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Qualitative analysis and evaluation of secrets present in the majestical *Diospyros malabarica* fruit extracts

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Abstract:

A member of the Ebenaceae family, *Diospyros malabarica* (Deshi Gab) relieves inflammation, toothaches, infection (bacterial and fungal), hypercholesterolemia, and other diseases. *Diospyros malabarica* fruit extracts were screened for phytoconstituents using numerous analytical methods including FTIR, UV, HPLC, AAS, density, polarimetry, refractive index, TLC, and qualitative and quantitative phytoconstituent screening. D. malabarica fruit extracts were produced in n-

hexane, methanol, and chloroform. The greatest fluorescence of powdered medicinal material was seen in methanolic extract. FTIR detected hydrocarbons, whereas UV and HPLC detected active components having pharmaceutical activity. Atomic absorption revealed Mn, Cu in all three extracts, Cd in methanolic and n-hexane, and Ni exclusively in methanolic. It was found to be 1.334 in water, 1.368 in methanol, 1.361 in n-hexane, and 1.354 in chloroform extracts. Extracts from methanol and chloroform were dextrotatory, whereas n-hexane was levorotatory. There were no alkaloids or triterpenoids found in any of the three Diospyros malabarica extracts either qualitatively or quantitatively. This fruit's methanolic extract has maximum phenolic content of 97.00.91g GAE/mg and flavonoid content of 970.8g QE/mg, respectively. This work will aid in future antibacterial, antifungal, alpha amylase inhibitory, and antioxidant investigations.

Keywords: *DIOSPYROS MALABARICA,* Florescence analysis, FTIR, UV, HPLC, AAS, TLC, Density, refractive index, polarimetery

1. Introduction:

In nature, medicinal plants have a great source of therapeutic compounds since dawn of civilization to cure ailments (Oli and Gautam 2022). The use of plants and plant-derived products as medicine has been a popular tradition as the beginning of human civilization. Plants are considered as the richest source of traditional and modern medications as well as food supplements, pharmaceutical intermediates and chemical entities for synthetic drugs (Petrovska 2012). In prehistoric age, man sought to alleviate his pain or treat his illnesses through the action of bioactive compounds present in plants, although in an intuitive way based on random discoveries (da Silva Martins *et al.* 2022). Such secondary metabolites of medicinal plants have proven to have antimicrobial activity due to the action of their bioactive compounds (Fernández *et al.* 2020). The extraction of bioactive compounds is an especially important step, not only for the separation of compounds, but also during the analysis of solid materials (Martins et al, 2022). Extracts of plants are widely used in formulation of medicines to cure ailments from headache to cancer (Yassine *et al.* 2020). Medicinal plants extracts are used as medicines from 100 of years and it is difficult to produce synthetically. Different parts of plants (roots, leaves, fruits, stem, and flowers) are used to treat ailments in terms of Ayurveda, Homeopathic, Unani treatment plans which categorize significance of pharmacological activities (Koehn and Carter 2005, Beghyn *et al.* 2008, Hunter 2008). Diospyros consist of large genus mainly having trees and

shrubs, belongs to family Ebenaceae, bark is febrifuge, bitter and astringent (Jadhav 2008). In current study *Diospyros malabarica* is focused due to its medicinal properties for analytical studies which belong to genus Diospyros (Kaushik *et al.* 2012).

Various parts of *Diospyros malabarica* are used for wood carvings, furniture, boats making and construction purposes. Air dried wooden density of plant is 0.70-1.12 gram/cm, which make it strong,tough and the palatability increases when fruits ripe. Tannin present in the immature fruits can be used for staining. Chronic dysentery and diarrhea can be treated with its seeds (Ravikumar *et al.* 2014). It is considered to be medicinally important because different parts of it, for instance-bark, showed anti-diabetic. (Mondal *et al.* 2006). Previous studies also reported that D. malabarica is traditionally used for the treatment of ulcer, dysentery, intermittent fever and irregularities in the menstrual cycle (Yoshihira *et al.* 1967, Mondal *et al.* 2006).

Since extensive studies on analytical evaluation of D. malabarica fruits have not been carried out yet, different parts of D. malabarica fruits may contain pharmacologically active phytoconstituents with excellent antioxidant propensity. Herein, we prepared D. malabarica fruit extracts to extract both polar and nonpolar phytochemicals. The phytochemicals of the extracts was investigated by following multiple analytical techniques such as; Florescence analysis, FTIR, UV, HPLC, AAS, TLC, Density, refractive index, polarimetery and density. The main aim of analytical evaluation is the provision of reproducible and reliable outcomes that are suitable for the desired purpose in a chosen analytical method.

2. MATERIALS AND METHODS:

2.1 Plant Material

The fresh fruits of *Diospyros malabarica* were gathered in the long stretch of January from the green-belt, University of Punjab, Lahore, Pakistan, whereby it was developed for decorative aims. The plant was identified by the Prof. Dr. Zaheer U Din Babar from Department of Botany, Govt. College University Lahore, Pakistan. The voucher number was GCU-BOT-HERB-3470, and it was given to the plant after validation. The plant sample was retained in the herbarium of Department of Botany, Govt. College University (GCU), Lahore, Pakistan.

2.2 Preparation of Extracts

The extraction of extracts from *Diospyros malabarica* was carried out by cold maceration under the predefined conditions. The fruits of *Diospyros malabarica* were taken and washed with cleaned water to expel all residue particles from the fruits. Fruits were disinfected by dipping

into 70% ethanol for one minute, and after that spread in laminar flow chamber which are already sterilized to completely evaporate the ethanol. The plant material was grinded by utilizing grinder. After drying, plant material was soaked for 7 days in different solvents of analytical evaluation of increasing polarity for example n-hexane, chloroform and methanol. After complete soaking process, the plant material was filtered by using Whatman filter paper (No. 1). The filtrate was added to rotatory evaporator under low pressure. The extract free of solvent was refrigerated at 2-4°C in screw scratched sterile containers for further use.

2.3 Fluorescence analysis:

Florescence method was used and evaluated at both sunlight and UV by treatment with multiple reagents. Sterilized test tubes were taken and fresh dried leaves powder was used in it. 1 gram powder was added in test tube and final volume was made up. UV short and long wavelength and visible lights were used for observation (Krüger and Schulz 2007).

2.4 Fourier transforms infrared spectroscopy (FTIR):

Functional group from the dilutions were identified by using FTIR with ATR (attenuated total reflection), the sample was scanned 10 times from the wave number range 600-2000 cm^{-1}. Sample was directly placed on the ATR plate (Krüger and Schulz 2007).

2.5 Ultraviolet – Visible Spectroscopy (UV):

The absorption maximum (ʎ max) of Diospyros malabarica was determined by scanning the working test solution in UV ranges, from 400-200 nm, against their respective blanks. Sample was subjected to Ultraviolet – Visible Spectroscopy to find the wavelength of absorption (Murugan and Mohan 2014).

2.6 High performance liquid chromatography (HPLC):

Took 5ml of sample and diluted it to 10ml with methanol. Sonicate it for 2 minutes after vigorous shaking. Used 0.45-micron filter paper to filter the sample solution and inject volume 20 µL into HPLC by following isocratic mode (Krüger and Schulz 2007).

2.7 Atomic absorption spectroscopy (AAS):

1 gram sample powder was taken and added 7ml nitric acid in it and allowed to stand for 10 minutes. Then added 3ml perchloric acid and heat this on digestion block heater at 150 Celsius for 30 minutes. Then raised the temperature to 250 Celsius for 20 minutes till the crystal clear/wine green color appeared and volume makeup was made 25ml with distilled water.

Digested sample was estimated on atomic absorption spectrophotometer for heavy metal detection (Krüger and Schulz 2007).

2.8 Refractive index

Refractive index widely known as index of refraction is a percentage of the twisting of a beam of light entering from one medium onto another. The refractive index of the working test solutions of concentration 10µg/ml was estimated (Krüger and Schulz 2007, Murugan and Mohan 2014).

2.9 Polarimetry:

To check whether compounds were optically active or not sample solution at concentration of 10µg/ml were added to polarimeter (Krüger and Schulz 2007).

2.10 Density:

Pycnometer was filled with distilled water weighted than the test sample was added and weighted again. Repeat the process for the sample with indefinite and unknown density (ρ) and conclude its weight m_L (measured weight - weight of empty pycnometer) (Krüger and Schulz 2007).

$$\rho_L = \frac{m_L}{m_{H_2O}} \times \rho_{H_2O}$$

Equation 1. Formula equation for density of a liquid

Where: -

m_{H_2O} is practically obtained mass of water (Empty pycnometer weight is subtracted from it). A proportional relation that concludes the density of estimated liquid (ρ)

2.11 Thin layer chromatography:

The plates were developed using methanol (M), ethyl acetate (EA) and n-hexane (N) and acetonitrile (ACN) in different ratios (M-ACN 3:7, N-EA 7:3, N-EA 1:9, M-ACN 7:3, N-EA 5:5 and M-ACN 7:3) as the mobile phase.

2.12 Qualitative and Quantitative phytoconstituent screening:

The differential qualitative and quantitative chemical tests can be performed aimed at establishing a profile of given plant extract for its composition. The subsequent tests may be performed on extracts to detect various phytochemicals present in them. Phytochemical tests were performed in the pharmacognosy department, faculty of pharmacy, The University of Lahore (Tüzen 2003, Verma and Trehan 2013).

2.12.1 Detection of Alkaloids

a) Mayer's Test

added one or 2 drops of Mayer's reagent from either side angle of test tube with few milliliters of test sample.

b) Wagner's Test

A few drops of Wagner's reagent were added to the filtrate. A positive test confirmed reddish brown precipitates.

c) Hager's Test

Infiltrate 2 milliliters of test solution with few drops of Hager's Reagent. The positive test was indicated by presence of white precipitates (Parker and Parker 1924).

2.12.2 Detection of Carbohydrates

Plant extracts were mixed with 5ml water and filtered. The filtrate was used for tests:

a) Molisch's Test

Alpha naphthol 2 droplets alcoholic solution was added to 2ml of test solution and shaken well than along the side of test tube mix slowly 1ml of sulphuric acid, violet ring appeared indicated presence of carbohydrates.

b) Fehling's Test

Boil 1ml of test solution on a water bath, then add 1ml each Fehling solution A and Fehling solution B appearance of red coloured precipitates showed that sugar was present.

***Fehling's solution A*:** copper sulphate 34.66g was mixed in few ml of deionized water and final volume was makeup.

***Fehling's solution B**:* Sodium hydroxide 50g and potassium sodium tartarate 173g was mixed in few ml of water and final volume was makeup.

c) Benedict's Test

0.5ml of test solution was mixed in 1ml of Benedict's reagent. The sample test mixture was heated for 5 minutes on boiling water bath. Characteristic coloured appearance indicated that sugar is present (Raaman 2006).

2.12.3 Detection of Glycosides

50mg of rotarized plant extract was hydrolyzed for 60 minutes with conc. HCl, filtered and was used for following tests.

a) Borntrager's Test

Test sample 2ml add 3ml of $FeCl_3$ and shake it well separate layer of chloroform is formed than add 10% solution of ammonia appearance of pink color indicated the presence of glycosides.

b) Legal's Test

In pyridine dissolve 50mg plant extract, 10% NaOH was used to made solution alkaline after addition of sodium nitro prusside solution. Appearance of pink colour indicated presence of glycoside (Raaman 2006).

2.12.4 Detection of Saponins

a) Froth Test:

The 50 mg rotarized extract was diluted with deionized water and completed at 20 ml. The sample dilution was stirred in a measuring cylinder for 20 minutes. A foamy layer indicated presence of saponins.

b) Foam Test

In 2ml of water extract of 0.5g was rigorously stirred, if foam layer formed remains for 10 minutes indicates the presence of saponins (Raaman 2006).

2.12.5 Detection of Proteins and Amino Acids

The 100 mg rotarized plant was mixed in 10 ml of distilled water and filtered was tested for proteins and amino acids**.**

a) Xanthoproteic Test

Some drops of conc. HNO_3 was mixed in *Diospyros malabarica* fruit extracts, formation of yellow colored indicated that proteins were present.

b) Ninhydrin Test

2 droplets of 0.25% w/v Ninhydrin preparation were added plant extract. Characteristic purple color showed presence of amino acids (Raaman 2006).

2.12.6 Detection of Phyto-sterols (Liebermann – Burchard's Test)

Extracts of *Diospyros malabarica* fruit were mixed with chloroform and was filtered. Then 2ml of acetic anhydrates was added in the filtrate, it was then boiled and cooled. Few drops of conc. H_2SO_4 was added on the edges of the test tube. Brown color in the middle indicated that phyto-steroids were present (Raaman 2006).

2.12.7 Detection of Fixed Oils and Fats (Spot Test)

A small fraction of extract was squeezed in two papers. Presence of oil on the paper indicated the presence of fixed oil (Raaman 2006).

2.12.8 Estimation of Tannins and Phenolic Compounds

a) $FeCl_3$ Test

In 2 milliliters of acidic *Diospyros malabarica* fruit extracts add few droplets of nonpartisan 5% ferric chloride solution. A dark blue shading showed the presences of phenolic mixes.

b) Lead Acetate Test

In hydrated extracts of *Diospyros malabarica* fruit add 3 ml of 10% lead acetic acid. A cumbersome white hastens showed the proximity of phenolic mixes (Raaman 2006).

2.12.9 Detection of Gum and Mucilage:

100mg extract was liquefied in 10 ml water and added with a steady ringing to this 25 ml absolute alcohol. The presence of mucilage gums was reported as white or cloudy precipitation.

2.12.10 Test for Steroids and Triterpenoid:

a) Salkowski's Test

In a test tube *Diospyros malabarica* fruit extract was mixed with 2 milliliters of chloroform, concentrated sulphuric acid was added by the walls of test tube carefully. Formation of reddish brown layer in the junction showed of steroid ring.

b) Libermann Burchard's Test

In a test tube chloroform mixed *Diospyros malabarica* extract add few drops of acetic anhydride, cool it after boiling and upon addition of conc. sulphuric acid brown colored ring formed at the interfaces (Tiwari *et al.* 2011).

2.13 Estimation of crude contents

Plants as a defensive mechanism against pathogens etc. produces many types of chemicals called phytochemicals. These chemicals in humans have special properties like antioxidant, antimicrobial DNA replication, and activation and deactivation of enzymes (Kumar *et al.* 2014). Wide range of bioactivities are expressed by these phytochemicals namely flavonoids, tannins, alkaloids, glycosides and many more (Tiwari *et al.* 2011). Different phytochemical screening methods have been applied for of medicinal herbs like total phenolics content and total flavonoids content assays to enumerate flavonoids and phenolics present in the plant (Monisha *et al.* 2017).

2.13.1 Estimation of total phenolics content (TPC)

Stock solution of extracts was prepared at concentration of 4 mg/ml dimethyl sulfoxide (DMSO). Stock solutions which were freshly formulated include: Folin-Ciocalteu (FC) reagent (10%), prepared in distilled water, positive control was 1mg gallic acid/1000 µl DMSO and 6% (6g in 100ml distilled water) sodium carbonate solution. A well stated protocol described by (Halliwell 2007) was used for the estimation of total phenolics content in test extracts. The positive control was gallic acid which was used at concentration of 3.12, 6.25, 12.5 and 25 µg/ml. Test dilutions (20 µl) were added to particular 96 well plate subsequently after addition of 0.009 µl FC reagent (10%). The reaction mixture was allowed to stand for 10 mins at 37°C. 0.009 ml of Na_2CO_3 dilution was than added. This reaction mixture was incubated at 37^{o}C for half an hour in the incubator. Now, the absorbance was estimated by using microplate reader at 630 nm. A calibration curve was drawn. The results were expressed as µg gallic acid equivalent (GAE)/mg extract.

2.13.2 Estimation of total flavonoids content (TFC)

The stock solutions which were used for this assay were prepared freshly and comprised of 10% aluminum chloride (10g in 100 ml DW), 1M potassium acetate solution (9.82 g of potassium acetate in 100 ml DW). Quercetin (4mg/ml was prepared in DMSO) acts as a standard. The test extract solutions were prepared at concentration of 4 mg/ml in DMSO. A well stated procedure was was performed for the estimation of total flavonoids in test samples (4.0 mg per ml of DMSO). Reaction mixture was allowed to stand for half an hour at 25°C. Now, the absorbance was measured by using microplate reader. The calibration curve was drawn. The results were expressed in µg quercetin equivalent (QE)/mg extract of plant (Joseph and Raj 2010).

2.13.3 Estimation of crude fiber

The powder of the air dried fruit of *Diospyros malabarica* was processed with the 1.25% aqueous sulphuric acid which is trailed by 1.25% sodium hydroxide arrangement in a clean disinfected china dish and residue was washed with water. Weight the residues and burn them at 550-650°C in furnace until white ash produced (Angelova *et al.* 2008).

$$crudefiber = weig\square tlossonignition\ (g)\ * 100$$

2.13.4 Estimation of crude fat

1 g of fresh powdered fruit were taken and homogenized with 20 milliliters of chloroform and 10 ml of methanol. The mixture was kept on an orbital shaker for 15-20 minutes at room

temperature. The mixture was filtered. The residue was weighed and the lipid content expressed in mg / g of powder was calculated (Angelova *et al.* 2008).

2.13.5 Total protein content

2.50 g of plant was macerated with 0.1ml of double distilled water containing few droplets of Triton-X for 1 day at 37°C. The mixture was filtered and the filtrate was centrifuged at 2800 rpm for 25 min. An aliquot of 0.20ml supernatant was mixed with 0.80 ml of deionized water, 4ml of reagent C (60 ml reagent A: 3% sodium carbonate in 0.10 N NaOH and 1000ul of reagent B: 0.80% $CuSO_4$ n 2% potassium sodium tartrate and 0.30 ml of Folin Ciocalteu reagent and incubated at 28˚ for 40 minutes. The absorbance was recorded with coloured complexation at 600 nm in contrast to a blank consisting all the components except the dilution. The positive control consists of Bovine serum albumin (0.01-100mg/ml). The proteins were estimated from linear regression equation of the standard curve (Fatima *et al.* 2015).

2.13.6 Estimation of total carbohydrate

The value of total carbohydrate was expressed as 100-(sum of % age ash, lipids, moisture and protein) (Khan *et al.* 2015).

3. Results

3.1 Fluorescence analysis

The fluorescence produced by the powder of *Diospyros malabarica* fruit upon treatment with various chemicals and solvents is given in Table 1a, 1b and 1c. The collective results of fluorescence showed that the fluorescence of the powdered drug material in NaOH, $FeCl_3$ and methanol is maximum so the sequential studies infers that the methanolic extract should be used for analytical and biological activities.

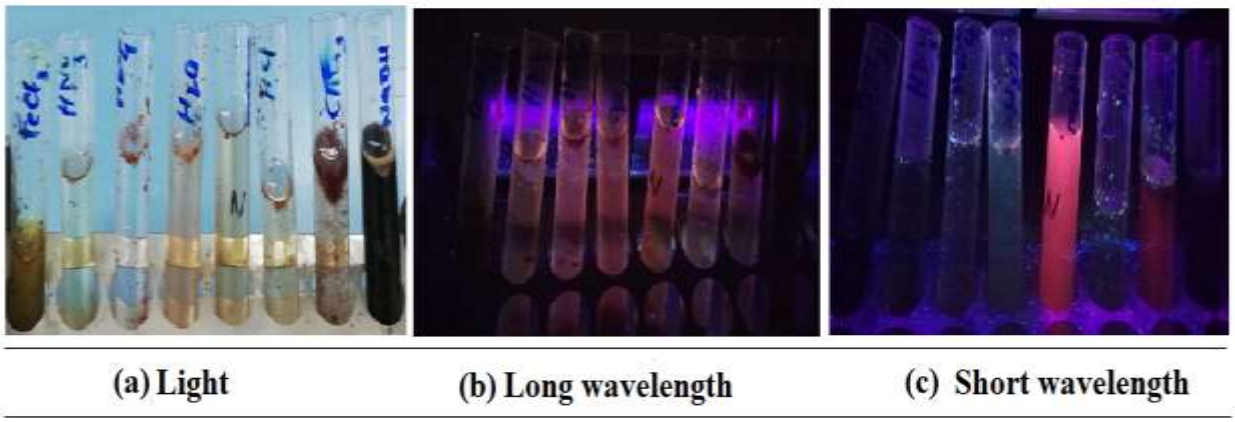

Figure 1. Fluorescence analysis of powdered *Diospyros malabarica* fruit

Table 1. Fluorescence properties of *Diospyros malabarica*

Sr. No	Reagent	Fluorescence properties of *Diospyros malabarica*		
		Visible light	Short wavelength (254 nm)	Long wavelength (366 nm)
1	Ferric chloride	Greenish Black	Black	Gray
2	Nitric acid	Light green	Tan	Crystal green
3	Sulphuric acid	No Florescence	Eagle gray	Pink
4	Water	Golden	Metallic Silver	Pink
5	Methanol	Brownish green	Pink	Lilac
6	Hydrochloric Acid	Light green	Shark teal	No fluorescence
7	Chloroform	Brown	Brown	Light maroon
8	Sodium hydroxide	Reddish black	Maroon	Black

3.2 Fourier transform infrared spectroscopy (FTIR)

FTIR spectrum of the compound indicated intense peaks in the region 1650-1495 cm^{-1} at 1552 cm-1 that correspond to the aromatic ring. A characteristic band at 3650-2850 cm^{-1} indicates amide group (Figure 2). So, the powder contains compounds with aromatic rings and amide group parentally in their structure.

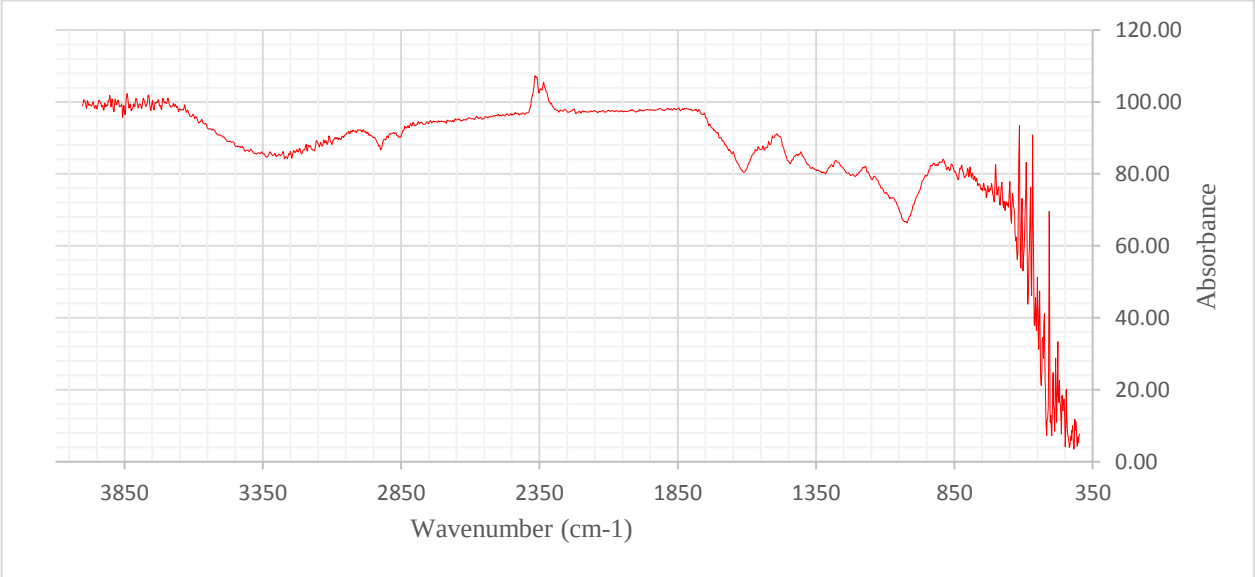

Figure 2. FTIR Spectroscopy of powdered *Diospyros malabarica* fruit

3.3 Ultraviolet – Visible Spectroscopy (UV)

The UV absorption spectra of the working solutions of *Diospyros malabarica*is shown in figure 3(a-c). The spectrum of *Diospyros malabarica*methanolic solution indicated the presence of two peaks showing absorbance maxima at 230 nm and 274 nm.

The UV scan of the n hexane extract solutions of *Diospyros malabarica* indicated the presence of one chromophore showing the absorbance maxima at 210 nm (Table 2). This absorbance behavior suggests that the quantification of the compound could be performed at this wavelength.

The UV scan of the methanolic extract solutions of *Diospyros malabarica* also indicated the presence of one chromophore showing the absorbance maxima at 230 nm (Table 2). This absorbance behavior suggests that the quantification of the compound could be performed at this wavelength.

The UV scan of the chloroform extract solutions of *Diospyros malabarica* also indicated the presence of one chromophore showing the absorbance maxima at 208 nm (Table 2). This absorbance behavior suggests that the quantification of the compound could be performed at this wavelength. The absorbance maxima of the n hexane, methanolic and chloroform solutions of *Diospyros malabarica* via UV spectroscopy are shown in figure 3 (a-c).

Table 2. Ultraviolet absorption spectra of *Diospyros malabarica* fruit extracts

Sr No	Extract	Absorbance maxima	
		230 nm	274 nm
A	(n) hexane extract	210	282
B	Methanolic extract	230	274
C	Chloroform extract	208	273

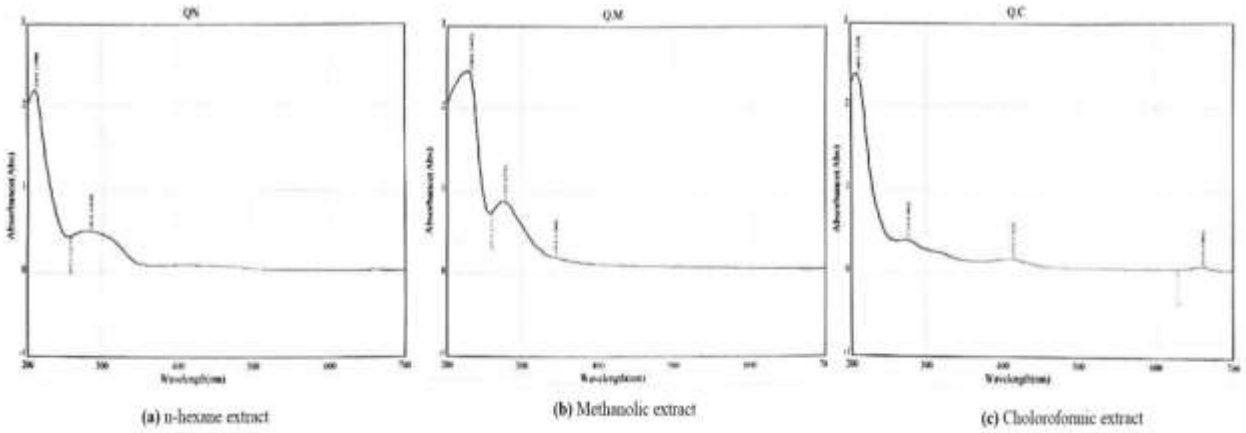

Figure 3. Absorbance maxima of the n-hexane, methanolic and chloroform extract of *Diospyros malabarica*

3.4. High performance liquid chromatography (HPLC)

The HPLC chromatograms of methanolic solutions of *Diospyros malabarica* are given as figure 4-5. A remarkably different peak of methanolic extract solution was observed at 2.256 min., injection volumes of 20 µl were used. These results were consistent with our previous finding on UV profile of *Diospyros malabarica*. The experiments for test dilution and standard solution were run at 370 nm wavelengths in triplicate by using its methanolic solution. The separation of components in the dilution was achieved in the form of different peaks in the chromatogram obtained by using the optimized conditions shown in the figure 4 and figure 5. The HPLC chromatogram of the methanolic extracts of the preparation are shown below

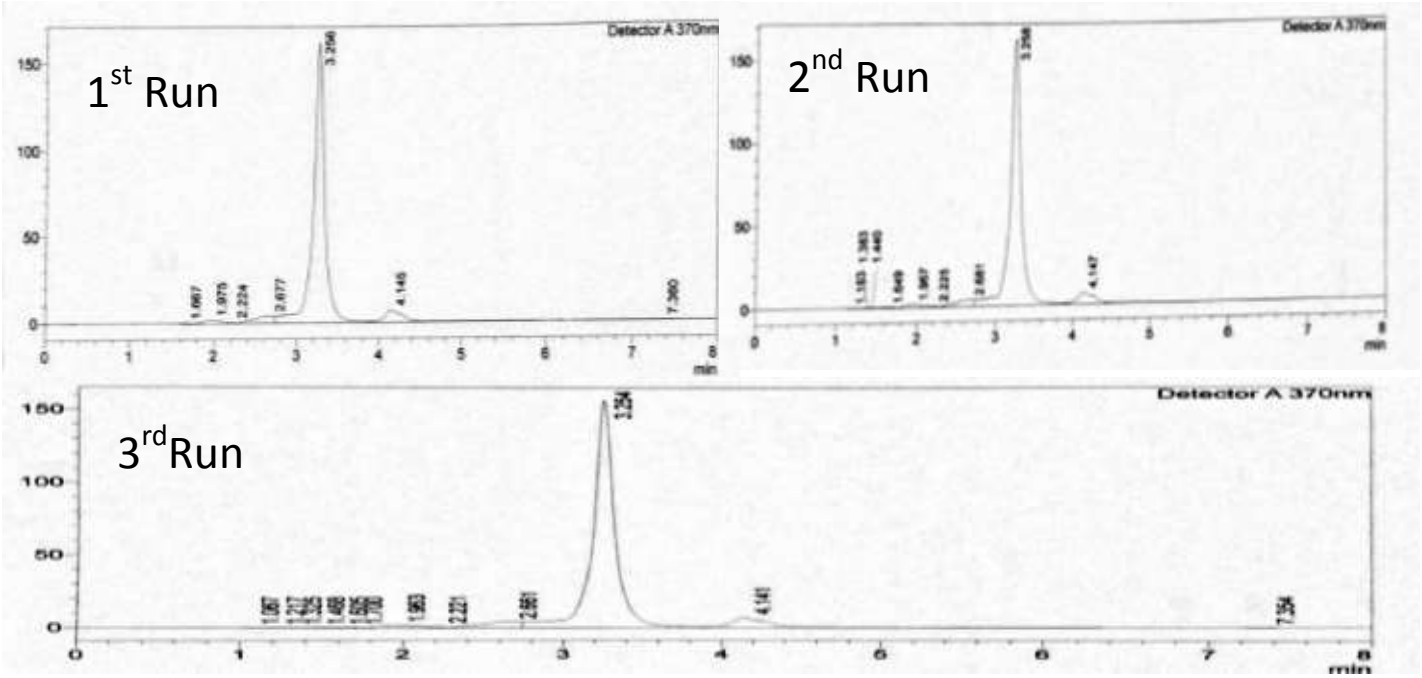

Figure 4. The HPLC Chromatogram of test sample at 370nm

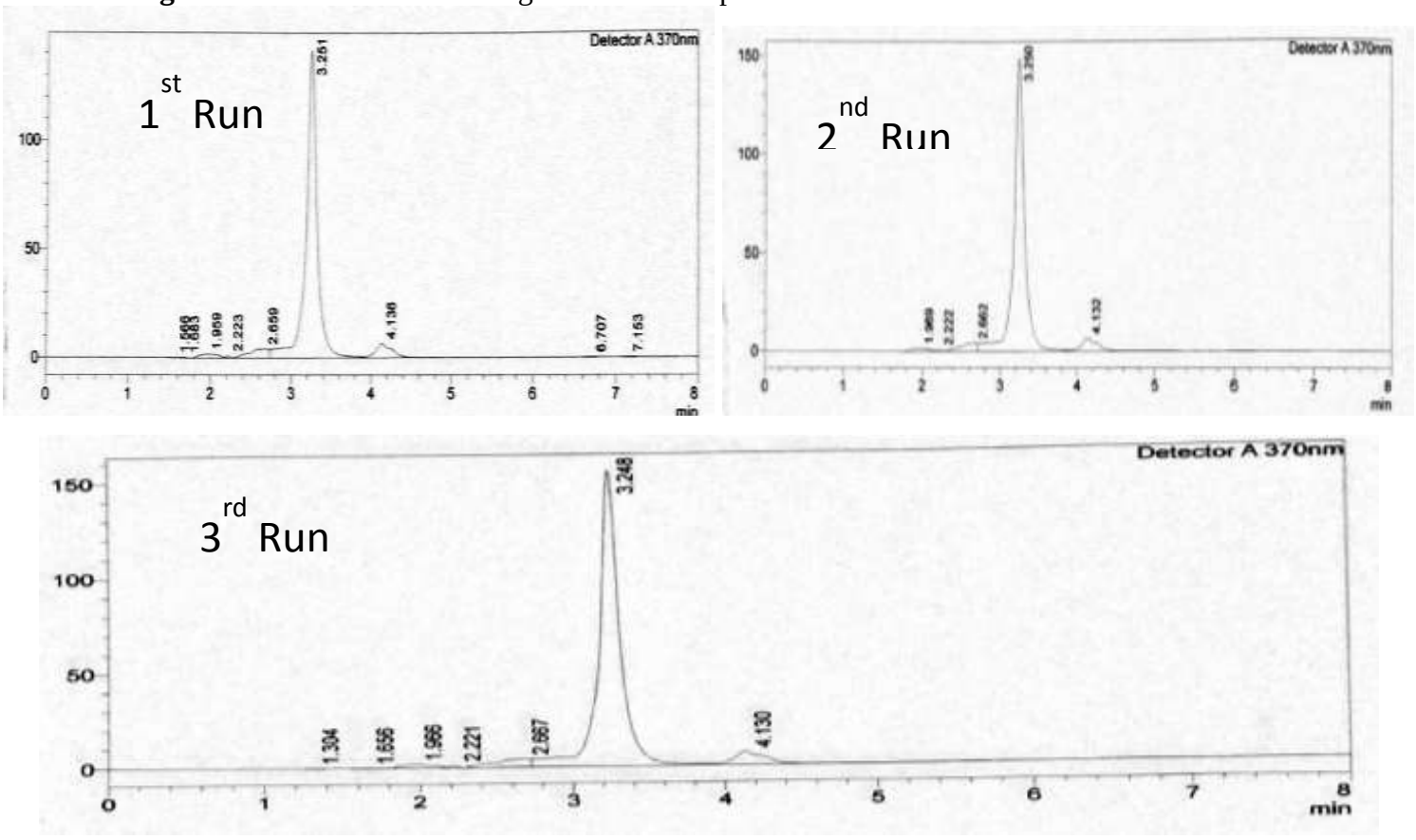

Figure 5. The HPLC Chromatogram of test Quercetin (standard) at 370nm

3.5 Atomic absorption spectroscopy (AAS)

The metal contents in the powder of *Diospyros malabarica* fruit were determined by using atomic absorption spectrophotometer are given in table 3. Atomic absorption showed the presence of Mn, Cu, in all three extracts, Cd was found in methanolic and n-hexane extract and Ni were only present in methanolic extract.

Table 3. Metal contents in the extracts of *Diospyros malabarica* fruit

Sr. No	**Sample Name**	**Mn mg/L**	**Cd mg/L**	**Cu mg/L**	**Pb mg/L**	**Ni mg/L**
1	n-Hexane extract	0.0589	0.0014	0.0614	ND	ND
2	Methanol extract	0.1389	0.0093	0.0427	ND	0.0053
3	Chloroform extract	0.1302	ND	0.0206	ND	ND

ND: Not detected

3.6 Refractive index

Refractive indices of the samples (*Diospyros malabarica*) are given the table 4. Refractive index in water, methanolic, n-hexane and chloroform extracts was detected as 1.334, 1.368, 1.361 & 1.354 respectively.

Table 4. Refractive indices of the extracts of *Diospyros malabarica* fruit

Test compounds	Refractive index
Water	1.334
Methanolic extract	1.368
(n) hexane extract	1.361
Chloroform extract	1.354

3.7. Polarimetry

The test sample extracts; methanolic, n hexane and chloroform extract showed optical rotation at 0.070, -0.088 (Levorotatory) and 0.076 respectively which indicates that the compounds were optically active and methanolic and chloroform extract were dextrorotatory whereas n hexane was levorotatory.

3.8. Density

Density of *Diospyros malabarica* has been illustrated in the table 5 given below

Table 5. Density of *Diospyros malabarica* fruit extracts

Reading no.	Density of water at 25˚C	Density of D.M at 25˚C
1	0.997 g/ml	0.815 g/ml
2	0.997 g/ml	0.814 g/ml
3	0.997 g/ml	0.816 g/ml
Average	0.997 g/ml	0.815 g/ml
S.D	0	0.00178
S.E.M	0	0.00057735

3.9 Thin layer chromatography

Different ratios of ethyl acetate, n-hexane and methanol were required to bring about complete resolution of the product from impurities and the un-reacted dilution. The Methanolic, Chloroform and N-Hexane extracts were best eluted with M-ACN 3:7, N-EA 7:3, N-EA 1:9, M-ACN 7:3, N-EA 5:5, and M-ACN 7:3 dilutions (Figure 6 A-F).

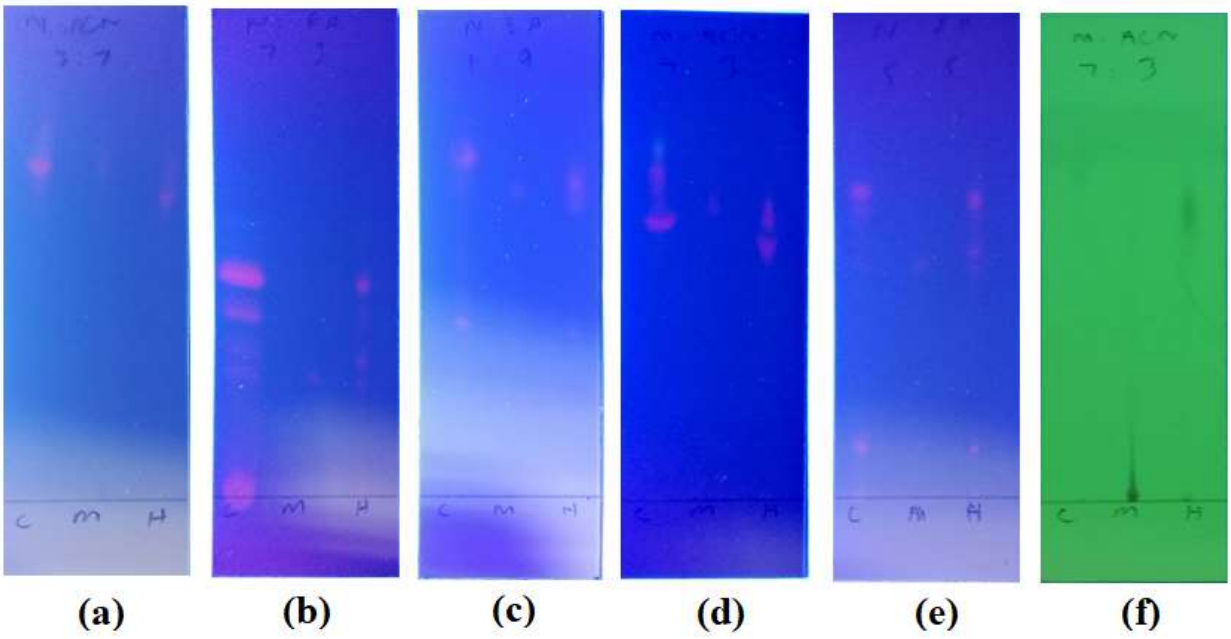

Figure 6 (a-f): Thin layer chromatograms of the extracts of *Diospyros malabarica* fruit.

The Rf values corresponding to the spots of desired products are given in Table 6. TLC of the extracts infers that the methanolic and Chloroform extracts showed the spots in the chromatograms and can further be used in studies.

Table 6. The retardation factors (Rf) of the extracts of *Diospyros malabarica* fruit extracts

Sr No.	Mobile Phase	Ratio	Rf(cm) Chloroform	Rf(cm) Methanol	Rf(cm) N-Hexane
A	M-CAN	3:7	0.79	0.72	0.69
B	N-EA	7:3	0.78	0.69	0.63
C	N-EA	1:9	0.83	0	0.75
D	M-CAN	7:3	0.51	0.26	0.47
E	N-EA	5:5	0.68	0.49	0.64
F	M-CAN	7:3	0	0.26	0.47

Where;

- M= Methanol
- EA= Ethyl Acetate
- N= N-Hexane
- ACN=Acetonitrile

3.10. Phytochemical studies

The phytochemical constituents in the ethanolic extracts of the preparations (*Diospyros malabarica*) are given in Table 7. The three extracts of D. malabarica seed prepared with chloroform, n-hexane and methanol showed the presence of high amount of sterols, tannins, glycosides, carbohydrates, saponins, lipids, gum and mucilage and proteins. On the other hand, Phytosterols, and Alkaloids showed negative results in almost all the three extracts which indicates the absence of these phytoconstituents in the *D. malabarica*.

Table 7. Phytochemical constituents in the chloroform, n-hexane & methanol extracts of the fruit (*Diospyros malabarica*)

Phytochemicals	Tests performed	Chloroform extract	n-Hexane Extract	Methanol extract
Sterols	Salkowaski test	+	+	+
	Liembermann Burchard test	+	+	+
Phytosterols	Liembermann Burchard test	-	-	-
Tannins	Ferric Chloride test	+	+	+
	Lead acetate test	+	+	+
Glycosides	Borntrager's test	+	+	+
	Legal's test	+	+	+
Carbohydrates	Molisch's test	+	+	+

	Benedict's test	+	+	+
	Fehling' test	+	+	+
Saponins	Foam test	+	+	+
	Froth test	+	+	+
Alkaloids	Mayer's test	-	-	-
	Wagner's test	-	-	-
	Hager's test	-	-	-
Lipids	Spot test	+	+	+
Protein	Xanthoproteic test	+	+	+
	Ninhydrin test	+	+	+
Total Crude Contents				
Total crude fiber		8 %		
Total crude fat		9%		
Total protein content		1.62%		
Total carbohydrate		83.88 %		

4. Discussion

The present study was carried out to investigate the phytoconstituents and antioxidant potential of *Diospyros malabarica* fruit extracts by using multiple analytical techniques. Qualitative phytochemical analysis was performed on all three extracts of *Diospyros malabarica*, sterols, tannins, glycosides, carbohydrates, saponins, lipids, gum, mucilage and proteins were present in all three extracts, whereas alkaloids and triterpenoids were not found in all three extracts. The *D. malabarica* tree grown in Indo-Pakistan has been reported to have antioxidant and numerous therapeutic applications (Bharadwaj *et al.* 2021). FTIR results in our study indicated the presence of hydrocarbons, UV and HPLC illustrated the occurrence of active constituents with probable pharmacological activities as illustrated by (Bharadwaj *et al.* 2021). Atomic absorption showed the presence of Mn, Cu, in all three extracts, Cd was found in methanolic and NH extract and Ni was only present in methanolic extract. Refractive index in water, methanolic, n hexane and chloroform extracts were detected as 1.334, 1.368, 1.361 & 1.354 respectively. D. malabarica extracts are known for restorative benefits. For instance, plant sterols and alkaloids are found to be effective for the prevention of various oxidative stress linked diseases such as chronic inflammation, cardiovascular diseases, cancer and anti-inflammatory activities (Deepa *et al.* 2007, Souto *et al.* 2011, Iqbal *et al.* 2015) and antimalarial (Dua *et al.* 2013) activity. Fluorescence study of the extracts determined here signifies standard parameters to ensure the quality and purity of the extracts (Zhao *et al.* 2011). Tannins are identified to have antiviral (Kumari and Jain 2012), antitumor activities (Kolodziej *et al.* 2005, Kumari and Jain 2012) and antibacterial (Akiyama *et al.* 2001). Saponins are well known for their wide range of

pharmacological and medicinal activities such as antimicrobial, anticancer, anti-ulcer and so on (Ezeabara *et al.* 2014). Furthermore, previous research on *D. malabarica* extract reported the latent bioactivity (e.g., antioxidant activity)(Mondal *et al.* 2006). According to (Hossain *et al.* 2019), antioxidant activity of plant extracts depend on the existence of particular secondary metabolites, such as tannins, flavonoids and phenolics. In one study, biochemical profiling was done on *Phoenix dactylifera* and it was estimated that *Phoenix dactylifera* mucilage could be possibly used as medicinal and pharmaceutical ingredient due to occurrence of bioactive compounds and its physicochemical possessions (Pasha *et al.* 2022). In another study, it was found that D. malabarica fruit is rich in different phytoconstituents with several therapeutic propensities and can be used as a possible source of natural antioxidants with other therapeutic uses including hepatoprotective effectiveness (Shubhra *et al.* 2019). Taken together, since these phytoconstituents are primarily accountable for the antioxidant activity, *D. malabarica* fruit extracts can be an important source of natural antioxidants. *D. malabarica* fruit can further be screened against various disease causing pathogens and can be a potential source of chemical and biologically important drug candidates.

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