IJPSR (2018), Volume 9, Issue 1 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 27 April, 2017; received in revised form, 07 July, 2017; accepted, 25 July, 2017; published 01 January, 2018

IN-VITRO ASSESSMENT, ISOLATION AND SPECTRAL ANALYSIS OF THE CHLOROFORM FRACTION OF *DIOSPYROS KAKI* FRUIT

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Keywords:

GC-MS, *Diospyros kaki*, Antioxidant, Anti-inflammatory, Phytochemicals

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ABSTRACT: Persimmon (*Diospyros kaki* Linn.), which belongs to the Ebenaceae family, have numerous bioactive compounds such as polyphenols, dietary fiber, carotenoids and minerals. This fruit possesses hypolipidemic and antioxidant properties and can be used in anti-atherosclerosis diets. The present paper reveals the phytochemical screening, Antioxidant, Anti-inflammatory activity and GC-MS Spectral analysis of the isolated fatty acids compounds of the plant *Diospyros kaki*. From the Antioxidant and Anti-inflammatory activity the chloroform fraction of ethanolic extract showed the good activity and proceeded for isolation. Six fatty acid compounds were isolated from the plant *Diospyros kaki* and analyzed using Gas chromatography-Mass Spectrometry. From the analysis 13 compounds were identified by comparing their retention time and peak area with that of literature and interpretation of mass spectra. The major phytoconstituents were Spiro [(tricyclo [6.2.2.0(2, 7)] dodeca and 1- monolinoleoylglycerol trimethylsilyl ether. Hence this study creates a platform to screen many bioactive components to treat many diseases.

INTRODUCTION: Oxidation is the process through which the living organisms produce their energy. Free radicals, including Reactive oxygen species (ROS) and reactive nitrogen species are formed as intermediates during the regular pathway of aerobic metabolism to oxidize the membrane lipids. As a consequence, many cellular components like proteins, lipids and DNA gets damaged [1]. Thus the cell damage caused by the free radicals appears to be the major contributor to aging, cardiovascular disease, cataracts, cancer, immune system decline and brain damage [2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.9(1).140-49

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(1).140-49

Free radicals are chief intermediaries that provoke or uphold inflammatory processes and as a result, their neutralization by antioxidants and radical scavengers can attenuate inflammation [3]. Antioxidant compounds from the plants can decrease the production of free radicals, and lessen the disease caused by the inflammation and oxidative stress [4].

Medicinal plants have been identified for many years and considered as potential sources of pharmaceutical agent. The beneficial effects of plant materials are due to their secondary metabolites present in them [5]. In traditional medicine the plant has a great potential for producing a new drug to treat chronic and infectious diseases. The Phyto-medicine present in the plant called the secondary metabolite is more important in the treatment of the various diseases [6].

So the use of plants is attractive and more essential not only for their anti-inflammatory properties, but also for their capacity to avoid cellular damage induced by free radicals [7]. Persimmon (*Diospyros Kaki* Linn.) is a vital horticultural crop which has numerous cultivated varieties. Persimmon fruit posses different nutrients and phtyochemicals such as carbohydrates, organic acids, Vitamins, tannins, polyphenols, dietary fibers and carotenoids, *etc*., which play an important role in the flavor, color, nutritive and pharmaceutical value of the fruit [8].

Persimmon fruits are coupled with a range of biological activities, including antioxidant effects, and their effective radical scavengers were found due to the flavonoid groups, namely catechin derivatives [9]. Based on the conventional knowledge of medicinal system, the present study was carried out to estimate the total phenol and flavonoid content of different solvents and their fractions. *In vitro* methods of assessment were used to determine the anti-oxidant and anti-inflammatory activity of the extracts and their fractions of the plant *Diospyros kaki.* The active chloroform fraction of ethanolic extract of the plant was subjected to isolation and analyzed for the GC-MS spectral studies. This work will facilitate in identifying the therapeutic importance of the plant.

MATERIALS AND METHODS:

Collection and Authentication of the Plant: The fresh persimmon fruit was collected from the Simpson Park, Conoor, Nilgiris. The plant was identified with the help of available literature and authenticated by Dr. S. Rajan, Field Taxonist Survey of Medicinal plant and Collection, Department of Ayush, Emerald, The Nilgiris. The fruits of the plant were washed with tap water followed by distilled water, dried in shade for 10 days prior to study and then stored in airtight glass jars, until in use.

Preparation of Extracts from Persimmon Fruits: [10] Fresh persimmon fruits were cut into four parts and the flesh was freeze-dried with freeze dryer. The dried persimmon fruit (*Diospyros kaki*) was ground and passed through a 25-mesh sieve. Each 1kg of the fine powder was subjected to triple maceration using 9 lit of ethanol and macerated for 72 hours and filtered. The pooled menstrum was subjected to vacuum distillation using rotar vapour (Buchi, R120). The concentrated extract was dried in vacuum desicator until a constant weight obtained.

Fractionation: 10gm of the Ethanolic extract was dissolved in 30ml of water using ultra sonication. The above portion was subjected to successive liquid-liquid fractionation using 150ml of Hexane, 200ml of Chloroform, 200ml of Ethyl acetate and 100ml of Acetone individually. All the obtained fractions were distilled under vacuum using rotar vapour (Buch, R120) and were subjected to drying using vacuum desicator until uniform weights were obtained. The percentage yield of the dried fractions is calculated. The mother extract and the fractions were subjected to *in vitro* antioxidant and anti-inflammatory using DPPH, Total antioxidant capacity, Superoxide dismutase, lipid peroxidation, Membrane stabilization, and Protein denaturation. The active fraction after *in vitro* studies was subjected to isolation [11].

Preparation of Fatty Acid Methyl Ester: The chloroform fraction was subjected to reflux for four hours using a mixture of methanol and acetyl chloride (95:5, 200ml). The obtained mixture was diluted with water and extracted thrice with the equal volume of n-hexane containing 0.01% butylated hydroxyl toluene. The combined n-hexane layer was evaporated to get fatty acid methyl ester (FAME) [12].

Urea Complexation: The above obtained 6gms of FAME was subjected to urea complexation in order to separate the saturated fatty acids from the unsaturated fatty acids. The 3ml of methanol and 1gm of urea were added to FAME and gently heated to get a clear solution and was cooled at room temperature and stored at 0 °C overnight. Then it was filtered to remove the crystals at the bottom [13]. The filtrate was again fractionated with 30ml of n-hexane, 30ml of chloroform and 30ml of acetone. The different sub-fractions were evaporated under vaccum. The dried 3gms of n-hexane fraction was dissolved in 10ml of n-hexane and was subjected again to liquid, liquid fractionation using methanol (30ml). The n-hexane and the methanol layer were evaporated leaving a fine yellow coloured. The obtained crystals were subjected to purification using methanol as a solvent in 10cm pen column.

The purified fractions were evaporated, leaving white colored crystal and named as a compound A and B. The 2gms of chloroform fraction was dissolved in 6ml of chloroform and was fractionated with 12ml of n-hexane and 10ml of methanol individually. The hexane and methanol sub-fractions were subjected to evaporation, leaving a white glittering needle shaped crystals and labelled it as compound C and D. The 1gm of methanol fraction was dissolved in 5ml of methanol and was subjected to liquid liquid fractionation using chloroform. The separated fractions were subjected to evaporation, leaving a white colored crystal and labelled as compound E and F. The purity of all the obtained 6 crystals was checked by its TLC and melting point profiles.

TLC and Melting Point Analysis: Each fraction was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel 60F254, 7X6cm (Merck). The solvent used is Chloroform and methanol in the ratio of (9:1). After the run, plates are dried and the spots were detected by using the UV spectrometer. The movement of the active compound was expressed by its retention factor (RF) values were calculated for different samples [14].

Chemicals Used: Ethanol-Hexane, chloroform, Ethyl acetate, Acetone, Methanol, Acetyl chloride, urea, 2, 2-diphenyl- 1- picrylhydrazyl radical (DPPH), ferric chloride, sodium phosphate, phosphate buffer saline, Ammonium molybdate (4mm), sodium sulphate (28mm), Egg lecithin, sulphuric acid, phosphate buffer (7.4) ascorbic acid (200mm), Ferric chloride (400mm), Trichloro acetic acid 15% w/v, Tributanol acetic acid (0.375% w/v), Trypsin, Dimethylsulphoxide, Trypsin, Rutin Gallic acid, Aspirin, Diclofenac sodium. All the chemicals were purchased from Sigma Aldrich.

Quantitative Analysis of Phytochemicals:

Determination of Total Phenolic Content: The total phenolic content was determined using spectrophotometric method. The reaction mixture was prepared by mixing 0.4ml (1 mg/ml) of extract, 0.2ml of 1mol/l Folin-Ciocalteu's reagent dissolved in water and 0.6ml of $NaHCO_3$ (20% w/v). The samples were incubated at 30 min in dark at room temperature to complete the reaction. The absorbance was determined at λ_{max} = 700nm. The samples were prepared in triplicate and the mean value of absorbance was obtained. Blank was concomitantly prepared with ethanol instead of extract solution. The same procedure was repeated for the gallic acid and the calibration line was constructed. The total phenolic content was expressed in terms of gallic acid equivalent (mg of GaA/g of extract) [15].

Determination of Total Flavonoids Content: The aluminium chloride colorimetric assay was used for total flavonoids determination, as described. Briefly, 0.5ml of the extract was mixed with 300µl of sodium nitrate (1:20 w/v). Then, it was incubated at room temperature for 5 minutes and 300µl of (1:10 w/v) aluminium chloride, 2ml of 1M sodium hydroxide and 1.9ml of distilled water were added. Then, absorbance of the reaction mixture was measured at 510nm, along with the standard, rutin and blank. The total flavonoids content was determined as microgram, rutin equivalent by using the standard rutin graph obtained by comparing the calibration curve prepared from a reference solution containing rutin (10-300µg/ml) [16].

DPPH Radical Scavenging Activity: [17] DPPH radical is a generally used technique to estimate the free radical scavenging capacity of natural compounds. This assay is based on the measurement of the scavenging ability of antioxidant substances toward the stable radical. DPPH solution was freshly prepared by dissolving 24mg of DPPH in 100ml ethanol and stored at -200 °C before use. The mixture containing 1.0ml of various concentrations of the extracts (2-10mg/ml) with 1.0ml of 0.8mmol/l DPPH solution was shaken vigorously allowed standing for 30 min in the dark condition. Absorbance was measured at 515nm. Ascorbic acid was used as standards. All determinations were performed in triplicate. The inhibition percentage for scavenging DPPH radical was calculated according to the equation:

$$\% \text{ of inhibition} = \frac{(\text{ABS Control} - \text{ABS Sample})}{\text{ABS Control}} \times 100$$

Where A_0 was the absorbance of the control and A_1 was the absorbance of the sample.

Phosphomolybdenum Assay (Total antioxidant capacity): The antioxidant activity of the sample was evaluated by the phosphor-molybdenum method. To 0.1ml of the extract and the fraction, 1ml of reagent solution (0.6M sulphuric acid, 28mM sodium phosphate, 4mM ammonium molybdate combined in eppendorf tube) was added. The tubes were capped and incubated at 350 °C for 90 min. After cooling to room temperature the absorbance was measured at 695nm against blank. Ascorbic acid was used as the standard and the total antioxidant capacity is expressed as equivalents of Ascorbic acid [18].

Hydrogen Peroxide Radical Scavenging Assay: The ability of the extract to scavenge hydrogen peroxide was determined according to the method given by Ruch *et al*., (1989) [16]. A solution of hydrogen peroxide (2mmol/l) was prepared in phosphate buffer (pH 7.4). To the fractions and extracts (1–10µg/ml), hydrogen peroxide solutions (0.6ml) were added. Absorbance of hydrogen peroxide at 230nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide and compared with ascorbic acid, the reference compound [19].

$$\% \text{ of inhibition} = \frac{(\text{ABS Control} - \text{ABS Sample})}{\text{ABS Control}} \times 100$$

Abs control is absorbance of the control; Abs sample is absorbance of the sample.

Superoxide Radical Scavenging Activity: To 0.5ml of different concentration of the extract and the fractions, 1ml alkaline DMSO and 0.2ml NBT 20mM (50mg in 10ml phosphate buffer pH 7.4) were added. The absorbance was measured at 560nm [20].

$$\% \text{ of inhibition} = \frac{(\text{ABS Control} - \text{ABS Sample})}{\text{ABS Control}} \times 100$$

Lipid Peroxidation Assay: The mixture (Egg phosphatidylcholine in 5ml saline) was sonicated to get a homogeneous suspension of liposome. Lipid peroxidation was initiated by adding 0.05mM ascorbic acid to a mixture containing liposome (0.1ml). The pink chromogen was extracted with a constant volume of n-butanol and absorbance of the upper organic layer was measured at 532nm. The experiment was performed in triplicate [20].

$$\% \text{ of inhibition} = \frac{(\text{ABS Control} - \text{ABS Sample})}{\text{ABS Control}} \times 100$$

***In-vitro* Anti-Inflammatory Activity:**

Inhibition of Protein Denaturation: [21] Test solutions having different concentration (50-250 µg/ml) of drug was taken with 1ml (1mM) of egg albumin solution. The mixture was incubated at 27 ± 1 °C for 15 min. Denaturation was induced by keeping the reaction mixture at 70 °C in a water bath for 10 min. After cooling the turbidity was measured spectrophotometrically at 660nm. The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated as follows:

$$\% \text{ of inhibition} = \frac{(\text{ABS Control} - \text{ABS Sample})}{\text{ABS Control}} \times 100$$

HRBC Membrane Stabilization Method: Blood was collected freshly and mixed with equal volume of Alsever's solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl). It was then centrifuged at 3000g for 15 minutes. The cells were washed with iso-saline and a 10% suspension was made with iso-saline. Different concentrations of ethanol extract (100-500µg/ml) were prepared in iso-saline. To 0.5ml of the extract, 1ml phosphate buffer, 2ml hyposaline and 0.5mL HRBC suspension was added and incubated for 30 minutes at 370 °C and then centrifuged at for 20 minutes. Absorbance was measured at 560nm. Aspirin was used as the standard and control was taken without the extract served as negative control [22].

Percentage of HRBC membrane stabilization or protection was calculated using the formula:

% Stabilization = 100 – [(Optical Density of Drug) ÷ (Optical Density of Control) × 100]

GC-MS Analysis: [23] The phytoconstituent present in the ethanolic and hydroalcoholic extract and the four fractions of *Diospyros kaki* were analyzed on a Shimadzu QP-2010 GC-MS. The following conditions were used: ZB-5MS column Phenomenex Zebron (30 mx 0.25 mm x 0.25 mm); helium (99.999%) carrier gas at a constant flow of 1.1ml/min; 1µl injection volume; injector split ratio of 1:40; injector temperature 240 °C; electron impact mode at 70eV; ion source temperature 280

°C. The oven temperature was automatic from 100 °C (isothermal for 5 min), with an increase of 10 °C/min to 250 °C (isothermal for 5 min) and 10 °C/min to 280 °C (isothermal for 15 min). The individual phytoconstituents were identified by comparing their mass spectra with the spectra of known compounds stored in the spectral database, NBS; WILEY and NIST attached to the GC-MS instrument and reported.

Statistical Analyses: The results were presented as mean ± SD. All analyses were carried out in triplicates. Statistical data were performed by one way analysis of variance. Significant differences between groups were determined at $P < 0.05$. Graph pad prism was used for the graphical and statistical evaluations.

RESULTS AND DISCUSSION: Since ancient time mankind is using medicinal plants or natural products to treat acute and chronic diseases. Natural antioxidants are the most important source present [2] in these plants to treat the diseases like cancer, cardiovascular diseases by scavenging the free radical which are the main reason for the pathogenesis of these diseases [3]. Phytochemical analysis revealed the presence of phenols, flavonoids, tannins, carbohydrates, and proteins is present in all the extracts. Alkaloids and Glycosides are absent in all the two extracts, steroids is present in ethanolic extract of *Diospyros kaki* but absent in hydroalcoholic extract.

TABLE 1: PHYTOCHEMICAL ANALYSIS

S. No	Name of the test	Ethanolic extract of *Diospyros kaki*	Hydro alcoholic extract of *Diospyros kaki*
1	Phenol	+	+
2	Flavonoid	+	+
3	Alkaloid	-	-
4	Glycosides	-	-
5	Steroids	+	-
6	Tannins	+	+
7	Carbohydrates	+	+
8	Proteins	+	+

Phenolics and flavonoids are the major group of compounds present in plants which have the subsequent effects like decreasing blood pressure, stimulating intestinal peristalsis, choleretic and diuretic functions, reducing the viscosity of the blood and as well as major antioxidation or free radicals scavenging activities [6]. The total phenol and flavonoid content of the ethanolic extract of chloroform is 80.48mg/ml and 110.3mg/ml. Thus the high total phenolic and total flavonoid content present in chloroform fraction of the plant *Diospyros kaki* could be considered as a traditional medicine for treating many acute and chronic ailments of different etiology.

TABLE: 2 TOTAL PHENOLIC AND FLAVONOID

Sample	TPC (mg gallic acid/100 ml)	TFC (mg rutin/100 ml)
Ethanolic	82.98±1.37	262.43±1.68
Chloroform	84.3±1.25	270.45±1.03
Ethylacetate	47.19±0.94	56.03±1.78
Acetone	67.23±1.39	72.26±1.34

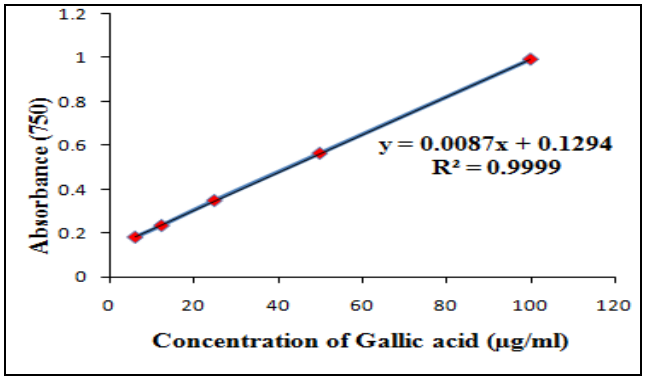

FIG 1: STANDARD GRAPH FOR PHENOL

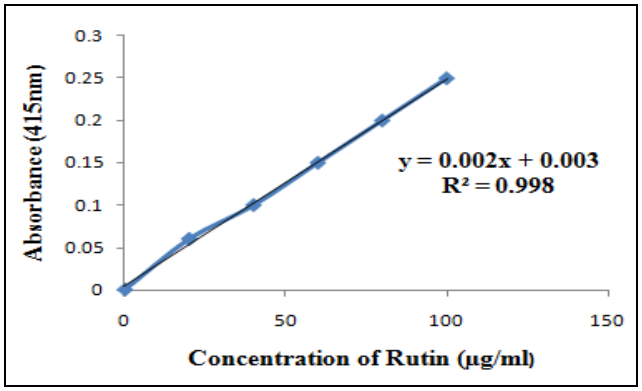

FIG 2: STANDARD GRAPH FOR FLAVONOID

***In vitro* Antioxidant Activity:**

DPPH Radical Scavenging Activity: To evaluate antioxidant activity DPPH radical was generally used as substrate antioxidants. In specific antioxidant reactions this 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) acts as a stable free radical. As a result at the 517nm, there is reduction of DPPH concentration by the antioxidant, which decreases the optical absorbance of DPPH. The free radical scavenging activity of the three plant extract was estimated by DPPH is shown in (**Table 3**).

The results specify that the chloroform fraction of the ethanolic extract of the plant has apparent effects on scavenging the free radicals. The scavenging outcome of the chloroform fraction is 90.43µg/ml at a concentration of 500µg/ml and the scavenging activity also increased in a dose dependent manner. At the concentration range of 100 to 500µg/ml, the standard ascorbic acid showed the maximum value of 150.5µg/ml in a dose dependent manner. When compared to the standard the inhibition value of the chloroform fraction of the ethanolic extract of the plant was found to be significant.

Total Antioxidant Capacity: The total antioxidant activity of the extracts was measured spectro-photometrically based on the development of phosphomolybdenum complex. Comparision of the total antioxidant capacity of the extract, fractions to standard ascorbic acid the antioxidant activity of the fraction exhibited increasing trend with the increasing concentration. While the standard antioxidant also showed the same trend as the concentration increased the activity also increase, 0.54 ± 0.01µg/ml and the standard is 1.02 ± 0.42µg/ml. This shows that the activity of the chloroform fraction of the ethanolic extracts having activity comparable to that of the standard.

Hydrogen Peroxide Radical Scavenging Activity: The chloroform fraction of the ethanolic extracts was capable of scavenging hydrogen peroxide in a concentration dependent manner. The The IC_{50} value of the chloroform fraction is 19.26µg/ml at a concentration range of 200µg/ml which is having a similar effect to that of ascorbic acid 41.1 µg/ml at a concentration of 200µg/ml. Scavenging of H_2O_2 by the plant extracts might be recognized by their phenolics, which contribute electron to H_2O_2, thus reducing it to water. The result shows that the radical scavenging activity of chloroform fraction is significant to the standard.

Superoxide Radical Scavenging Activity: In superoxide anion scavenging assay, the chloroform fraction of the ethanolic extract of the plant showed maximum superoxide anion scavenging activity and the results are presented in **Table 3**. The IC_{50} value of the chloroform fraction of the ethanolic extract is 93.15µg/ml which is significant to that of the standard. The chloroform fraction and the standard suppressed dexyribose degradation in a concentration-dependent manner. Ascorbic acid was used as reference standard. This shows that the choloroform fraction is having significantly better activity compared to the mother extract.

Lipid Peroxidation Scavenging Activity: The lipid per oxidation inhibition activity of the extract and its fraction was compared with standard ascorbic acid. From the results the chloroform fraction of the ethanolic extract is giving a significant activity with that of the standard (29.68µg /ml)exhibited inhibition at 250µg/ml, respectively (**Table 3**). The high inhibitory effect of lipid per oxidation of the extracts could be due to the abundant presence of antioxidant active compounds like flavonoids, saponins, sterols, terpenes, tannins which has high inhibition of lipid per oxidation property. A positive correlation of lipid per oxidation inhibition with free radical scavenging activities was observed.

TABLE 3: ANTIOXIDANT ACTIVITY OF *DIOSPYROS KAKI* (IC_{50} VALUE)

Name of the extracts	DPPH	Total antioxidant capacity (TAC)	Hydrogen Peroxide(H_2O_2)	Superoxide dismutase (SOD)	Lipid peroxidation (LPO)
Alcoholic extract	153±1	1.24±0.0152	38.2±1.07	97.24±0.77	49.3±1.19
Chloroform Fraction	90.43±1.40	0.54±0.01	19.26±0.90	93.15±1.17	29.68 ±1.01
Ethylacetate Fraction	109.6±1.52	0.92±0.01	26.56±1.38	82.49±0.56	32.6±1.04
Acetone Fraction	153.65±1.70	1.35±0.46	41.08±0.83	103±1.68	48.45±8.45
Ascorbic acid	150.5±1.2	1.02±0.4220	41.12 ±0.691	100.5±0.69	50.23±0.93

Each value is expressed as mean ± SD n = 3

***In-vitro* Anti-inflammatory Activity:**

Protein Denaturation Method: In protein denaturation method, percentage inhibition with respect to control is a measure of protein stabilization. The present findings exhibited a concentration dependent inhibition of protein (albumin) denaturation by alcohol extract and the fractions like chloroform, ethyl acetate, acetone of *Diospyros Kaki* throughout the concentration range of 1000 to 15.625µg/ml. Ibuprofen (at the concentration range of (1000 to 15.625µg/ml) was used as reference drug which also exhibited

concentration dependent inhibition of protein denaturation. Chloroform fraction possessed IC_{50} value 66.9µg/ml at the concentration range of 125µg/ml whereas that of Ibuprofen was found to be 118.33µg/ml.

However, the effect of Ibuprofen and the fraction was found to be moderate activity.

Membrane Stabilization: Membrane stabilization is an additional mechanism to provide the results for the anti inflammatory effect [44]. Chloroform fraction of *Diospyros kaki* prevents hypotonicity induced membrane lysis (HRBC membrane stabilization method) to extent of 18.86µg/ml at the concentration of 500µg/ml which is comparable to that of the standard drug Diclofenac sodium 55.56 (500µg/ml). The anti-inflammatory activity of the both alcoholic and standard were concentration dependent.

TABLE 4: ANTI-INFLAMMATORY ACTIVITY OF *DIOSPYROS KAKI* (IC_{50} VALUE)

Name of the extract	Protein denaturation	Membrane stabilization
Alcoholic extract	100.56±1.63	47.22±1.85
Chloroform fraction	66.97±1.77	18.86±1.34
Ethyl acetate fraction	98.35±0.89	33.22±1.07
Acetone fraction	120.33±1.28	57.24±1.34
Ibuprofen	118.3333±0.946	-
Diclofenac	-	55.562±0.5513

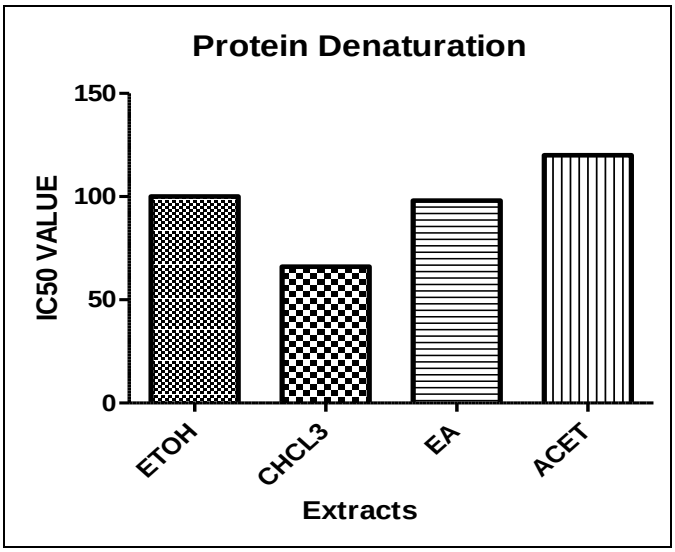

FIG. 3: EFFECT OF ETHANOLIC EXTRACT AND THEIR FRACTION ON PROTEIN DENATURATION

GC-MS is one of the most excellent techniques to spot the constituents of volatile matter, long and branched chain hydrocarbons, alcoholic acids, esters etc [45, 46].

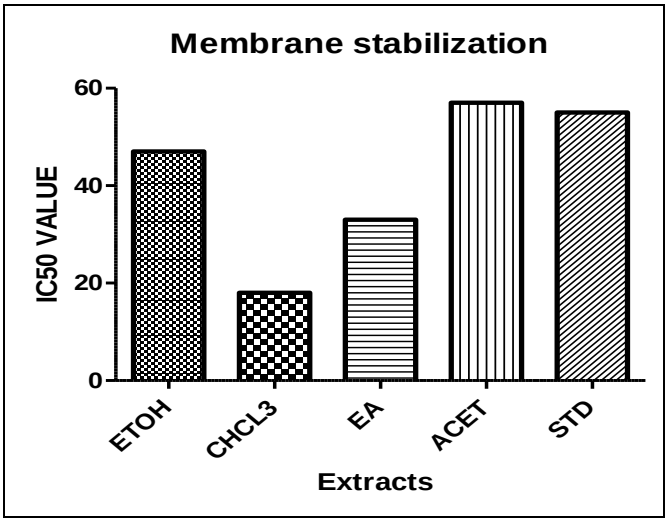

FIG. 4: EFFECT OF ETHANOLIC EXTRACT AND THEIR FRACTION ON MEMBRANE STABILIZATION
Where ETOH-Ethanolic extract, $CHCl_3$ Chloroform extract, EA-Ethyl acetate extract, ACET-Acetone extract, STD-Standard

The GC-MS analysis leads to the detection of the number of compounds from the GC fractions of the ethanol extract of *Diospyros kaki* through mass spectrometry attached with GC. The mixtures of components present in the fruit were detected by the GC-MS and which are shown in **Tables 4**.

The compounds identified were

1. Spiro[(tricyclo[6.2.2.0(2,7)]dodeca5,9diene)4,1' cyclobutane]11,2'dione,1,3,3,5,12,12hexameth,
2. 3-Methyl-6, 7- benzoisoquinoline,
3. 3- Isopropy- l6a, 10bdimethy- l8- (-2-oxo-2-phenylethyl) dodecahydrobenzo [f] chromen- 7-one,
4. 4,11Dispiro(2'cyclobutanone)tricyclo[6.2.2.0(2,7)] dodeca5, 9 diene, 1,3,3,5,12,12hexamethyl,
5. a- N- Normethadol, Monolinoleoylglycerol trimethylsilyl ether,
6. Azabicyclo [3.3.0] octane, 2acetyl1, 5dimethyl8 8ethylenedioxy4methoxycarbonylmethyl,
7. 2à-Bromo-17- áhydroxy- 5à- androstan- 3- one,
8. N-(-O-Nitrophenylthio)-l-leucine,
9. Corynan-17-ol, 18, 19-didehydro-10-methoxy-acetate (ester),
10. 2- Methyl- 3, 5- dinitrobenzyl alcohol, tert-butyldimethylsilylether,
11. 5HCyclopropa[3,4]benz[1,2e]azulen5one,9,9ab is(acetyloxy)3[(acetyloxy)methyl]1,1a,1b,4,4a, 7a,7b,8,9,9adecahydro7bhydroxy1,1,6,8tetrame thyl,[1aR(1aà,1bá,4aá,7aà,7bà,8à,9á,9aà),

12. 3-Isobutoxycarbonylmethylamino- 2- (4 chloro phenyl) thioacrylomorpholide,

13. 3- Isopropyl- 6a, 10- bdimethyl- 8- (-2-oxo-2-phenyl-ethyl)-dodecahydro-benzo[f]chromen-7-one.

TABLE 5: GC-MS ANALYSIS OF CHLOROFORM FRACTIONS OF *DIOSPYROS KAKI*

S. no	Compound Name	Retention time	Nature of the compound	Molecular formula	Activity of the compound
1	Spiro[(tricyclo[6.2.2.0(2,7)]dodeca5,9diene	6.90-15.43	Fatty acid	C21H28O2	No activity reported
2	3-Methyl-6,7- benzoisoquinoline	16.93	Alkaloid	C14H11N	No activity reported
3	3-Isopropy-l6a,10bdimethy-l8-	27,19	Fatty acid	C26H36O3	No activity reported
4	4,11Dispiro(2'cyclobutanone)tricyclo	12.44	Fatty acid	C24H32O2	No activity reported
5	a-N-Normethadol	11.69	Fatty acid	C20H27NO	Antibacterial activity
6	1Monolinoleoylglycerol trimethylsilyl ether	29.01-33.04	Steroid	C27H54O4Si2	Antimicrobial Antioxidant Anti-inflammatory Antiarthritic Antiasthma, Diuretic
7	2Azabicyclo[3.3.0]octane	6.06	Amino acid	C16H25NO5	No activity reported
8	2à-Bromo-17-áhydroxy-5à-androstan-3-one	6.33	Steroid	C19H29BrO2	No activity reported
9	N-(-O-Nitrophenylthio)-l-leucine	6.64	Amino acid	C12H16N204S	No activity reported
10	Corynan-17-ol,18,19-didehydro-10-methoxy-acetate (ester)	6.74	Fatty acid	C22H28N2O3	Anti-diarrhoeal activity
11	2-Methyl-3,5-dinitrobenzyl alcohol,tert-butyldimethylsilylether	8.03	Alkaloid	C14H22N2O5Si	No activity reported
12	5HCyclopropa[3,4]benz[1,2e]azulen5one	13.12	Ketone compound	C26H34O8	Inhibits IgE synthesis
13	3-Isobutoxycarbonylmethylamino-2-(-4chlorophenyl)thioacrylomorpholide	16.09	Amino acid	C19H25ClN2O3S	No activity reported

GC-MS Results:

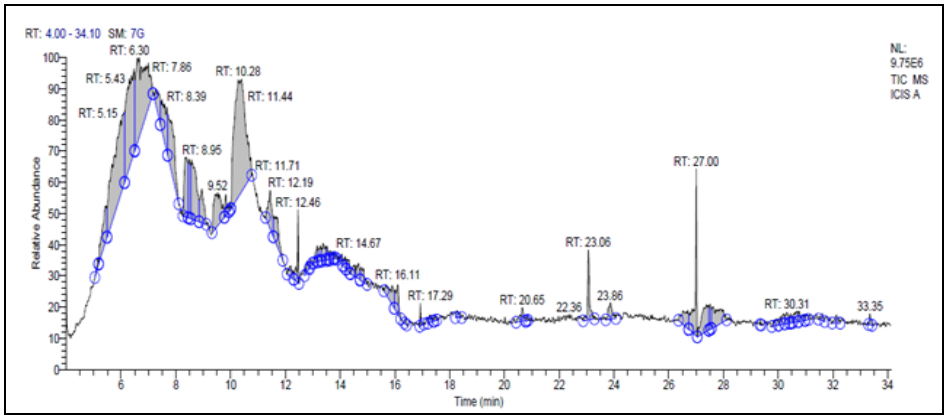

FIG. 5: GC-MS CHROMATOGRAM OF COMPOUND A

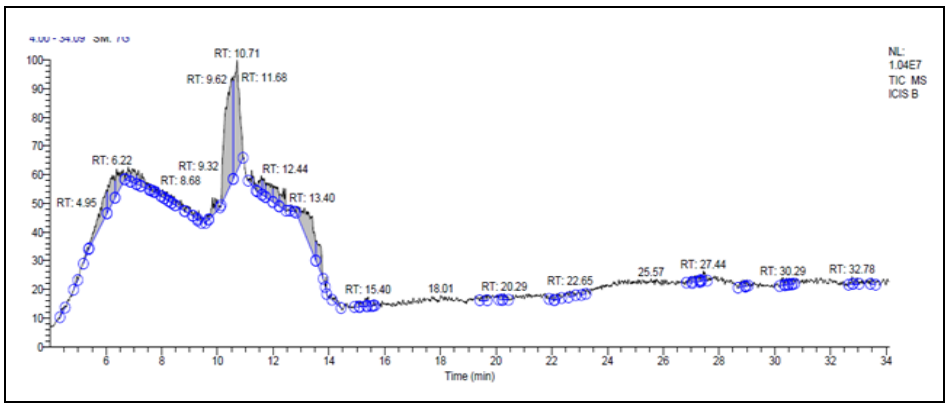

FIG. 6: GC-MS CHROMATOGRAM OF COMPOUND B

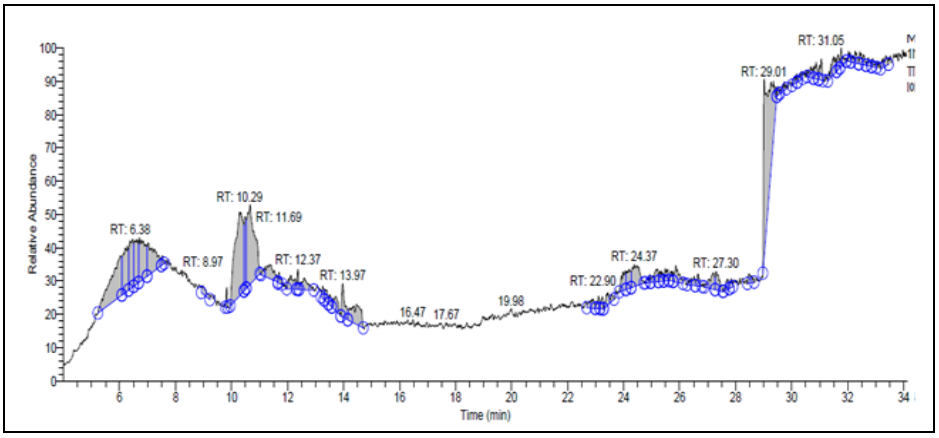

FIG. 7: GC-MS CHROMATOGRAM OF COMPOUND C

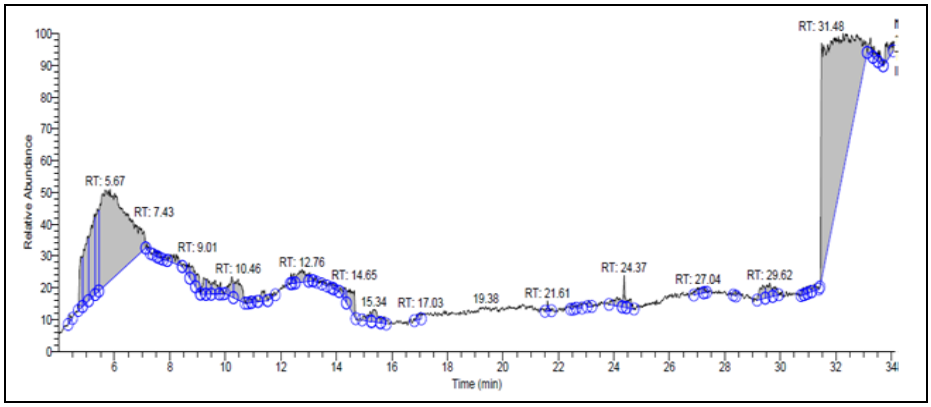

FIG. 8: GC-MS CHROMATOGRAM OF COMPOUND D

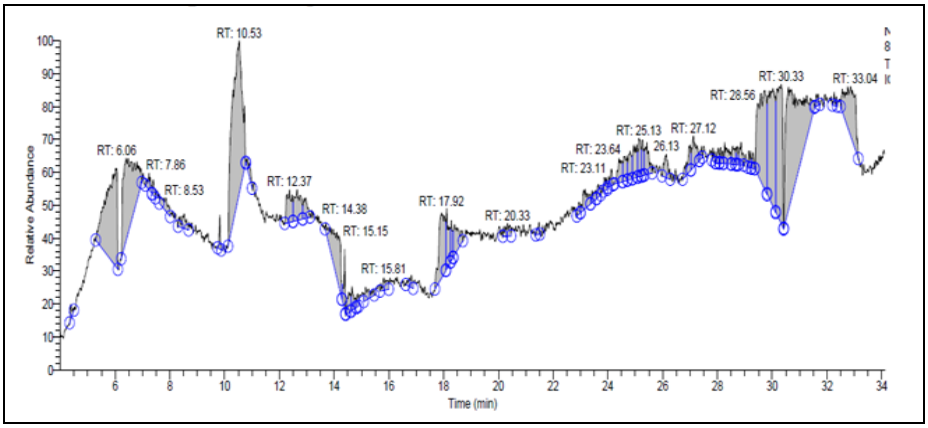

FIG. 9: GC-MS CHROMATOGRAM OF COMPOUND E

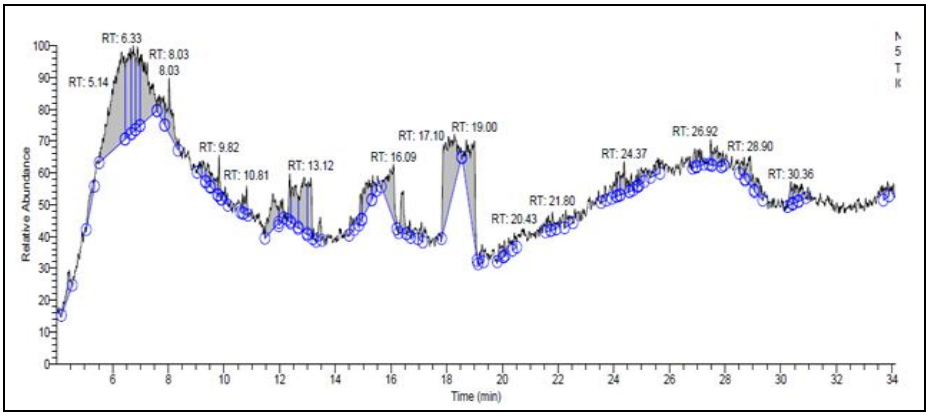

FIG. 10: GC-MS CHROMATOGRAM OF COMPOUND F

CONCLUSION: From the results of our study the *Diospyros kaki* fruit has been revealed to contain both antioxidant and anti-inflammatory activity which is responsible for many diseases. The existence of diverse bioactive compounds in *Diospyros kaki* proved the pharmaceutical importance. By isolating and identifying these bioactive compounds, novel drugs can be formulated to treat different diseases. Further studies will be required to find out the molecular level.

ACKNOWLEDGEMENT: I would like to thank Dr. B. Duraiswamy, Head and Professor, Department of pharmacognosy and phyto-pharmacy, JSS College of Pharmacy, Ooty for his great support and help.

CONFLICT OF INTEREST: No conflict of interest regarding this publication.

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How to cite this article:
Vanitha B and Duraisamy B: *In-vitro* assessment, isolation and spectral analysis of the chloroform fraction of *Diospyros kaki* fruit. Int J Pharm Sci & Res 2018; 9(1): 140-49. doi: 10.13040/IJPSR.0975-8232.9(1).140-49.