ISSN:2229-6107

INTERNATIONAL JOURNAL OF PURE AND APPLIED SCIENCE & TECHNOLOGY

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www.ijpast.in

INTERNATIONAL JOURNAL OF PURE AND APPLIED SCIENCE & TECHNOLOGY
IJPAST

ISSN 2229-6107 www.ijpast.in
Vol 12,Issuse 3.Aug 2022

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

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ABSTRACT: The fruit of the ebenaceae family, the persimmon (Diospyros kaki Linn.), contains a wide variety of beneficial substances, including minerals, dietary fiber, carotenoids, and polyphenols. Atherosclerosis diets may benefit from this fruit's hypolipidemic and antioxidant characteristics. The isolated fatty acid components of the plant Diospyros kaki are revealed in this research using phytochemical screening, antioxidant, anti-inflammatory, and GC-MS spectral analysis. We isolated the chloroform fraction of the ethanolic extract because it exhibited strong antioxidant and anti-inflammatory properties. The were six different fatty acid molecules extracted from thegas chromatography-mass spectrometry was used to examine the plant Diospyros kaki. Analysis of retention times and peak areas, in conjunction with literature and mass spectral interpretation, led to the identification of thirteen chemicals. Tricyclo[6.2.2.0(2,7)] dodeca and 1-monolinoleoylglycerol trimethylsilyl ether were the primary phytoconstituents. This research lays the groundwork for future screenings of bioactive components with the potential to cure a wide range of disorders.

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

INTRODUCTION:

The energy that all living things get from is oxidation. In the normal course of aerobic metabolism, free radicals such reactive nitrogen species and reactive oxygen species (ROS) are produced as intermediates on the road to oxidizing the lipids in the cell membrane. Many parts of the cell, including DNA, lipids, and proteins, are so damaged 1. Therefore, it seems that free radical cell damage is the primary cause of aging, heart disease, cataracts, cancer, a weakened immune system, and brain impairment 2. Because free radicals are key players in initiating and maintaining inflammatory processes, using antioxidants and radical scavengers to neutralize them may reduce inflammation. 3. Inflammation and oxidative stress are known to cause illness, however plant-based antioxidants may reduce free radical generation and alleviate some of these symptoms. For a long time, people have looked to medicinal plants as possible sources of pharmacological agents. The presence of secondary metabolites in plant materials is responsible for their positive effects. 5. The plant has tremendous promise as a novel medicinal ingredient for the treatment of infectious and chronic disorders, according to traditional medicine. In the treatment of many ailments, the phyto-medicine found in the plant, known as the secondary metabolite, is more significant. 6.

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INTERNATIONAL JOURNAL OF PURE AND APPLIED SCIENCE & TECHNOLOGY
IJPAST
ISSN 2229-6107 www.ijpast.in
Vol 12,Issuse 3.Aug 2022

Both the anti-inflammatory effects and the ability to protect cells from free radical damage make plant-based solutions increasingly appealing and necessary. One of the most important horticultural crops, persimmon (Diospyros Kaki Linn.) comes in a wide variety of cultivars. The taste, color, nutrition, and medicinal value of persimmon fruit are influenced by its many nutrients and phtyochemicals, which include carbs, organic acids, vitamins, tannins, polyphenols, dietary fibers, and carotenoids, among others. 8.

The flavonoid groups in persimmon fruits, especially catechin derivatives 9, are responsible for their antioxidant benefits and other biological activities, such as their role as efficient radical scavengers. The current investigation aimed to determine the overall phenol and flavonoid content of various solvents and their fractions by using previously established medical system information. The anti-inflammatory and antioxidant properties of the plant Diospyros kaki extracts and their fractions were evaluated using in vitro techniques. Isolation and analysis of the active chloroform fraction from the plant's ethanolic extract was performed for the GC-MS spectrum investigations. The medicinal significance of the plant may be better understood with the help of this study.

DATA AND PROCEDURES:

The fresh persimmon fruit was sourced from Simpson Park in Conoor, Nilgiris, as part of the plant's collection and authentication process. Dr. S. Rajan, of the Department of Ayush at Emerald, The Nilgiris, verified the plant's identity using the literature that was already accessible. After rinsing with tap water and then distilled water, the plant's fruits were left to dry in the shade for ten days before being studied. They were then preserved in sealed glass containers until needed.

Harvesting Persimmon Fruits for Extract: 10 The flesh of fresh persimmon fruits was freeze-dried after being chopped into four pieces. Using a 25-mesh sieve, the powdered dried persimmon fruit (Diospyros kaki) was filtered. After being macerated for 72 hours in 9 lit of ethanol, each 1 kilogram of fine powder was filtered. Rotar vapor vacuum distillation was used on the combined menstrual fluids.

(R120, Buchi). A vacuum desiccator was used to dry the concentrated extract until its weight remained constant.

To prepare the fractions, 10 grams of the ethanolic extract were ultrasonicated into 30 milliliters of water. Hexane (150 ml), chloroform (200 ml), ethyl acetate (200 ml), and acetone (100 ml) were used in sequence for the liquid-liquid separation of the aforementioned component. After collecting the various fractions, they were all dried using a vacuum desiccator and distilled under vacuum with rotar vapour (Buch, R120) to ensure consistent weights. The dried fractions' % yield is determined. Protein denaturation, lipid peroxidation, total antioxidant capacity, superoxide dismutase, membrane stabilization, and mother extract and fraction in vitro antioxidant and anti-inflammatory testing was conducted. Following in vitro investigations, the active fraction was separated by separation 11.

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IJPAST
ISSN 2229-6107 www.ijpast.in
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Fatty acid methyl ester preparation: 200 milliliters of a 95:5 ratio of methanol to acetyl chloride was refluxed with the chloroform fraction for four hours. The resulting blend was rinsed with water and then extracted three times using n-hexane of the same volume that contained 0.01% butylated hydroxyl toluene. By evaporating the mixed n-hexane layer, FAME 12 was obtained.

In order to isolate the saturated and unsaturated fatty acids, the 6 grams of FAME that were previously collected underwent urea complexation. After adding 3 milliliters of methanol and 1 gram of urea to FAME, the mixture was heated gradually until it became transparent. After cooling to room temperature, it was kept at 0 degrees Celsius for the night. After that, the crystals at the bottom thirteen were removed by filtering it. Another round of fractionation was performed on the filtrate using 30 ml each of n-hexane, chloroform, and acetone. Vacuum evaporation was used to separate the various components. Once again, the dried 3 grams of n-hexane fraction was dissolved in 10 milliliters of n-hexane and submitted to liquid fractionation with 30 milliliters of methanol. A thin film of yellow was left after the n-hexane and methanol layers evaporated. The crystals that were produced were then purified on a 10cm pen column using methanol as the solvent.

A and B, two compounds, were formed when the evaporated portions left behind a white crystal. After dissolving 2 grams of chloroform fraction in 6 milliliters of chloroform, the mixture was separately fractionated with 12 milliliters of n-hexane and 10 milliliters of methanol. Compounds C and D were created by evaporating the hexane and methanol portions, which resulted in the formation of white, dazzling needle-shaped crystals. Chloroform was used for liquid fractionation on a 1gm methanol fraction that had been dissolved in 5ml of methanol. Compounds E and F were identified after evaporation left behind a white crystal from the separated fractions. Using TLC and melting point profiles, we confirmed that each of the six crystals we got was pure.

Melting point analysis and thin layer chromatography (TLC) were performed on each fraction according to the standard one-dimensional ascending technique using silica gel 60F254, 7X6cm (Merck). Chloroform and methanol, in a 9:1 ratio, are the solvents used. Plates are dried after the run, and spots are spotted using a UV spectrophotometer. The retention factor of the active chemical was used to represent its mobility.

relative frequencies (RF) were determined for several samples. temperature at which the reaction will end. The absorbance was measured at a maximum wavelength of 700 nm. The samples were made three times to ensure accuracy, and the average absorbance value was calculated. In place of the extract solution, ethanol was used to simultaneously prepare the blank. To generate the calibration line for gallic acid, the same technique was done. In milligrams of gallic acid equivalents per gram of extract, the total phenolic content was measured.

The total flavonoid content was determined using the aluminum chloride colorimetric test, following the procedure outlined. To summarize, 0.5 milliliters of the extract was combined with 300 microliters of sodium nitrate at a ratio of 1:10 weight-to-volume. The next step was a 5-minute incubation period at room temperature, followed by the addition of 300μl of aluminum chloride (1:10 w/v), 2ml of sodium hydroxide (1M), and 1.9ml of distilled water. Next, the absorbance of the reaction mixture, standard, rutin, and blank were all measured at 510 nm. The microgram equivalent of rutin was used to calculate the total flavonoid concentration, which was then compared to the calibration curve made from a reference solution containing rutin (10-300 μg/ml).

Chemicals Used: Ethanol-Hexane, chloroform, Ethyl acetate, Acetone, Methanol, Acetyl chloride, urea, 2, 2-diphenyl- 1- picrylhydrazyl

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IJPAST

ISSN 2229-6107 www.ijpast.in
Vol 12,Issuse 3.Aug 2022

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. Sigma Aldrich was the source for all of the compounds.

Analyzing Phytochemicals Quantitatively: Total Phenolic Content Determination: The spectrophotometric technique was used to determine the total phenolic content. We made the reaction mixture by combining 0.4 ml of extract (1 mg/ml), 0.2 ml of Folin-Ciocalteu's reagent diluted in water (1 moles/liter), and 0.6 ml of NaHCO3 (20% weight/volume). At room temperature, the samples were left to incubate for 30 minutes in a dark environment.

One common method for estimating a compound's free radical scavenging capability is to look at its DPPH radical scavenging activity. The capacity of antioxidant compounds to scavenge the stable radical is the basis of this test. We made the DPPH solution by dissolving

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

24 mg of DPPH in 100 ml of ethanol, and then we kept it at -200 for later use.

(°C) before to consumption. In a dark environment, a mixture was agitated vigorously for 30 minutes with 1.0 ml of a 0.8 mmol/l DPPH solution and 1.0 ml of extracts at concentrations ranging from 2 to 10 mg/ml. The absorption wavelength was 515 nm. Standard compounds included ascorbic acid. We ran each test three

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

times to be sure. The equation was used to compute the inhibition % for DPPH radical scavenging:

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

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 thetotal 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].

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].

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IJPAST
ISSN 2229-6107 www.ijpast.in
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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].

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ISSN 2229-6107 www.ijpast.in
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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

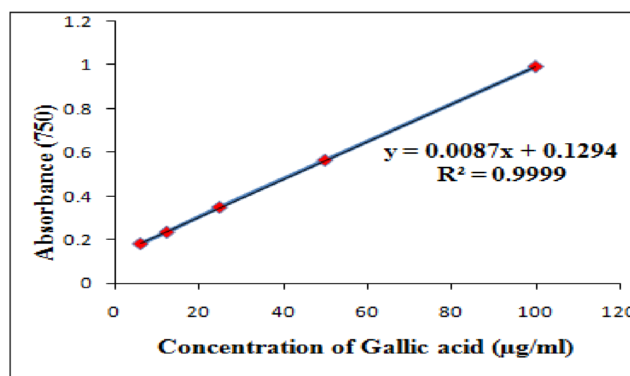

FIG 1: STANDARD GRAPH FOR PHENOL

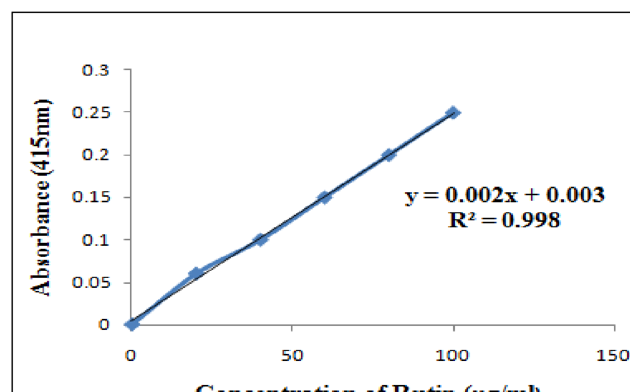

FIG 2: STANDARD GRAPH FOR FLAVONOID

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,

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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

INTERNATIONAL JOURNAL OF PURE AND APPLIED SCIENCE & TECHNOLOGY
IJPAST
ISSN 2229-6107 www.ijpast.in
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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 theconcentration 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

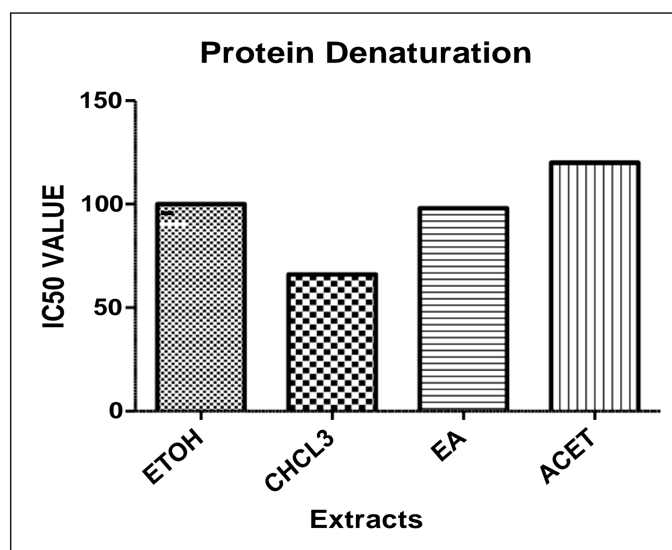

FIG. 3: EFFECT OF ETHANOLIC EXTRACT ANDTHEIR FRACTION ON PROTEIN DENATURATION

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].

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IJPAST
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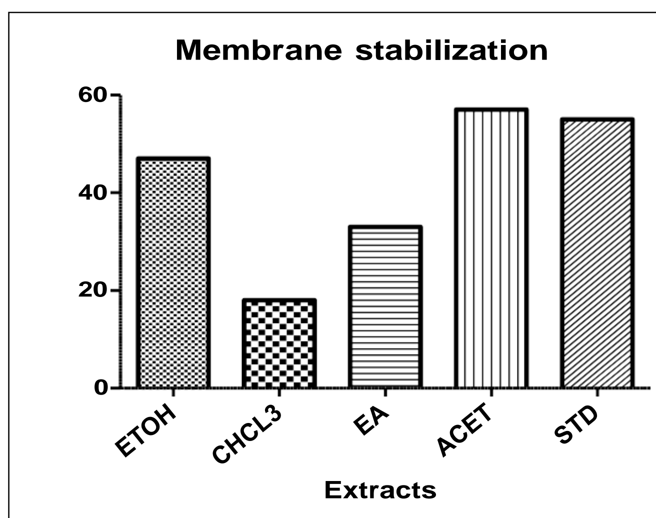

FIG. 4: EFFECT OF ETHANOLIC EXTRACT AND THEIR FRACTION ON MEMBRANE STABILIZATION
Where ETOH-Ethanolic extract, $CHCl_3$ Chloroform extract, EA-Ethyl acetate extract, ACET-Acetone extract, STD-Standard

The GC-MS analysis leads to the detection of the number of compounds from the GC fractions of theethanol extract of *Diospyros kaki* through mass spectrometry attached with GC. The mixtures of components present in the fruit were detected bythe 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, Monolinoleoylglyceroltrimethylsilyl ether,
6. Azabicyclo [3.3.0] octane, 2acetyl1, 5dimethyl88ethylenedioxy4methoxycarbonylmethyl,
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,8,9,9adecahydro7bhydroxy1,1,6,8tetramethyl,[1aR(1aà,1bá,4aá,7aà,7bà,8à,9á,9aà),
12. 3-Isobutoxycarbonylmethylamino- 2- (4 chlorophenyl) 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

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8	2à-Bromo-17-áhydroxy-5à-androstan-3-one	6.33	Steroid	$C_{19}H_{29}BrO_2$	No activity reported
9	N-(-O-Nitrophenylthio)-l-leucine	6.64	Amino acid	$C_{12}H_{16}N_{204}S$	No activity reported
10	Corynan-17-ol,18,19-didehydro-10-methoxy-acetate (ester)	6.74	Fatty acid	$C_{22}H_{28}N_2O_3$	Anti-diarrhoeal activity
11	2-Methyl-3,5-dinitrobenzyl alcohol,tert-butyldimethylsilylether	8.03	Alkaloid	$C_{14}H_{22}N_2O_5Si$	No activity reported
12	5HCyclopropa[3,4]benz[1,2e]azulen5one	13.12	Ketone compound	$C_{26}H_{34}O_8$	Inhibits IgE synthesis
13	3-Isobutoxycarbonylmethylamino-2-(-4chlorophenyl)thioacrylomorpholide	16.09	Amino acid	$C_{19}H_{25}ClN_2O_3S$	No activity reported

GC-MS Results:

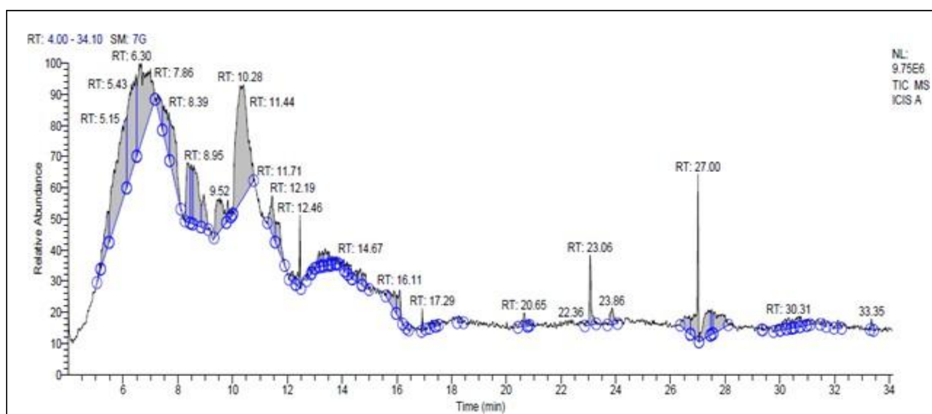

FIG. 5: GC-MS CHROMATOGRAM OF COMPOUND A

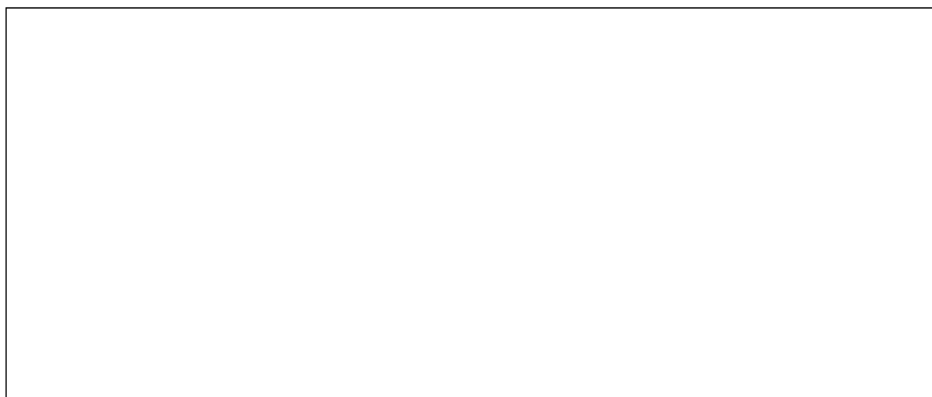

FIG. 6: GC-MS CHROMATOGRAM OF COMPOUND B

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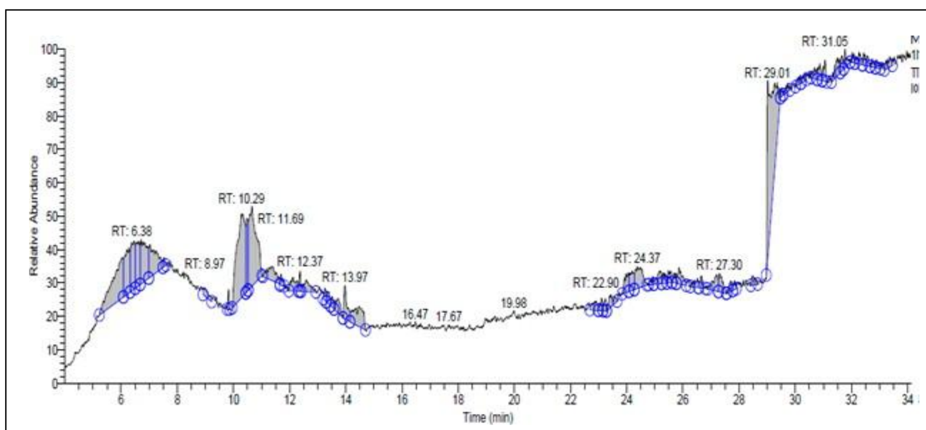

FIG. 7: GC-MS CHROMATOGRAM OF COMPOUND C

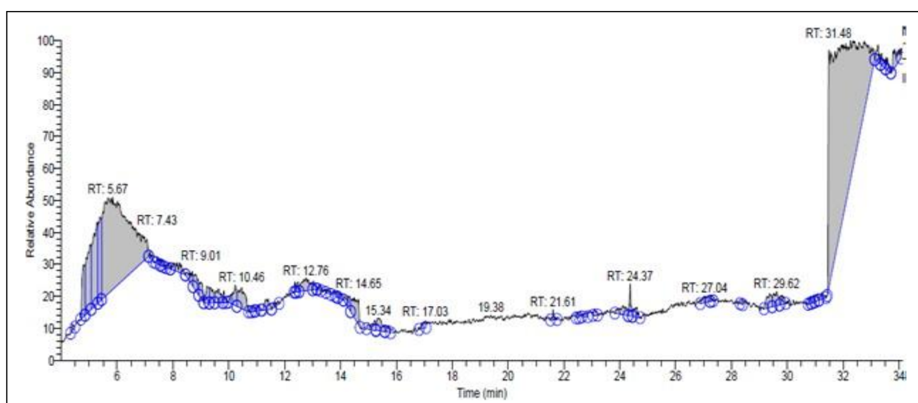

FIG. 8: GC-MS CHROMATOGRAM OF COMPOUND D

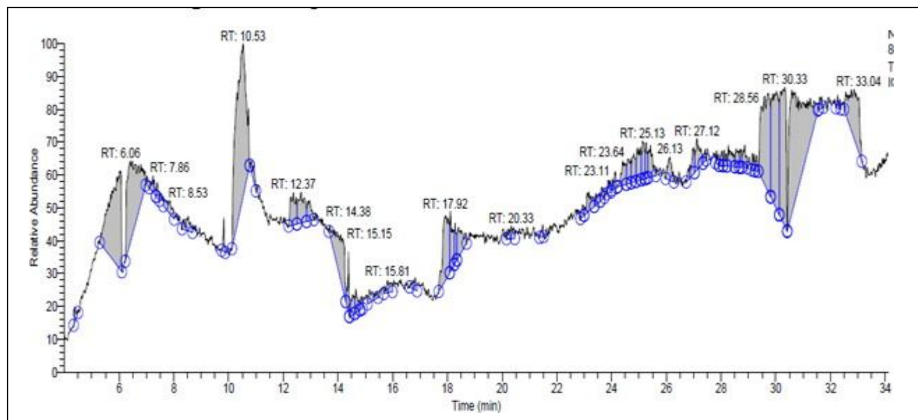

FIG. 9: GC-MS CHROMATOGRAM OF COMPOUND E

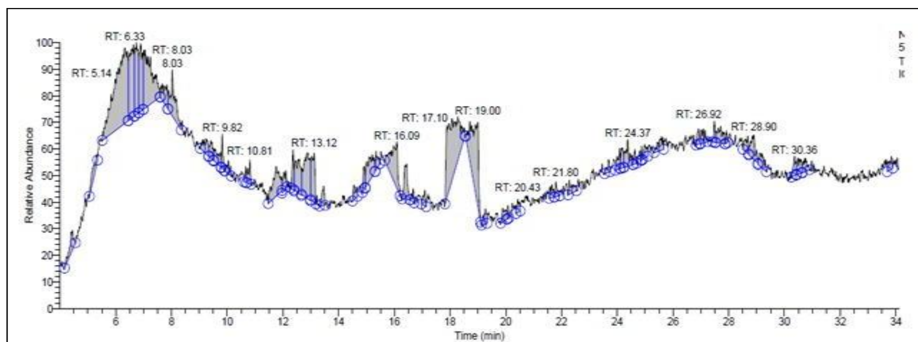

FIG. 10: GC-MS CHROMATOGRAM OF COMPOUND F

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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. Theexistence 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.

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