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ISOLATION, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF BIOMOLECULES FROM *MADHUCA LONGIFOLIA* SEEDS

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ABSTRACT: Natural herbs have had to show profound action for many years. The phytochemicals present in plants responsible for preventing disease and promoting health have been studied extensively to establish their efficacy and understand their action's underlying mechanism. Such studies have included identifying and isolating the chemical components and establishing their biological potency both by *in-vitro* and *in-vivo* studies in experimental animals and through epidemiological and clinical-case control studies in man. The present work describes "Isolation, Characterization and Biological Evaluation of *Madhuca longifolia* seeds". The seeds were collected, authenticated, shade dried and powdered using a mechanical grinder. The powdered material was extracted by the Soxhletion method by using solvents in increasing order of polarity *i.e*, hexane, ethyl acetate and methanol. The percentage yield of hexane extract was found to be (57.8%), ethyl acetate extract (4.6%) and methanolic extract (18.9%) was respect to the powdered crude drug**.** Each extract was subjected to preliminary phytochemical screening using standard phytochemical methods. The hexane, ethyl acetate and methanol extracts were found to be rich in many phytoconstituents like fatty acids, lipids, terpenoids, saponins, tanins, aminoacids. The structure of the compound was elucidated by NMR spectra (^{1}H NMR) and IR spectra are used to determine the presence of various functional group in compounds. And the compounds of hexane extract were elucidated as Oleic Acid, Stearic Acid and Shpingosine. Screening of biological activities like antioxidant activity and Anti-Cancer activity of different extracts was done by DPPH assay method and using 6 different cell lines, respectively. The *In-vitro* antioxidant activity and anti-cancer activity was observed to be more effective in methanolic extract than ethylacetate extract, Hexane extract and pure compound. Study findings also suggest that phytochemicals may reduce risk of toxic effects. A *Madhuca longifolia* seed has several pharmacological activities and potential to provide Anti cancer activity in methanolic extract of 18.9 % yield.

INTRODUCTION: Plants are considered awesome in cause and were loved as Mother (Goddess). They have assumed a critical job in keeping up human wellbeing and improving the nature of human life for a great many years.

Over the most recent couple of years, there has been exponential development in natural medicine. These medications are gaining fame both in creating and created nations due to their root and fewer symptoms. *Madhuca longifolia* is profoundly viewed as an all-inclusive panacea in ayurvedic medication.

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Madhuca normally known as mahua or butternut tree. It has a place with the family Sapotaceae [1]. It has a critical spot in innate culture. The bark is yellowish dim to dim darker red in shading and smooth inside. The bark is prescribed for mucus and in stiffness bark chips are somewhat warmed and tied on joints. The bark is a decent solution for tingle, growing, breaks and snake-nibble harming. Preliminary phytochemical investigations of stem bark with ethanol, water and chloroform remove demonstrated the nearness of starch, terpenoids, proteins, adhesive, anthraquinone glycosides, heart glycosides, saponins and tannins. Madhuca or the Butter nut tree is a medium to enormous estimated deciduous tree conveyed in Nepal, India and Sri Lanka [2]. *Madhuca longifolia* is an enormous tree, about 17m high with a huge top [3]. Mahua is an enormous, obscure, deciduous tree hovering a great part of the focal Indian scene, both wild and developed. Mahua seeds are of monetary significance as they are the acceptable wellspring of eatable fats [4]. The blossoms have been generally utilized as cooling operator, tonic, sexual enhancer, astringent, demulcent and for the treatment of helminthes, intense and ceaseless tonsillitis, pharyngitis just as bronchitis [5]. *Madhuca longifolia* leaves are expectorant and furthermore utilized for interminable bronchitis and Cushing's malady [6]. The refined juice of the blossom is viewed as a tonic, both dietary and cooling, and furthermore, in the treatment of helminthes, intense and incessant tonsillitis, just as bronchitis. The leaves are applied as a poultice to calm skin inflammation. The ethereal parts are utilized for the treatment of aggravation [7].

1.1 Introduction of *Madhuca longifolia*: *Madhuca longifolia* is an Indian tropical tree found largely in the central and north Indian plains and forests.

1.1.1 Scientific Classification: The botanical name of Mahua is *Madhuca indica* and it belongs to the family Sapotaceae. The synonyms of species are M. Latifolia, Macbride, *Bassia latifolia* Roxb. [(8)]

1.1.2 Useful Parts of Plant: Every part of any plant possesses some medicinal properties, either in small or large proportions. Different parts of a plant often contain different active ingredients, so one part may be toxic and another quite harmless [9].

The plant consists of several parts; they may be classified according to their function. They are root, bark, leaves, flowers, fruits, seeds, oil.

FIG. 1: *MADHUCA LONGIFOLIA*

Traditional uses [10]: Flowers are consumed as raw, cooked to survive. Major quantity of flowers used in preparation of distilled liquors Pest of mahwa tree bark is used to cure fracture of bone Traditionally, *Madhuca Indica* bark has been used against diabetes, rheumatism, ulcers, bleeding, and tonsillitis. The seed oil massage is very effective in alleviating pain. It is also beneficial as a nasya (nasal drops) in diseases of the head due to pitta, like sinusitis. Flowers are used to treat bronchitis, increase production of breast milk [10]. The leaves ash mixed with ghee to make a dressing to wounds and burns. Bark extract used to treat dental problems. Seed fat is used to treat skin problems, headache, laxative, piles. Seed cake as insecticidal and pesticide.

Industrial Application [11]: It can be used to manufacture laundry soaps and lubricants. The fat which is obtained from Mahua fruit oil is used in cooking, frying and manufacturing chocolates. The seed fat has emulsion properties so it is mostly used as an emulsifying agents in a few pharmaceutical industries. *Madhuca Indica* has several pharmacological activities [12-14] and the potential to provide health to society. It is used as Anti-

diabetic, antiulcer, hepato-protective, anti-pyretic, anti-fertility, analgesic, antioxidant, swelling, inflammation, piles, emetic, dermatological, laxative, tonic, anti-burn, anti earthworm, wound healing headache and many more problems. The different ailments treated with these parts include tuberculosis, rheumatoid arthritis, cholera, paralysis, snake-bite, debility, tonsillitis, influenza, piles, arthritic pain, helminthiasis, low semen count, headache, flatulency and infections, besides being used as a blood purifier and as an antidote to poison.

The seeds of the tree contain about 40% pale yellow oil. This oil is used as cooking oil by most of the tribes in Odessa, Chhattisgarh, and Maharashtra. After the oil extraction, the residue is used as fish poison. The Bheel tribe of Madhya Pradesh burns this residual cake inside the room to keep the snakes away. The other uses of Mahua oil are hair oil, skin care, and vegetable butter, and making of soaps. Seed oil is galactogenic (stimulating breast milk), pain-relieving, and vomiting-inducing in action. These are used in pneumonia, skin diseases, and piles [15].

The Project's main aim is to Isolate, Purify and Characterize the Chemical Constituents from the medicinal plant *Madhuca longifolia* (Sapotaceae), Commonly known as Mahwa. Reasons to choose *Madhuca longifolia*: *Madhuca longifolia* is an important medicinal plant used traditionally as well as in modern medicine, Every part of the plant possess some medicinal property, Active constituents present in different parts of the plant; easy available in Telangana and Andhra Pradesh

MATERIALS AND METHODS:

2.1 Plant Identification and Collection: According to the literature review, the plant has important ethnomedicinal uses and active constituents, which are medicinally significant. Therefore, the seed of *M*adhuca *longifolia* was selected for the present study. The plant material was identified and authenticated by Dr . U.V. Mallavadhani, senior principal scientist, Natural product chemistry, CSIR-IICT, Research center, hubsiguda, Hyderabad. The plant was collected from the field area of the Tuticorin. The voucher specimen was stored at IICT for future reference. The plant material (seeds) was shed and dried to avoid the decomposition of bioactive constituents. About 650gms of *Madhuca longifolia* seeds were powdered by pulverizer; thus, the powder was packed in air-tight plastic bags.

2.2 Extraction:

2.2.1 Soxhlet Extraction: In the present work, the extraction of powdered plant material was done by soxhelation process, also known as hot extraction. It is a hot percolation extraction method.

It has three Main Sections:

- A percolator (boiler and reflux) circulates the solvent.
- A thimble (usually made of thick filter paper) that retains the solid to be laved.
- A periodic siphon mechanism empties the thimble.

Soxhlet Extraction [16]: Soxhlet extraction is also known as hot continuous extraction. In this method, a solid material containing some of the desired compounds is normally placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the Soxhlet extractor. The extraction solvent to be used is taken into a distillation flask and the Soxhlet extractor is now placed onto this flask.

The Soxhlet is then equipped with a condenser. The solvent is heated to reflux. The solvent vapour travels up a distillation arm and floods into the chamber, housing the thimble of solid. The condenser ensures that any solvent vapour cools and drips back down into the chamber housing the solid material.

The chamber containing the solid material is slowly filled with a warm solvent. Some of the desired compounds will then dissolve in the warm solvent. When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. The thimble ensures that the rapid motion of the solvent does not transport any solid material to the still pot. This cycle may be repeated many times over hours or days. During each cycle, a portion of the non-volatile compound dissolves in the solvent.

After many cycles, the desired compound is concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled.

2.2.2 Extraction Procedure: From 650gms powdered plant material, 550 gms was taken to carry out the Soxhlet extraction process. Plant material was subjected to Soxhlet extraction by using 3 different solvents hexane (non-polar), Ethyl acetate (medium polar) and methanol (polar) until about 15 cycles are completed with each solvent.

After extraction, the solvent is removed, typically by means of a rotary evaporator, yielding the extracted compound. And their percentage yield is calculated respectively.

Percentage of extract (%) = Weight of extract / Total wt of the compound to be extracted × 100

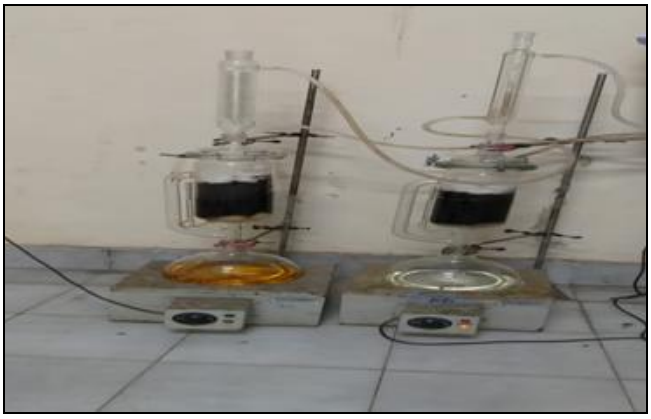

FIG. 2: SOXHLET EXTRACTION

2.3 Chromatographic Material: Silica gel was used for column and thin layer chromatographic (TLC) techniques. Silica gel (60-120 and 100-200 mesh size) for column chromatography. Pre-coated thin layer chromatography (TLC) aluminum plates (Silica gel 60 F254) for TLC analysis.

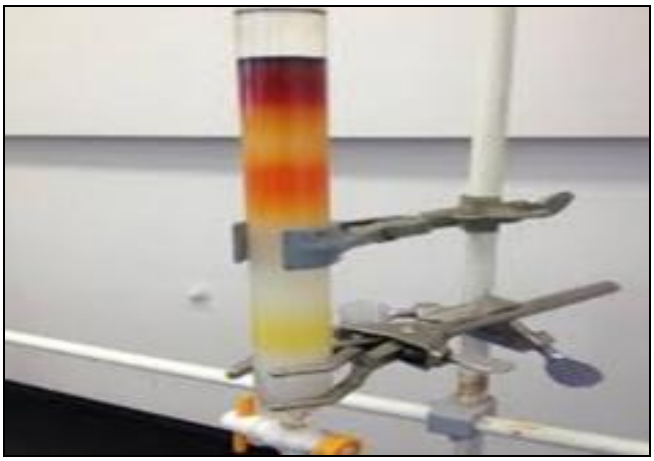

FIG. 3: COLUMN CHROMATOGRAPHY

FIG. 4: DISTILLATION UNIT

Selection of Solvent System: Thus, obtained extracts are subjected to TLC using different mobile phases for eluting the column, it includes

1. n-hexane: ethyl acetate
2. Ethylacetate: benzene
3. Chloroform: methanol
4. Acetone: Hexane
5. Benzene: Hexane
6. Ethyl acetate :methanol:water(1 drop):acetic acid (1-2drops).

Clear demarked spots were observed in hexane extract in hexane: ethyl acetate (20%). Therefore, hexane extract was selected to carry out column chromatography.

2.3.1 Isolation fo Various Phytochemical Constituents morf Hexane Extract: About 50gms is kept for column chromatography using silica gel (60-120 mesh) as an observant and hexane: ethylacetate as mobile phase

The Isolation Process: The column was packed with silica gel adsorbent with the help of hexane solvent by wet packing method. About 50 gms of hexane extract was dissolved in chloroform and adsorbed on 100 gms of silica gel (60-120 mesh).

The slurry was prepared by constant mixing and was loaded onto the prepared silica bed column. Initially, the column was run in pure hexane, and 13 fractions were collected, and then polarity

increased to 5% ethylacetate: hexane from 14th fraction 3 spots were obtained up to 40th fraction. The singlespotis seen in 41-52 fractions. The obtained sample was given for H NMR, IR. The compound was found to be unsaturated fatty acid. Than 5% hexane: ethyl acetate is continued for 53-62 fractions where with minute impurity is seen on TLC along with the same compound of 41-52. Then polarity is increased to 7.5% ethyl acetate: hexane and fractions 63-72 are collected where impurity along a single spot is observed.

Isolation of Compounds: Hexane extract was purified by column chromatography. The column was eluted with hexane and hexane: ethyl acetate mixtures as mobile phase to get oleic acid, Stearic acid and Shpingosine from hexane extract of *Madhuca indica* compounds in pure state. The structure of the compounds was established on the basis of various NMR, Mass spectral data and IR spectroscopy.

2.4 Preliminary Phytochemical Screening of Extracts:

2.4.1 Detection of Alkaloids

A. Dragendorff's test: To 1 ml of test filtrate, two drops of Dragendorff's reagent (Potassium bismuth iodide solution) was added and observed for the formation of prominent reddish brown precipitate.

B. Mayer's test: 1 ml of test filtrate was taken into a test tube and added two drops of Mayer's reagent (Potassium mercuric iodide solution) along the sides of the test tube and observed for white or creamy precipitate.

C. Wagner's test: 1 ml of test filtrate was taken into a test tube, added two drops of Wagner's reagent (Iodine-Potassium iodide solution) along the sides of the test tube and observed for reddish brown precipitate.

D. Hager's test: To 1 ml of filtrate, two drops of Hager's reagent (Picric acid) was added and observed for prominent yellow precipitate.

2.4.2. Detection of Carbohydrates:

A. Molisch's test: 1 ml of the test solution was taken and two drops of alcoholic solution of α–naphthol (Molisch's reagent) was added. The mixture was shaken and 1 ml of conc. H_2SO_4 was added slowly from the sides of the test tube. The test tube were cooled in ice water and allowed to stand. Then the test tubes were observed for violet ring formation at the junction.

B. Fehling's test: 1 ml of test filtrate was boiled in a water bath with a mixture of 1 ml each of Fehling's solutions A and B and allowed to be oil for 1min and observed for the formation of red precipitate.

C. Benedict's test: To 0.5 mL of filtrate, 0.5 mL of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 minutes and observed for the formation of a yellow, green, or red colored precipitate.

D. Barfoed's test: To 1 ml of test filtrate, 1 ml of Barfoed's reagent was added and heated on a boiling water bath for 2 minutes and observed for the formation of a red precipitate.

3. Detection of Proteins and Amino acids

A. Biuret test: To 3 mL of test filtrate, two drops of 4% NaOH were added and treated with two drops of 1% $CuSO_4$ solution and observed for the formation of pink color.

B. Ninhydrin test: To 3 mL of test filtrate, three drops of 5% Ninhydrin reagent were added and heated in a boiling water bath for 10 minutes, and observed the formation of a characteristic purple color.

2.4.3. Detection of Steroids and Terpenoids:

A. Salkowski test: To the test filtrate, 2 ml of chloroform and 2 ml of concentrated sulphuric acid were added, shaken well, and observed the coloration of chloroform and acid layers. Chloroform layer as red in color, and acid layer as greenish-yellow fluorescence.

B. Liebermann – Burchard's test: To the test filtrate, 2 ml of acetic anhydride 2 ml of chloroform were added and heated to boiling and cooled. Then 1 ml of concentrated sulphuric acid was added along the sides of the test tube and observed for the formation of color at the junction.

2.4.4. Detection of Phenolic Compounds and Tannins:

A. Ferric Chloride test: The test filtrates were taken and two drops of neutral 5% ferric chloride solution were added and observed for blue, green or violet color.

B. Lead Acetate test: The test filtrates were taken, and 3 ml of 10% lead acetate solution were added And observed for the formation of bulky white precipitate.

C. Bromine Water test: The test filtrates were taken and 1ml of bromine water was added and observed for discoloration of bromine water.

2.4.5. Detection of Glycosides:

A. Test for Cardiac Glycosides:

Legal test: The test filtrates were taken and added few drops of pyridine and 1 drop of 2% sodium nitroprusside and a drop of 20% sodium hydroxide solution was added and observed for the formation of deep red color.

Keller - Killiani test: The test filtrates were taken and added 2 ml of glacial acetic acid and two drops of 5% ferric chloride solution and mixed. Then 1 ml of sulphuric acid was added. The reddish brown colour appears at the junction of the two liquid layers and upper layer appear bluish green color.

B) Test for Saponin Glycosides:

Foam test: Filtrates were taken and 20 mL of distilled water was added and shaken for 15 min in a graduated cylinder and observed for the formation of a layer of stable foam.

2.4.6. Tests for Fats and Fixed Oils (Petroleum Ether Extract): Fixed oils and fats can be confirmed by chemical tests for glycerin, which is produced by hydrolysis.

Saponification test: Add a few drops of 0.5N alcoholic potassium hydroxide to a small quantity of various extracts along with a drop of phenolphthalein separately and heat on water for 1-2 hrs.

The formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats. Treat 5 drops of the sample with 1mL of 1% copper sulphate solution. Then add 10% sodium hydroxide solution. A clear blue solution is obtained which shows glycerin is present in sample. The cupric hydroxide formed in the reaction does not precipitate out as it is soluble glycerin. To the 5 drops of sample add a pinch of sodium hydroxide sulphate; pungent odor emanate, indicating the presence of glycerin [17].

2.5 H-NMR, C-NMR & Mass Spectroscopy: In ^{1}H-NMR signals, the area under a ^{13}C-NMR signal cannot be used to determine the number of carbons to which it corresponds. This is because the signals for some types of carbons are inherently weaker than for other types – peaks corresponding to carbonyl carbons, for example, are much smaller than those for methyl or methylene (CH_2) peaks. Peak integration is generally not useful in ^{13}C-NMR spectroscopy, except when investigating molecules that have been enriched with ^{13}C isotope [18]. In mass spectrometry, one generates ions from a sample to be analyzed. These ions are then separated and quantitatively detected. Separation is achieved based on different trajectories of moving ions with different mass/charge (m/z) ratios in electrical and/or magnetic fields [19].

2.6 Bio Activity:

2.6.1. Antioxidant Activity: Antioxidant activity of the test compounds was performed using two assays: DPPH assay and ABTS assay [20].

A. DPPH Assay:

Principle: DPPH (1,1-diphenyl-2-picrylhydrazyl) is a nitrogen radical that changes from purple to yellow on accepting electrons. The DPPH scavenging activity measures the reducing ability of antioxidants by inhibiting the pre-formed free radical, which can be calculated by measuring the reduction in the absorbance at 517nm.

Procedure: The compounds were dissolved in DMSO to obtain a stock solution of 10 mg/ml. The following compounds were not soluble: mahua hexane extraxt and compound –I. Assay was performed by adding sample to 0.5 mM DPPH solution and measuring the absorbance at 517nm. Screening was done for all the compounds starting at a concentration of 2 mg/ml and by taking Vitamin C (2 mg/ml) and Rutin (2 mg/ml) as reference standards.

B. ABTS Assay:

Principle: The ABTS assay is based on the principle of the action of antioxidants on a blue /green ABTS radical, reducing it into a colorless compound.

Procedure: The compounds were dissolved in DMSO to obtain a stock solution of 10 mg/ml. The following compounds were not soluble: mahua

hexane extract and compound –I. An assay was performed by adding the sample to 7mM ABTS solution and measuring the absorbance at 734nm. The screening was done for all the compounds starting at a concentration of 2 mg/ml and by taking Vitamin C (2 mg/ml) and Rutin (2 mg/ml) as reference standards.

2.6.2. Antidiabetic Activity:

A. α-Glucosidase Assay:

Principle: The alpha-glucosidase inhibitory activity of the compound depends upon the ability of the compound to block the action of an α-glucosidase enzyme on the substrate p-nitophenyl α-D-glucoside (PNP-Gluc).

Procedure: The compounds were dissolved in DMSO to obtain a stock solution of 10 mg/ml. The following compounds were not soluble: mahua hexane extraxt and compound –I. An assay was performed by adding sample to 0.1 U/ml enzyme and incubating along with 2.5 mM of substrate at 37ºC for 45 min and the reaction was stopped by the addition of 100 mM of sodium carbonate. The absorbance of the final product was measured at 405 nm. Screening was done for all the compounds starting at a concentration of 1 mg/ml and making further serial dilutions. Acarbose was used as a reference standard.

2.6.3 Anti-cancer Activity:

Procedure: Anti-cancer activity of the above-received extracts was performed using 6 different cell lines, including one normal cell line (A549 (Lung Cancer), MDAMB231 (Breast Cancer), U87MG (Glioblastoma), HT29 (colon Cancer) and L132 (Normal cell line). The compounds were dissolved in DMSO to obtain 1mg/ml stock solution. The following compounds were not soluble: mahua hexane extraxt and compound –I.

RESULTS AND DISCUSSION: *Madhuca indica* seed plant powder was subjected to continuous hot extraction with hexane. ethylacetate and methanol solvents. The results of percentage yield, phytochemical screening, column chromatography and biological activities of the extracts were shown below in respective tables.

3.1 Physical Status of Hexane, Ethyl Acetate and Methanolic Extracts of *Madhuca indica:* From the above results obtained summarised in Table 1 the % yield of hexane, ethylacetate and methanolic extracts of *Madhuca indica* was found to be 51.2, 4.07 and 16.8% w/w respectively. The Hexane extract was found to be more than ethyl acetate and methanolic extracts.

TABLE 1: PERCENTAGE YIELD OF HEXANE, ETHYL ACETATE AND METHANOLIC EXTRACTS OF *MADHUCA INDICA*

S. no.	Extract	Method of extraction	Colour	% yield (w/w)	Weight (gm)
1	Hexane	Soxhelation	Yellow	57.8%	318
2	Ethylacetate	Soxhelation	Yellow	4.6%	25.3
3	Methanol	Soxhelation	Brownish black	18.9%	104.4

Percentage Yield of the Isolated Compound from the Seed of *Madhuca indica:*

TABLE 2: PERCENTAGE YIELD OF OLEIC ACID, STEARIC ACID AND SHPINGOSINE FROM HEXANE EXTRACT OF *MADHUCA INDICA*

S. no	Extract	Compound	Wt	Yield (w/w)	Color
1	Hexane	Oleic acid	500mg	3.75%	Colorless
2	Hexane	Stearic acid	2gms	15.8.0%	White powder
3	Hexane	Shpingosine	10mg	1.23%	colorless

TABLE 3: PROPERTIES OF THE ISOLATED COMPOUNDS

Compound	Oleic acid	Stearic acid	Shpingosine
Weight	500mg	2gms	10mg
Solubility	Hexane, Chloroform and Isopropyl alcohol	Hexane, Chloroform and Isopropyl alcohol	Hexane, chloroform, methanal and Isopropyl alcohol
Mobile point	20% Hexane: Ethylacetate	20% Hexane: Ethylacetate	20% Hexane: Ethylacetate
Visibility	anisaldehyde –spray reagent	anisaldehyde –spray reagent	Anisaldehyde –spray reagent
Rf	0.82	0.98	1.2

3.2 Preliminary Phytochemical screening: Preliminary Phytochemical screening includes performing various tests to check the presence or absence of certain compounds and to confirm under which class they belong.

TABLE 4: RESULT OF THE PRELIMINARY PHYTOCHEMICAL SCREENING OF THE HEXANE EXTRACT, ETHYL ACETATE EXTRACT, AND METHANOL EXTRACT OF *MADHUCA INDICA*

S. no.	Chemical constituents	Hexane extract	Ethyl acetate extract	Methanol extract
1	Alkaloids	–	–	–
2	Saponins	–	+	+
3	Terpenoids	–	+	+
4	Steroids	+	+	+
5	Glycosides	–	–	–
6	Fats and oils	+	–	–
7	Tannins	–	+	+
8	Phabotannis	–	–	–
8	Phenols	–	+	+
9	Flavonoids	–	–	–
10	Proteins	–	–	–
11	Aminoacids	–	+	+
12	Carbohydrates	–	–	–

(+) Confirms presence and (-) confirms absence of above shown category of Phytoconstituents Preliminary phytochemical tests of the Hexane extract of *Madhuca indica* showed the presence of following phytochemicals such as Steroids and Fats and oils. Preliminary phytochemical tests of the Ethyl acetate extract of *Madhuca indica* showed the presence of following phytochemicals such as saponins and Steroids. Preliminary phytochemical tests of the Methanol extract of *Madhuca indica* showed the presence of following phytochemicals such as saponins, Terpenoids, Steroids, Tannins, Phenols and Amino acids.

3.3 Spectral Data and Structural Features of Isolated Compounds: Structural elucidation of the isolated compound- Oleic acid.

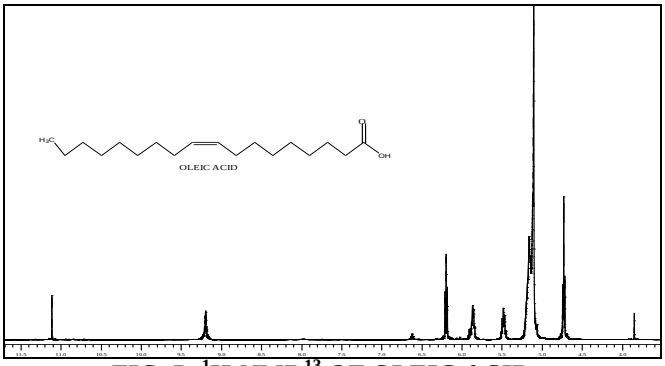

FIG. 5: ^{1}H-NMR^{13} OF OLEIC ACID

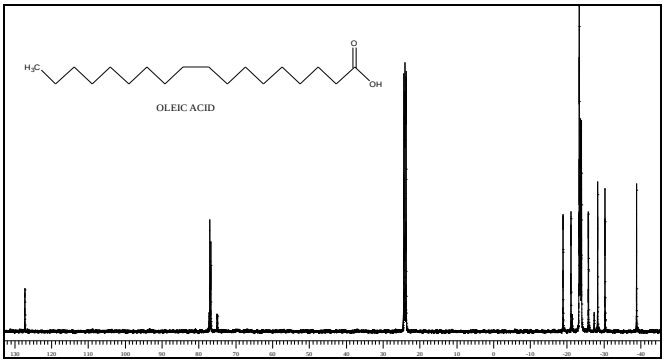

FIG. 6: C-NMR OF OLEIC ACID

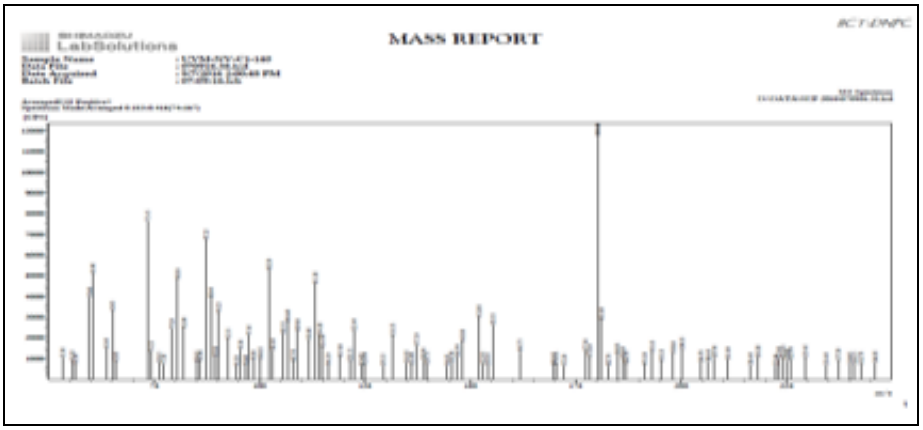

FIG. 7: MASS REPORT OF OLEIC ACID

TABLE 5: PROPERTIES OF THE ISOLATED COMPOUND-OLEIC ACID

Weight	500mg
Solubility	Hexane, Chloroform and Isopropyl alcohol
Mobile point	20% Hexane: Ethylacetate
Visibility	anisaldehyde –spray reagent
Rf	0.82

Structural Elucidation of Isolated Compound: Stearic Acid

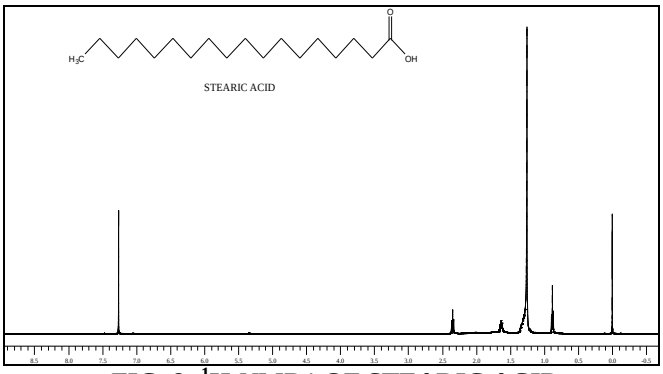

FIG. 8: ^{1}H-NMR1 OF STEARIC ACID

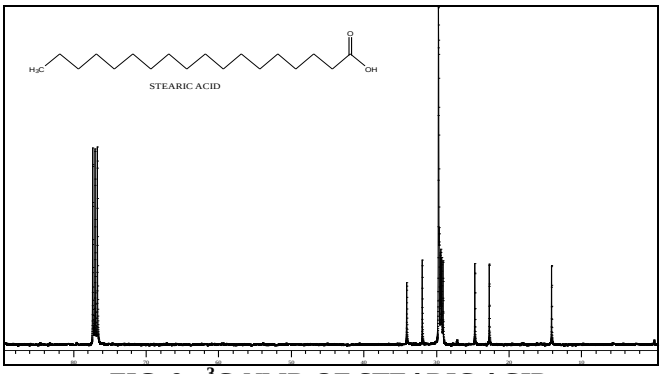

FIG. 9: ^{3}C-NMR OF STEARIC ACID

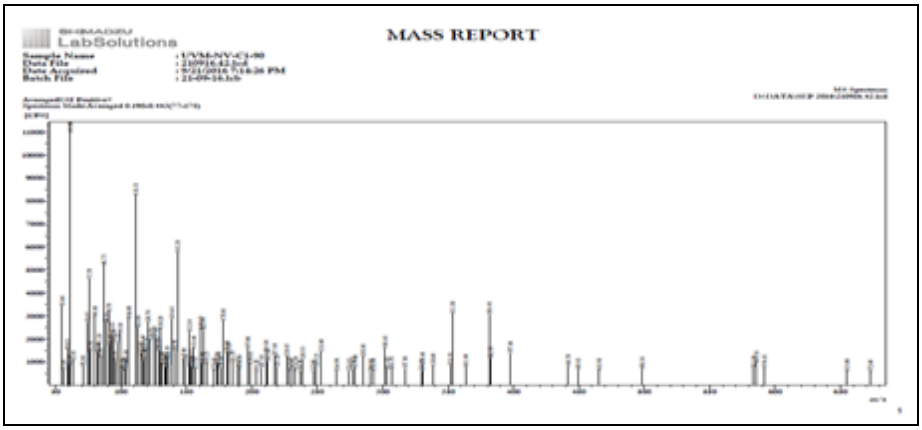

FIG. 10: MASS REPORT OF STEARIC ACID

TABLE 6: PROPERTIES OF THE ISOLATED COMPOUND-STEARIC ACID

Weight	2gms
Solubility	Hexane, Chloroform and Isopropyl alcohol
Mobile point	20% Hexane: Ethylacetate
Visibility	anisaldehyde –spray reagent
Rf	0.98

Structural Elucidation of Isolated Compound Shpingosine:

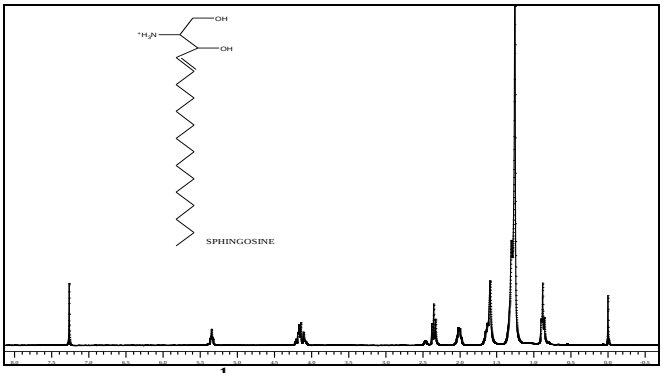

FIG. 11: ^{1}H-NMR1 OF SHPINGOSINE

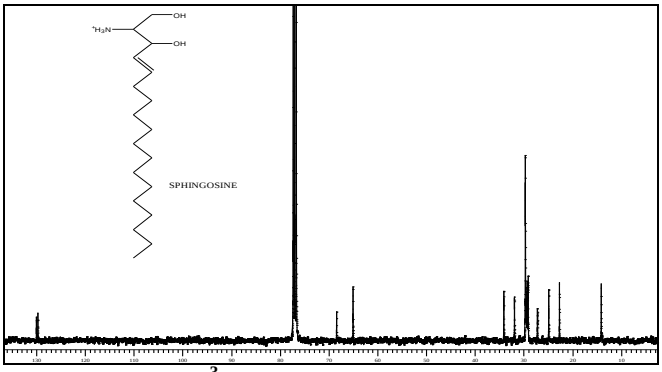

FIG. 12: ^{3}C-NMR OF SHPINGOSINE

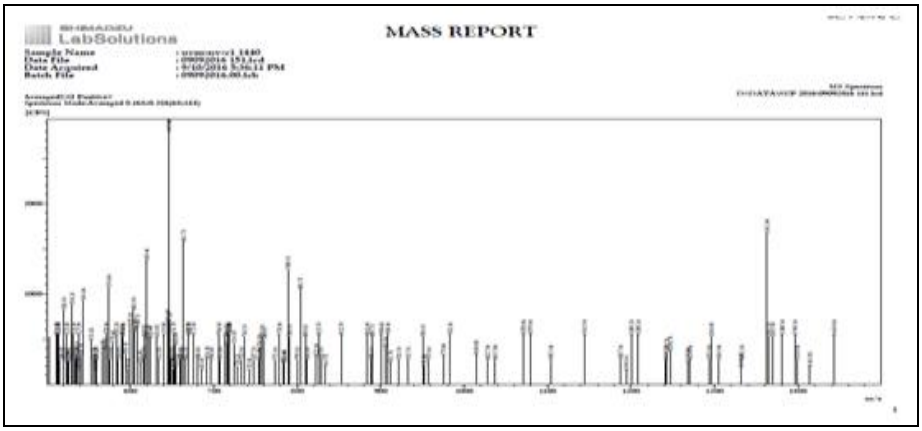

FIG. 13: MASS REPORT OF SHPINGOSINE

TABLE 7: PROPERTIES OF THE ISOLATED COMPOUND-SHPINGOSINE

Weight	10mg
Solubility	Hexane, chloroform, methanal and Isopropyl alcohol
Mobile point	20% Hexane: Ethylacetate
Visibility	Anisaldehyde –spray reagent
Rf	1.2

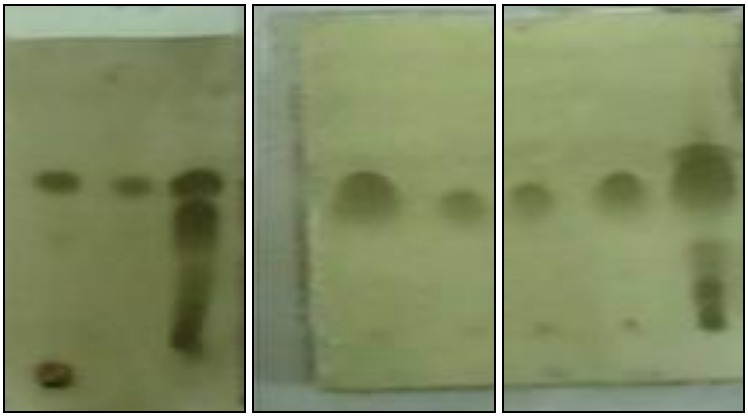

FIG. 14: TLC IMAGES OF OLEIC ACID, STEARIC ACID AND SHPINGOSINE

3.4 Bioactivity:

3.4.1. Antioxidant Activity: Antioxidant activity of the test compounds was performed using two assays: DPPH assay and ABTS assay.

DPPH Assay:

Results:

Table 8: Preliminary screening:

S. no.	Compound (2 mg/ml)	% Inhibition
S1	Vitamin-C	88.67
S2	Rutin	81.21
1	Mahua ethylacetate extract	11.49
2	Mahua methanolic extract	84.83
3	Stearic acid	0.58

Total number of active compounds: 01

TABLE 9: IC_{50} VALUES

S. no.	Compound	IC50 (mg/ml)±SEM
1	Mahua methanolic extract	0.24±0.03
S1	Vitamin C	0.08±0.0001
S2	Rutin	0.14±0.002

B. ABTS Assay: The IC_{50} of all the compounds obtained was tabulated and the results obtained are as follows:

TABLE 10: RESULTS: PRELIMINARY SCREENING

S. no.	Compound (2 mg/ml)	% Inhibition
S1	Vitamin-C	93.71
S2	Rutin	92.50
1	Mahua ethylacetate extract	44.92
2	Mahua methanolic extract	92.10
3	stearic acid	10.73

Total number of active compounds: 01

TABLE 11: IC_{50} VALUES

S. no.	Compound	IC_{50} (mg/ml)±SEM
1	Mahua methanolic extract	0.8±0.006
S1	Vitamin C	0.13±0.001
S2	Rutin	0.05±0.002

3.4.2. Anti-diabetic Activity α-Glucosidase Assay [21]: The results obtained were tabulated and are as follows

Results:

TABLE 12: PRELIMINARY SCREENING

S. no.	Compound (1 mg/ml)	% Inhibition
1	Mahua ethylacetate extract	-0.85
2	Mahua methanolic extract	-7.26
3	stearic acid	27.44
4	Acarbose (2 mg/ml)	71.85

The total number of compounds found to be active: 05.5.3

3.4.3. Anti-cancer Activity [22]:

TABLE 13: THE IC 50 OF ALL THE COMPOUNDS OBTAINED WAS TABULATED AND THE RESULTS OBTAINED ARE AS FOLLOWS

Anti-Cancer Activity (IC_{50} in µg/ml)						
S. no.	**Code**	**HT29**	**A549**	**MDAMB231**	**U87MG**	**L132**
1	Mahua ethylacetate extract	NA	NA	NA	NA	NA
2	Mahua methanolic extract	37.35 ± 0.11	51.5 ± 1.5	39.37 ± 1.67	64.8 ± 1.8	59.1 ± 1.1
5	stearic acid	42.38 ± 1.46	NA	NA	88.7 ± 6.8	89.8 ± 2.9
NA, Not Active; Other compounds are under study						

The total number of compounds found to be active: **1**

CONCLUSION: Medicines derived from plants have immensely contributed to improving human health and act as a source of inspiration for novel drug compounds. According to the Literature review *Madhuca longifolia*, this plant immense potential to be used in the area of pharmacology and as a prospective source of valuable drugs. Due to the presence of various compounds essential for good health, it can also be used to improve the health status of society. The extracts showed a significantly high antioxidant activity. The data clearly depicts the presence of compounds used for treating various bacterial diseases, indicating its use in the traditional system of medicine since ancient times. Further, the broad-spectrum activity of hexane, ethyl acetate, and methanol extracts proves to be encouraging in developing novel anti-cancer formulations shortly.

Antioxidants derived from *Madhuca longifolia* plants possess vast curative properties since they have fewer side effects as compared to synthetic antioxidants. *Madhuca longifolia* is of utmost importance for ethnobotanical purposes. It has been placed in the priority list of medicinal plants by The National Medicinal Plants Board of Govt. of India. The present study contributes to the current knowledge of various phytochemical active compounds of *Madhuca longifolia* possessing significant broad-spectrum antioxidant activity. Further fractionation and purification will elucidate the potential compound, which is a pressing need because of the upcoming resistance of the currently available antioxidants.

The present thesis describes "Isolation, Characterization and Biological Evaluation of*madhuca longifolia* seeds". The seeds was collected, authenticated, shade dried and powdered using mechanical grinder. The powdered material was extracted by Soxhletion method by using solvents in increasing order of polarity i.e, hexane, ethyl acetate and methanol. The percentage yield of hexane extract was found to be (51.2%), ethyl acetate extract (4.07%) and methanolic extract (16.8%) was respect to the dried plant material

Each extract was subjected to preliminary phytochemical screening using standard phytochemical methods. The hexane, ethyl acetate, and methanol extracts were found to be rich in many phytoconstituents like fatty acids, lipids, terpenoids, saponins, tannins, and amino acids. The structure of the compound was elucidated by NMR spectra (^{1}H NMR), and IR spectra were used to determine the presence of various functional groups in compounds. And the compound was elucidated as Oleic Acid, Stearic Acid, and Shpingosine. Screening of biological activities like antioxidant activity and Anti-Cancer activity of different extracts was done by the DPPH assay method. The

in-vitro antioxidant activity using DPPH assay method was observed to be more in methanolic extract than ethylacetate extract. Hexane extract and pure compound.

The extracts were known to be rich in phytochemical constituents; methanol extracts showed better antioxidant activity. According to the literature review, any pharmacologically significant chemical constituents and even new chemical constituents were isolated. Therefore these extracts can be further processed to yield beneficial compounds that show good pharmacological activity.

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CONFLICT OF INTEREST: Nil

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