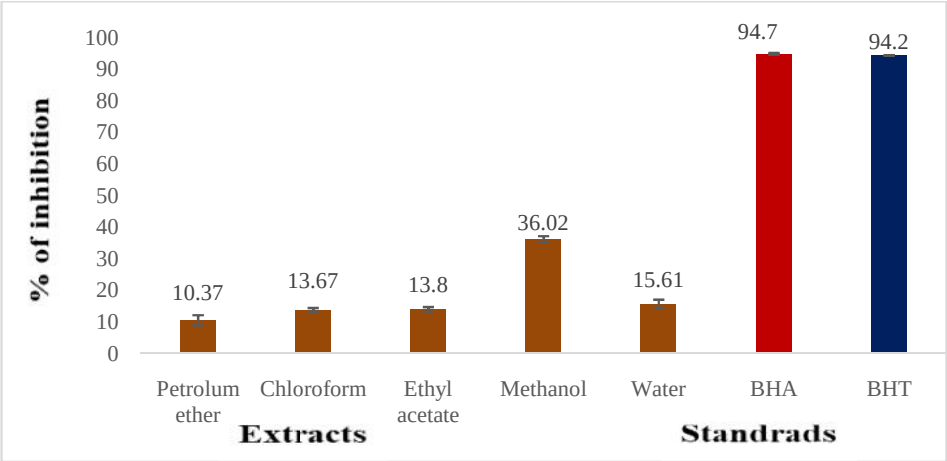
TABLE 4: ABTS radical cation scavenging activity, Phosphomolybdenum assay, Ferric reducing antioxidant power assay of *F. amplocarpa*

Samples	Extracts	ABTS (µg TE/g extract)	Phosphomolybdenum (mg AAE/g extract)	FRAP m M Fe (II)/mg extract)
Fruit	Petroleum ether	23416.67± 908.1039	9±1.05	38.7± 1.85
	Choloroform	28347.22± 2362.387	12.73± .61	76.41± 4.31
	Ethyl acetate	64111.11± 492.2725	32.93± 2.00	263.76± 3.01
	Methanol	32340.28± 1661.232	28.06± 1.22	246.79± 4.54
	Water	54284.72± 1414.265	9.13± 0.98	153.88± 4.55
Standard	Rutin	151833.3±416.6667	-	246.41±2.23
	BHT	153569.4±1048.588	-	412.59±1.85

TE – Trolox Equivalents; AAE ascorbic acid equivalents.Fe (II) - Ferric Equivalents, EDTAE - EDTA equivalents.
Values are mean of triplicate determination (n=3) ± standard deviation.

Super oxide radical scavenging activity

Superoxide anion plays an important role in formation of reactive oxygen species. Although superoxide is a relatively weak oxidant, it decomposes to form stronger ROS, such as singlet oxygen and hydroxyl radicals, which initiate peroxidation of lipids. Various extracts of *F. amplocarpa* fruit was subjected in order to find out the activity against this harmful radical. The ethyl acetate extract of fruit (36.02%) was effectively scavenged superoxide in a concentration-dependent manner than the other extracts (Fig -2). The scavenging activity of extracts was compared with the standards BHT and BHA.



Values are mean of triplicate determination (n=3) ± standard deviation
FIGURE 2: Superoxide radical scavenging activity of *F. amplocarpa*

Reducing power assay

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. In this assay, the presence of reductions (antioxidants) in tested samples would result in the reduction of Fe^{3+} ferricyanide complex to the ferrous form. The measurement of the reductive ability from Fe^{3+} to Fe^{2+} in the presence of *F. amplocarpa* extracts was investigated and shown in (Fig-3). The reducing power of all the extracts showed a concentration dependent activity from 100 to 500 µg/mL of samples. Increase in absorbance of the reaction mixture indicated the reducing power of the

samples. The reductants present in the extract acts by preventing the peroxide formation or reacting with free radicals and terminating the free radical chain reaction. In the present study such termination could greatly be contributed by the ethyl acetate extracts.

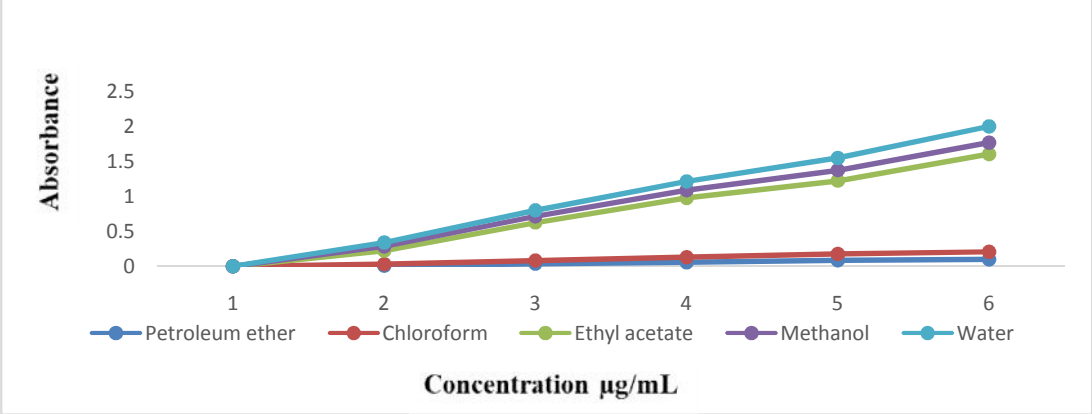


FIGURE 3: Reducing power assay of *F. amplocarpa* fruit extract

Identification of Bioactive compounds

There are several biologically active compounds present in *F. amplocarpa* fruit that are responsible for the antioxidant, anti-inflammatory and anti-microbial activity. The result of the antioxidant activity of studied fruit sample showed highest activity in ethyl acetate extract and also it has appreciable flavonoid content. Hence the extract selected for GC-MS analysis in order to find out volatile compound. GC-MS chromatogram showed about 25 peaks indicating presence of 25 chemical components (Table 5 and Fig-4).

TABLE 5: Chemical compounds of Ethyl acetate extract of *F. amplocarpa*

Peak	Compound Name	Retention time	Molecular weight	Area %
1	Sulfide, sec-butyl isopropyl	5.16	132	4.62
2	1,2,3-Propanetriol, monoacetate	6.81	134	4.82
3	Catechol	8.62	110	7.28
4	2-Hydroxy-3-methylsuccinic acid	9.60	148	3.65
5	Butanoic acid, heptafluoro, methyl ester (CAS)	10.01	228	0.91
6	9-Octadecenoic acid (Z)- (CAS)	13.69	282	0.51
7	1,1'-Biphenyl, 4-methyl- (CAS)	15.12	168	2.99
8	4,5-Dimethoxy-2-methylphenol	15.39	168	2.99
9	Desulphosinigrin	16.77	279	5.02
10	Decanoic acid, 2,3-dihydroxypropyl ester (CAS)	17.04	246	5.02
11	9-Octadecenoic acid (Z)- (CAS)	19.42	282	1.23
12	Isopropyl myristate	20.24	270	8.02
13	Myristic acid isobutyl ester	22.23	284	8.02
14	Bacteriochlorophyll-c-stearyl	23.54	840	1.95
15	Xanthotoxin	25.08	216	1.42
16	9-Octadecenoic acid, (E)-	27.32	282	1.47
17	5-Octadecenal (spectrum disagrees) (CAS)	28.85	266	1.47
18	Erucic acid	29.99	338	1.47
19	cis-11-Eicosenoic acid	30.14	310	1.56
20	Dotriacontane (CAS)	32.79	450	1.77
21	Octadecanoic acid, 4-hydroxy-, methyl ester	34.44	314	2.16
22	9-Hexadecenoic acid, 9-octadecenyl ester, (Z,Z)-	36.17	504	3.77
23	Octadecane, 1-[2-(hexadecyloxy)ethoxy]- (CAS)	36.49	538	1.51
24	Tetrapentacontane, 1,54-dibromo-	39.04	914	1.15
25	Guanidine, N,N'-diphenyl- (CAS)	40.41	211	1.04

DISCUSSION

Qualitative phytochemical screnning

The present study for preliminary phytochemicals showed the presence of tannins, saponins, steriods, alkaloids, flavonoids, phenolic and terpenoids (Ghosh *et al*., 1998). Saponins are reported to have anti-ageing activity which is related to their free radical scavenging action (Jun *et al*., 1989). Tannins were reported to exhibit antiviral, antibacterial and antitumor activity (Sajeesh *et al*., 2011). Flavonoids have been shown to prevent liver cancer (hepatoma) and to prevent the liver from lipid per oxidative effects in experimental hyperlipidaemia

(Blazovics *et al*., 1993). The higher amount of alkaloids present in all the part of the plant can make the plant to be a good source of alkaloids which can be isolated and then purified. The phenolic and flavonoids have got much attention in the day to day life due to their antimutagenic and antioxidant activities. Therefore the estimation and characterization of fruit extracts of *F. amplocarpa* for phenolic and flavonoids should be done to explore bioactive principle of such compounds.

Quantification of total phenolic, tannin and flavonoids

The presence of phenol had pharmacological properties such as anti-apoptosis, anticarcinogen, anti-inflammation, anti-aging, anti-atherosclerosis, inhibition of angiogenesis, cell proliferation activities, and cardiovascular protection (Han *et al*., 2007). Tannins involve in protein synthesis. It is a large polyphenolic group of secondary compound. It contains sufficient hydroxyls groups and other suitable groups to form a strong complex with other macromolecules. The presence of tannin used as astringents against diarrhea (Yoshida *et al*., 1991), as diuretics (Hatano *et al*., 1991), anti-inflammatory, antiseptic, and hemostatic pharmaceuticals (Haslam, 1996). Flavonoids group helps in plant metabolites to provide cell signalling pathways and antioxidant activity. Fruit extract shows the presence of flavonoids compounds. Flavonoids are the important antibiotics group. These antibiotic principles are effective in defensive mechanism of the plants against different microbes (Hafiza, 2000). The presences of flavonoids are used as pharmacological activity such as antimicrobial activity (Cowan, 1996), antioxidant (Salah *et al*., 1995), and anticancer activities (Okwu, 2004).

DPPH radical scavenging activity

This model of scavenging the stable free radical is widely used method to evaluate antioxidant activities in a relatively short time compared with other methods. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability (Saikumar *et al*., 2017). The DPPH assay is commonly used because it is technically simple and gives accurate and repeatable results. The assay is valid to quantify samples with hydrophilic or lipophilic antioxidants (Perez-Jimenez *et al*., 2008). The antiradical scavenging activity of different extracts of *F. amplocarpa* would be related the nature of phenolics, thus contributing to their electron transfer/ hydrogen donating ability (Blios, 1958). Thus it can be assumed that *F. amplocarpa* may contain antioxidants of higher molecular value which may not be able to counteract with DPPH. Therefore, further antioxidant assays should be carried out to assess the quantity of antioxidants in *F. amplocarpa*.

$ABTS^{\bullet+}$ radical scavenging activity

The ABTS radical is soluble in both aqueous and organic solvents, is not affected by ionic strength and can be used to measure the antioxidant capacity of hydrophilic and lipophilic compounds in test samples Roginsky and Lissi (2005). Total antioxidant activity (TAA) of samples having higher phenolic content seems to be efficient for functioning as potential nutraceuticals or antioxidants when they are ingested along with nutrients. The radical is suitable for evaluating antioxidant capacity of phenolics due to their comparatively lower redox potentials (0.68V). Many phenolic compounds can thus react with the ABTS radical because of this thermodynamic property (Osman *et al.,* 2006). As (Schleisier *et al.,* 2002) reported, this method is simpler, more productive, and less expensive as compared with the traditional ABTS test. Hagerman *et al*. (1998) have reported that the high molecular weight phenolics (tannins) have more ability to quench free radicals (ABTS+). Since, the extracts from different methods of extraction have the ability to scavenge free radicals, thereby preventing lipid oxidation via a chain breaking reaction; they could serve as potential nutraceuticals when ingested along with nutrients. The total antioxidant activity shown by the extracts of *F. amplocarpa* points out that it may due to the higher amount phenolics and tannins which seemed to act as good radical scavengers.

Phosphomolybdenum assay

Phosphomolybdenum assay is successfully used to quantify vitamin E in seeds, being simple and independent of other antioxidant measurements commonly employed, it was decided to extend its application to plant extracts (Re *et al.,* 1999). A spectrophotometric method has been developed for the quantitative determination of antioxidant capacity by the phosphomolybdenum method. The assay is based on the reduction of Mo (VI) to Mo (V) by the sample and the subsequent formation of a green phosphate/ Mo (V) complex (Prieto *et al*., 1999). From the results, antioxidant capacity of the extracts was able to inhibit the Mo complex. From this, total antioxidant capacity (TAA) of *F. amplocarpa* may be due to the presence of phenolics and flavonoids in the plant extracts.

FRAP assay

The ferric reducing antioxidant power assay is used to measure the antioxidant effect of any substance in the reaction medium as its reducing ability. (Yen and Duh, 1993) and (Siddhuraju *et al*., 2002) have reported that the reducing power of bioactive compounds, mainly low and high molecular phenolics, was associated with antioxidant activity, specifically scavenging of free radicals. Thus the ferric reducing power the different extracts of *F. amplocarpa* reveals that there are compounds in the ethyl acetate extracts which have high affinity to the ferrous ions and thereby quench/ scavenge them through redox reactions.

Superoxide radical scavenging activity

Superoxide is also one of the most harmful radicals and its scavenging is necessary because it is a precursor for other major ROS, like hydrogen peroxide, hydroxyl and singlet oxygen. Numerous biological reactions generate superoxide radicals (Rao *et al*., 2010). Numerous biological reactions generate superoxide radical which is a highly toxic species. Although they cannot directly initiate lipid oxidation, superoxide radical anions are potent precursors of highly reactive species such as hydroxyl radical and thus the study of scavenging of this radical is important (Kannat *et al*., 2007). Since, the ethyl acetate extract of fruit *F. amplocarpa* showed appreciable percentage of scavenging activity against superoxide radical, it can be used against adverse effects caused by superoxide radical in the body. The active principles in the plant extracts may eliminate the radical by its reduction to

attain the octant stage or through the formation of water molecule.

Reducing power assay

The reducing properties are generally associated with the presence of reductions which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom. Reducing power is widely used in evaluating antioxidant activity of plant polyphenols. However, the presence of reductants (antioxidants) in the herbal extract causes the reduction of Fe^{3+}/ ferricyanide complex to Fe^{2+}/ ferrous form, so the reducing power of the sample or the Fe^{2+} complex can be monitored by measuring the formation of Perl's Prussian blue (Singh *et al*., 2004). The concentration dependent power of reduction shown by *F. amplocarpa* can be correlated with the content of phenolics and flavonoids which may appear to function as good electron or hydrogen donors.

GC-MS

The compounds present in the fruit sample Isopropyl myristate and Myristic acid isobutyl ester contain highest peak area (8.02) followed by Catechol (7.28). Decanoic acid, Octadecanoic acid, 9-Hexadecenoic acid, 9-octadecenyl ester, (Z, Z) - has the property of larvicidal effect (Falodun *et al.,* 2009). 9-Octadecenoic acid (Z) - (CAS) has been reported to have antioxidant and anti-inflammatory activity by Iniyavan *et al.,* 2012. Catechol belongs to flavonol compound, Erucic acid belongs to fatty acid and it has reported to have anti-microbial activity. Xanthotoxin has a property of Anti-tumour effect (Abdel Hafez *et al.,* 2009). Desulphosinigrin (glycoside) is reported to have anticancer, anti-inflammatory, anti-oxidant and antimicrobial activity (Wagay and Rothe, 2016).

CONCLUSION

F. amplocarpa exhibits a strong antioxidant activity in the following assay studied including DPPH, $ABTS^{\bullet+}$, Phosphomolybdenum, FRAP and Superoxide radical scavenging assay. The antioxidant activity of *F. amplocarpa* might be indorsed to effective hydrogen donating ability. The phytochemical analysis showed that there are many non-polar compounds like fatty acids (Decanoic acid, 2, 3-dihydroxypropyl ester (CAS)), Phenolic components Myristic acid isobutyl ester and flavonoid compounds such as Catechol. It can be concluded that *F. amplocarpa* fruit is a prominent source to Polyphenolic and antioxidant compounds.

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