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TOPIC: *IN VITRO* ANTIOXIDANT, ANTIMICROBIAL POTENTIAL, ANTI DIABETIC AND ANTI INFLAMMATORY OF CRUDE EXTRACTS OF *MALLOTUS RHAMNIFOLIUS.*

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INTRODUCTION

1.1 Medicinal vegetation – records and context

India has a wealthy lifestyle of medicinal herbs and spices from the olden days inclusive of greater than 2500 species and has a tremendous geographical place with excessive ability abilties for unani, siddha, ayurvedic, conventional drug treatments. But only a few species had been studied chemically and pharmacologically for his or her ability medicinal price [1, 2]. Human beings have used vegetation for the remedy of numerous illnesses for hundreds of years [3, 4]. According to the World Health Organization, maximum populations nevertheless trust on conventional drug treatments for his or her mental and bodily fitness requirements [5], considering the fact that they can't purchase the drug treatments of western pharmaceutical industries [6] and that they lack healthcare centers [7]. Rural regions of many growing international locations nevertheless agree with on conventional remedy for his or her healthcare desires and feature determined an area in everyday lifestyles.

1.2 Importance of medicinal vegetation Some medicinal plant compounds have determined use as drug entities or as version compounds for synthesis and semi-synthesis of medicine. A survey of the present day pharmaceutical use found out that, of the entire prescription, 25% are plant derived [20]. Plant molecules are extraordinarily numerous in shape, many are fragrant materials, maximum of which can be phenols or their oxygen-substituted derivatives. There is an extended significance on extracts and biologically lively compounds remoted from medicinal plant species utilized in natural remedy, because of the resistance that pathogenic micro-organisms construct towards the antibiotics and facet outcomes.

1.3 Natural antibiotic homes of plant secondary metabolites

Plant chemical compounds may be categorised as number one or secondary metabolites. Primary metabolites are extensively unfold over in nature, going on in a single shape or another, nearly in all organisms. In better vegetation, such molecules are regularly focused in seeds, bark and vegetative garage organs and are required for physiological improvement due to their significance in fundamental mobile metabolism. Primary metabolites acquired from better vegetation for business use are excessive extent-low price bulk chemical compounds (e.g. vegetable oils, fatty acids, carbohydrates etc.).

1.4 Extraction strategies of medicinal vegetation

Extraction, the time period utilized in pharmaceutically, includes the separation of medicinally

lively quantities of plant or animal tissues from the inactive or inert additives through the use of selective solvents in general extraction tactics. The strategies of extraction of medicinal vegetation are follows:

1.5 Antioxidant ability of medicinal vegetation

Oxidation is a fundamental a part of the everyday metabolic system in residing structures. In the oxidative system, reactive oxygen species (hydrogen peroxide and hypochlorous acid) and plenty of loose radicals (hydroxyl radical (OH) and superoxide anion) are generated [80, 81]. Rapid introduction of loose radicals may also purpose alternate withinside the shape, feature of mobile parts and membranes. It can bring about human neurologic and different problems along with most cancers, diabetes, cardiovascular, neurodegenerative illnesses, inflammatory ailment, asthma, and untimely getting old.

1.5.1 The mechanism of motion of antioxidants Low molecular weight antioxidants (LMWAs) are small molecules that often infiltrate cells, accumulate (at excessive concentrations) in particular cubicles related to oxidative harm, after which are regenerated through the mobile. In human tissues, mobile LMWAs are acquired from numerous reassets.

Initiation step

LH + R· ⟶ L· + RH LH represents the substrate molecule (mlipid), with R· because the beginning oxidizing radical. In oxidation the lipid generates a extraordinarily reactive allyl radical (L·) react with oxygen to shape a lipid peroxyl radical (LOO·). Propagation step L· + O2 ⟶ LOO· LOO· + LH ⟶ L· + LOOH In this response the peroxyl radicals are the chain providers of the response. They oxidizes the lipid similarly to generating lipid hydroperoxides (LOOH), which in flip damage right all the way down to a extensive variety of compounds, inclusive of alcohols, aldehydes, alkyl formates, ketones and hydrocarbons, and radicals, inclusive of the alkoxyl radical (LO·).

Branching step

LOOH ⟶ LO· + HO·

2 LOOH ⟶ LOO· + LO· + H2O The breakdown of lipid hydroperoxides regularly includes transition steel ion catalysts, in reactions much like the ones regarding hydrogen peroxide, yielding lipid peroxyl and lipid alkoxyl radicals. 18

Termination step In termination step reactions contain the aggregate of radicals to shape non-radical merchandise. LO· + LO· LOO· + LOO· LO· + LOO· The number one antioxidants (AH) are affords in hint quantities, it reasons both put off or inhibit the initiation step through reacting with a lipid radical or inhibit the propagation step through reacting with peroxyl or alkoxyl radicals. L· + AH ⟶ LH + A· LOO· + AH ⟶ LOOH + A· LO· + AH ⟶ LOH + A· Preventative antioxidants or secondary antioxidants are compounds that retard the price of oxidation. This can be carried out in some of ways, inclusive of elimination of substrate or singlet oxygen quenching .

2.0 Morphology

Habit: Trees as much as five m tall.

Trunk & Bark

Bark grayish, lenticellate.

Branches and Branchlets

Branchlets terete, stellately tomentose.

Leaves

Leaves simple, contrary to sub opposite, decussate; stipules caduceus; petiole 1-five cm long, plan convex in go section, swollen at each ends, stellate furry; lamina 10.five-23×five.three-thirteen cm, ovate, apex acute-acuminate, base rounded, whole or barely sinuate, yellow-glandular beneath; trinerved at base; secondary nerves ca. 6 pairs; tertiary nerves horizontally percurrent.
Inflorescence / Flower Flowers unisexual, in axillary spikes.

Fruit and Seed

Capsule, 3-valved, minutely stellate furry; seeds three, brown.

Fig:1 Species of Mallotus rhamnifolius

2.1 Review of literature on Phytochemistry with unique connection with Mallotus genus

The genus call is from the Greek word 'Mallotus' that means wooly in connection with the furry leaves, shoots and spiny culmination observed on a few plant life on this genus. They are focused in mountainous regions with an altitude underneath 1,000 m, however a few species can develop at an altitude of 2,000 m, which include Mallotus oreophilus Mull.

2.2 Reports on our centered species of Mallotus rhamnifolius (Willd.) Mull. Arg.

Mallotus rhamnifolius is normally known as as Buckthorn-leaved kamala. In Tamil- Marai-Yirdiyam, in Malayalam pee-tsjerou-ponnagam and Telugu- Konda-Kunkumu .Mallotus rhamnifolius is a small tree as much as five m tall. Bark is greyish, warty. Branchlets are round, velvety. Leaves are simple, contrary to sub opposite, decussate; stipules caducous; leaf-stalk 1-five cm long, plan convex in go section, swollen at each ends, stellate furry.

Taxonomic classification
Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Euphorbiales
Family: Euphorbiaceae

Genus: Mallotus
Species: Rhamnifolius

CHAPTER – III MATERIALS AND METHODS

3.1 Chemicals used

Most of the chemical substances and solvents used on this research had been bought from Merck chemical substances, Siga aldrich, Chemlabs and Sdfine chemical substances. All the chemical substances used on this Test had been > 95% natural or analytical grade.

3.2 Physiochemical evaluation of M. rhamnifolius

3.3 Determination of moisture (loss on drying)

About 1.five g of the powdered leaves became taken in a weighed skinny porcelain box with lid. It became dried it withinside the oven at a 100 °C to 105 °C. Then, it became allowed to chill in a desiccator and the loss in weight became observed.

Calculation of moisture content

Moisture content (%) = (W2 - W3 / W2-W1) × 100

which, W1 is weight of box with lid W2 is weight of box with lid and pattern earlier than drying W3 is weight of box with lid and pattern after drying.

3.4 Determination of ash price

Ash values are very vital in figuring out the nice and purity of crude pills, withinside the powder shape. In this ashing test of natural pills is to do away with all of the natural rely, in any other case it will likely be intervene in an analytical dedication. The overall ash, acid insoluble ash and water-soluble ash values had been decided from air-dried samples.

Principle

Principle of this Experiment is that once a regarded weight of feed is ignited to ash, the load of ash hence acquired is calculated in phrases of percent.

Apparatus

Silica crucible
Tongs
Weighing balance
Electric Bunsen burner
Muffle furnace

Desiccator

Asbestos sheet

A) Total ash price

Procedure

The following steps are worried withinside the dedication of ash price.

1. The weight of the smooth dry cloth in a formerly ignited and tarred silica crucible is found.
2. About 2 g of the air-dried cloth is unfold as a fair layer withinside the crucible.

3. The weighed crucible is cautiously located over an electric powered burner.

4. The crucible is saved partly open.
5. The pattern gets charred with preliminary expulsion of smoke.
6. The silica crucible is ignited via way of means of steadily growing the warmth to 500-600°C and hold for two h, till the cloth turns into white, indicating the absence of carbon. At this temperature, all natural rely may be burnt leaving at the back of minerals.
6. The residue is permitted to chill in a desiccator for 30 min after which weighed with none time gap.

B) Acid insoluble ash

This is acquired after whole combustion of the pattern.

It contains of portions:

1. The element this is soluble in dilute acids carries all crucial minerals and this is the beneficial part of the ash.
2. Other element, insoluble in dilute acids includes specifically sand and silica. For the maximum part, it represents impurity or adulteration.

Determination of acid insoluble ash price

Procedure

The following steps are worried withinside the dedication of acid insoluble ash price. 1. To the crucible containing the whole ash, 25 mL of 2N hydrochloric acid is brought, closed with a watch-glass and boiled lightly for five min.
2. The watch-glass is washed with five mL of warm water and that is brought to the crucible.
3. The insoluble rely is accumulated on an ash-much less clear out out paper and washed with warm water till the filtrate is neutral.
4. The clear out out-paper containing the insoluble topics is transferred to the unique crucible, dried on a warm plate and ignited to consistent weight.
5. The residue is permitted to chill in a desiccator for 30 min after which weighed with out time gap.

The percent of acid-insoluble ash in mg in keeping with g of air-dried cloth is calculated as follows.
Acid-insoluble ash % (w/w) = (B-C)/A × a hundred

A- Sample weight in g
B- Weight of dish + contents after drying (g)
C – Weight of empty dish (g)

C) **Water soluble ash** To the crucible containing the whole ash, 25 mL of water is brought and boiled for five min. The insoluble topics are accumulated in a sintered-glass crucible. They are washed with warm water and ignited in a crucible for 15 min at a temperature now no longer exceeding 450 °C. Percentage of water-soluble ash became calculated almost about air-dried substance. water-insoluble ash % (w/w) = (B-C)/A × a hundred

A is Sample weight in g
B is Weight of dish + contents after drying (g)
C is Weight of empty dish (g)

3.5 Plant series and processing Fresh leaves of M. rhamnifolius (in Figure-1) plant had been accumulated from environment of the Ramakrishna puram (range eight° 18' and eight° 32' N and longitude 77° 02' and 77° thirteen' E) close to Kalakad, located withinside the southern a part of Western Ghats, Tirunelveli district, Tamil Nadu. The accumulated plant substances had been recognized and authenticated for that reason to the Herbarium of French Institute of Pondicherry (specimen No.: HSJCSSR 003). All the accumulated plant substances had been tested for insect harm and fungal infections to do away with bad flowers.

3.6 Hot non-stop extraction (Soxhlet extraction)
Soxhelt equipment
In this technique, eighty g of the pulverized dried leaves are located in a porous bag or "thimble" made from robust clear out out paper, that's located in chamber (three) of the Soxhlet equipment (in Figure-2). The extracting solvent in flask (1) is heated, and its The gain of this technique, in comparison to different techniques, is that massive quantities of drug may be extracted with minimal amount of solvent.
% Extractive yield (w/w) = weight of dried extract /weight of dried depart × a hundred

3.7 Qualitative initial phytochemical screening of M. rhamnifolius The qualitative initial phytochemical exams had been done for trying out extraordinary chemical companies found in extracts. The number one metabolites like proteins, carbohydrates and glued oils and fat had been analyzed for his or her presence as in keeping with the same old procedures. Similarly, the secondary metabolites like, alkaloids, flavonoids, saponins, phenolic, tannins, terpenoids and glycosides had been additionally assessed withinside the leaves extracts of Mallotus rhamnifolius.

3.8 Pharmacological evaluation of M. rhamnifilous

3.8.1 Anti-microbiol screening

A) Nutrient agar
Nutrient Agar is a medium for the boom of bacterial organisms. This medium is ready from dehydrated powder, to be had from maximum providers commercially.
B) **Potato Dextrose Agar** (PDA) PDA is used for the boom of fungi. It is likewise used for plate rely techniques for food, dairy merchandise and trying out cosmetics. PDA may be used for developing clinically great yeast and molds. The nutritionally wealthy base (potato infusion) facilitates pigment manufacturing and mould sporulation in a few dermatophytes.

Principle PDA is contained of dehydrated potato infusion and dextrose that helps luxuriant fungal boom. Agar is brought because the solidifying agent. The sterile tartaric acid (20%) is brought to decrease the pH of this medium to a few. four ± zero. three, inhibiting bacterial boom.

Preparation from business medium powder

1. Commercial PDA powder (39 g) is brought to one L of distilled water.
2. It is boiled to dissolve the powder absolutely.
3. Then the answer is autoclaved for 15 min at 121°C.

C) Culture and upkeep of microorganisms

Pure cultures of all experimental micro organism and fungi had been acquired from the Department of microbiology, university, Delhi. The natural bacterial cultures had been maintained on nutrient agar medium and fungal lifestyle on potato dextrose agar (PDA) medium. Each bacterial and fungal lifestyle became similarly maintained via way of means of sub-culturing frequently at the equal medium and saved at 4oC earlier than use in experiments.

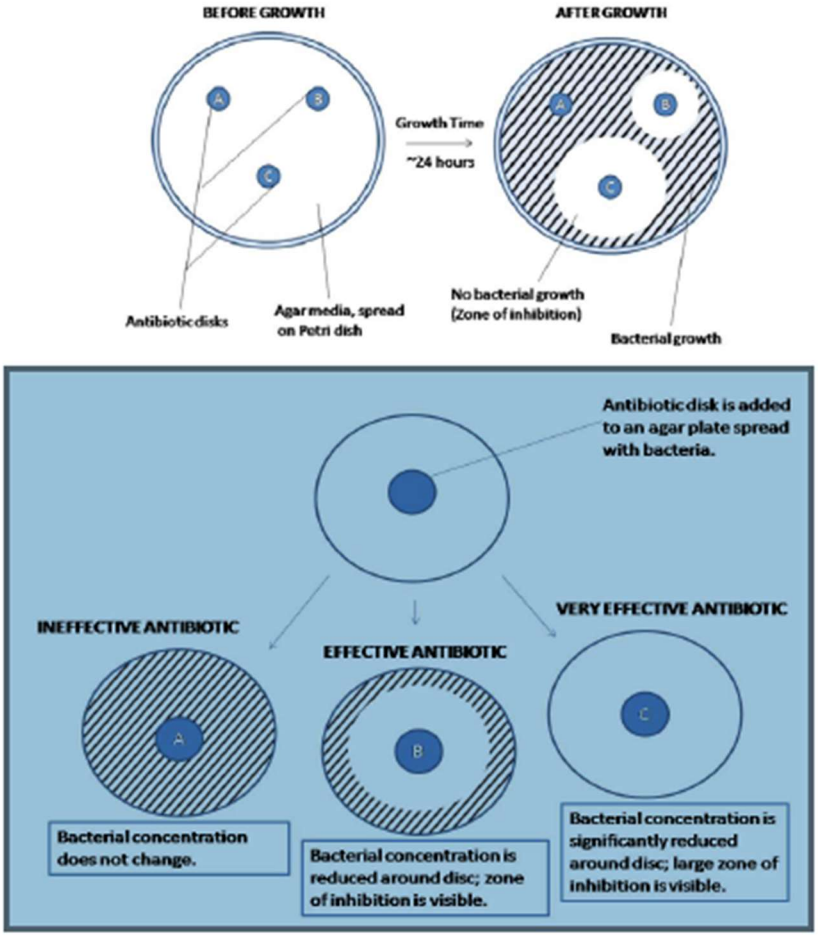


Figure-2: The sector of inhibition in disk diffusion technique

In this technique, wafers having antibiotics are located on an agar plate, in which micro organism had been located, and the plate is located to incubate. If an antibiotic stops the micro organism from developing or kills the micro organism, there may be a place across the wafer, in which the micro organisms have now no longer grown sufficient to be visible [11].

D) Culture and upkeep of microorganisms

Pure cultures of all experimental micro organism and fungi had been acquired from the Department of microbiology, university, Delhi. The natural bacterial cultures had been maintained on nutrient agar medium and fungal lifestyle on potato dextrose agar (PDA) medium.

E) **Experimental technique** Antimicrobial sports of various extracts had been evaluated via way of means of the agar nicely diffusion technique (Murray et al.) [263] changed via way of means of (Olurinola) [12].

Media instruction and its sterilization

In agar nicely-diffusion technique antimicrobial susceptibility became examined on stable (agar-agar) media in petri plates. For bacterial assay, nutrient agar (NA) (forty g/L) and for fungus PDA (39 g/L) became used for generating floor colony boom.

Agar nicely diffusion technique

Agar nicely-diffusion technique became hired to degree the antimicrobial pastime. Potato dextrose agar (PDA) and Nutrient agar (NA) plates had been swabbed (sterile cotton swabs) with eight hour-antique broth lifestyle of respective micro organism and fungi. Wells (10 mm diameter and approximately 2 cm a part) had been shaped in every of those plates the usage of sterile cork borer. Stock answer became organized at a attention of 10 mg/10 mL via way of means of ethanol with extraordinary extracts viz. petroleum ether, chloroform, ethyl acetate and ethanol: water.

F) Measurement of antimicrobial pastime the usage of agar nicely diffusion technique of M. rhamnifolius extracts.

The antimicrobial cappotential of the experimental flowers became calculated in line with their sector of inhibition towards numerous pathogens and the outcomes (sector of inhibition) had been in comparison with the pastime of the standards. Gentamicin and Ketoconazole. (a hundred µg/disc) had been used as fine manipulate for micro organism and fungi respectively.

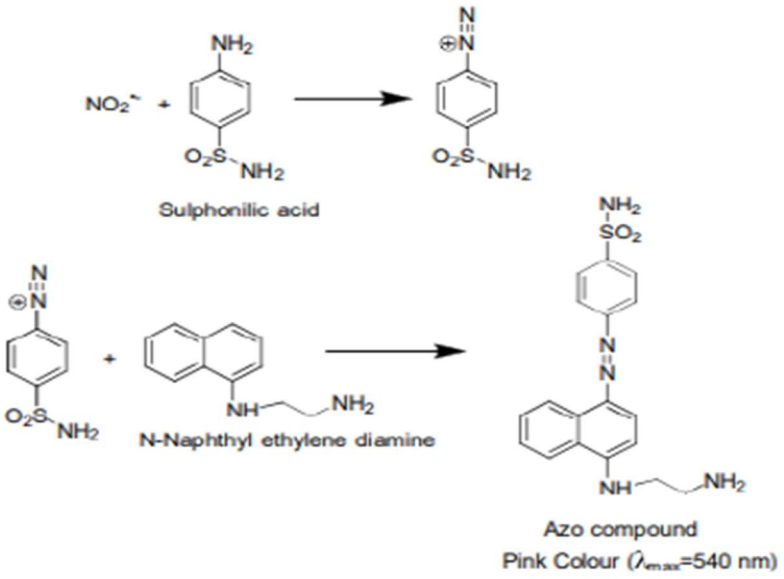
3.8.2 In vitro antioxidant pastime of M. rhamnifilous

All In vitro antioxidant sports had been performed for all of the extraordinary solvent crude extracts of M. rhamnifilous. All experiments had been done in triplicate.

3.8.2.1 Nitric oxide radical scavenging pastime

Principle

On the idea of Griess response, nitrite became first dealt with with a diazotizing reagent, e.g., sulfanilamide, in acidic media to shape a temporary diazonium salt. This intermediate became then allowed to react with a coupling reagent, N-naphthyl ethylenediamine to shape a strong ago compound.



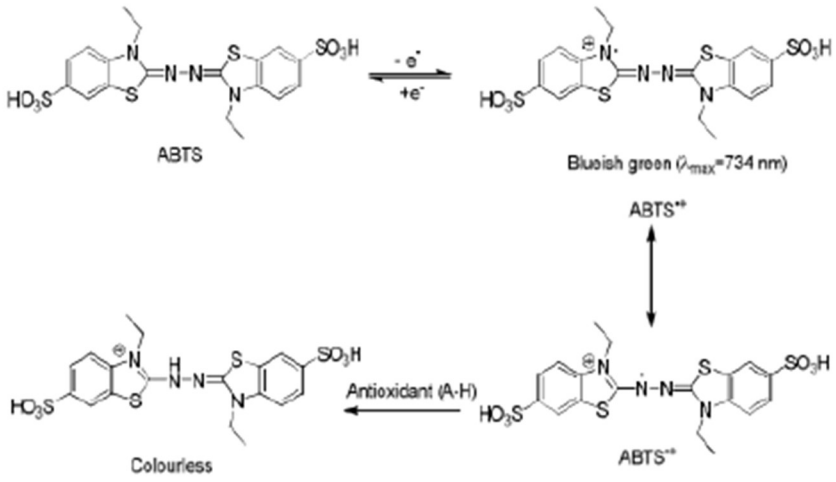
Experimental technique

The nitric oxide scavenging pastime of various solvent extracts of leaves of Mallotus rhamnifolius on nitric oxide radical became measured in line with the literature technique [13]. Sodium nitroprusside (10 mm) in phosphate buffered saline, became blended with 500 µl of various Mallotus rhamnifolius extracts and incubated at room temperature for one hundred fifty min.

3.8.2.2 ABTS radical scavenging pastime

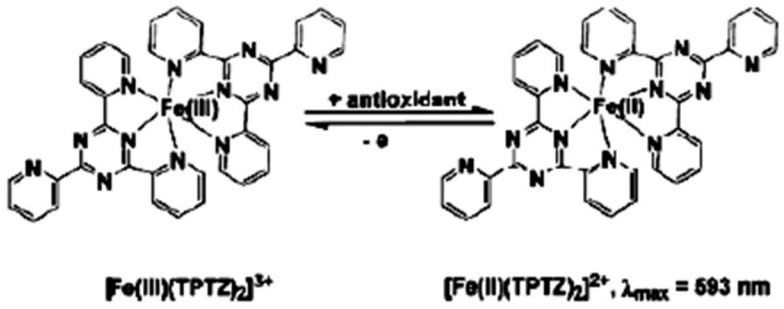
Principle

The ABTS (2, 2'-azino-bis (three-ethylbenzothiazoline-6-sulphonic acid) reacts with ammonium persulphate beneathneath darkish situation to shape ABTS unfastened radicals. The persulphate ions are worried in a Nucleophile assault on ABTS to generate greenish blue ABTS radicals (ABTS●+)



Experimental techniques

ABTS (2, 2'-azino-bis (three-ethylbenzothiazoline-6-sulphonic acid)) assay For ABTS assay [13] the system accompanied is as follows. The 500 µL inventory answers blanketed 7.four mm ABTS ● Solution and a couple of.6 mm potassium persulfate answer. Then the running answer became organized via way of means of blending identical portions of the 2 inventory answers and letting them react for 12 h at room temperature withinside the darkish. The answer became then diluted via way of means of blending 1 mL ABTS

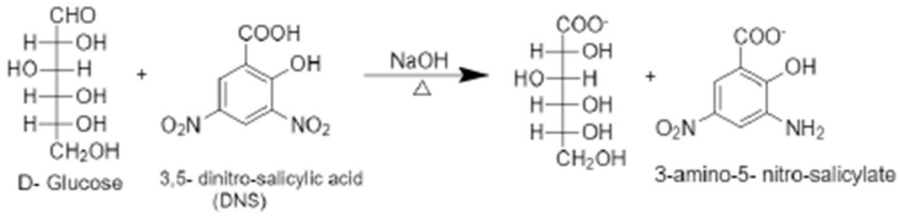


Total antioxidant potential became measured via way of means of the ferric decreasing antioxidant power (FRAP) assay [13]. The precept of this technique is primarily based totally at the discount of a ferric tripyridyl triazine complicated to its coloured ferrous from withinside the presence of antioxidants. Briefly, the FRAP reagent contained 2.five ml of a ten mmol/L, TPTZ (2, four, 6- tripyridyl-s-triazine) answer in forty mmol/L HCl with 2.five ml of 20 mmol/L FeCl3.6H2O and 20 ml of zero.25 mol/L acetate buffer (pH three. eight). It became organized freshly and warmed at 37°C.

3.8.3 In vitro anti-diabetic assay
Extraction of wheat α-amylase

Malted wheat flour (500 g) became brought to one thousand ml of zero.2lcium acetate answer at room temperature with consistent stirring for two h on a stirrer. Then the suspension became centrifuged at 40 °C at one thousand rpm for 10 min. The resultant clean brown supernatant became saved at 2 to a few °C previous to warmness remedy.

3.6.3.1 Determination of wheat α-amylase inhibitor pastime Principle Amylase pastime became expected via way of means of measuring both the arrival one of the produces or the disappearance of the substrate over time.



Experimental technique

The experimental technique became accompanied primarily based totally on "In vitro pharmacology research on Alocasi sanderiana W.Bull" [14]. The assay combination containing 2 hundred µl of zero.02 M sodium phosphate buffer, 20 µL of enzyme and the ethanol: water extracts of leaf of the M. rhamnifolius in attention variety a hundred-500 µg/ml had been incubated for 10 min at room temperature accompanied via way of means of addition of 2 hundred µl of starch in all Test tubes.

3.9.1 In vitro anti inflammatory pastime

Membrane stabilization property

Preparation of crimson blood cells (RBCs) suspension

Fresh human blood (20 mL) became accumulated and transferred to the heparin centrifuged tubes. The tubes had been centrifuged at 2500 rpm for 15 min and had been washed instances with identical extent of ordinary saline. The extent of the blood became measured and reconstituted as 10% v/v suspension with ordinary saline.

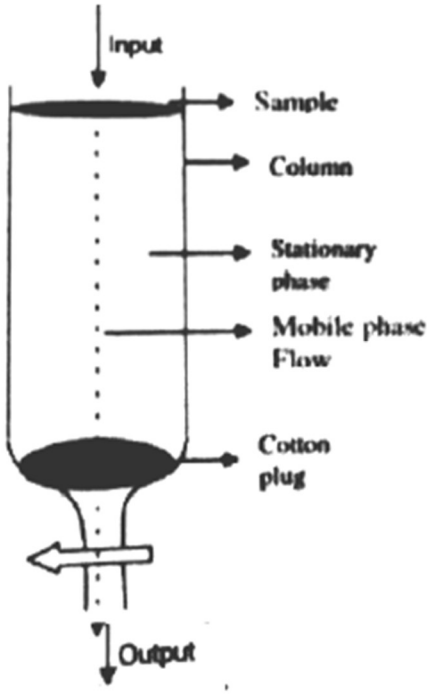


Figure-3: Coloumn chromatography

CHAPTER – IV
RESULTS AND DISCUSSIONS
4.1 Hot continuous extraction (Soxhlet method)

The extracts (petroleum ether, chloroform, ethyl acetate and ethanol extracts) of *Mallotus rhamnifolius* were prepared by the hot continuous extraction method. The color of the extracts was found out to be brownish dark green in petroleum ether and chloroform extracts, then reddish brown in ethyl acetate and ethanol: water extracts. The physical characteristics and % yield of *Mallotus rhamnifolius* are listed in Table-2 and Figure-1.

Solvent	**Color of extract**	**Sense of touch**	**Amount of extract (g)**	**% yield**
Petroleum ether	Brownish dark green	Sticky	4.67	5.83%
chloroform	Brownish dark green	Sticky	10.21	12.76%
Ethyl acetate	Reddish brown	Sticky	14.93	18.66%
Ethanol water (95:5)	Reddish brown	Sticky	28.45	35.56%

Table-2: Physical characteristics and % yield of *Mallotus rhamnifolius* on soxhlet extracts.

In the present work, the percentage yield (w/w) of crude extracts was also analyzed, wherein the highest yield (35.56%) was obtained with ethanol: water (95:5) and least (5.83%) with petroleum ether media.

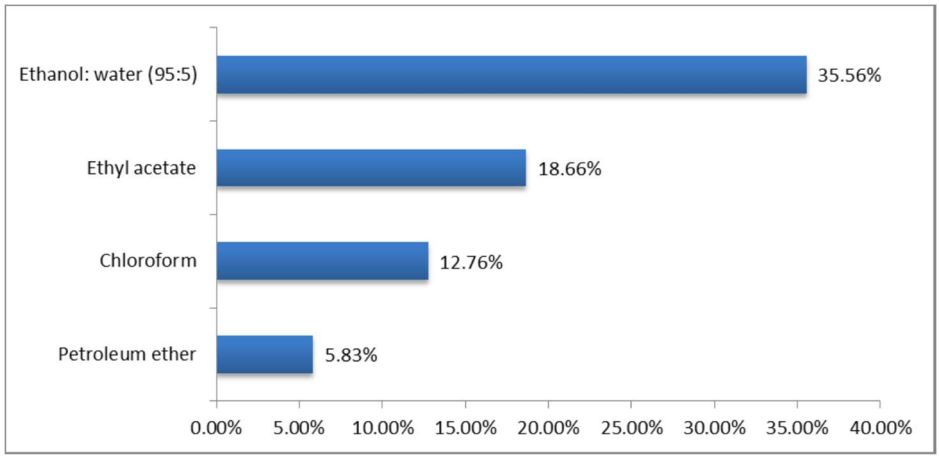


Figure-3: Percentage yield of *Mallotus rhamnifolius* on soxhlet extracts

Compared the value of the percentage yield (w/w) to the *Mallotus* tetracoccus [280], the extracts of petroleum ether, chloroform, ethyl acetate, acetone and ethanol were 25.3, 13.72, 12.87, 21.01, and 27.01 % respectively. According to the values, *Mallotus rhamnifolius* were more extracts on ethanol solvent

4.2 Phytochemical analysis of *M. rhamnifolius*

4.2.1 Qualitative phytochemical analysis

Phytochemical evaluation in exclusive solvent leaf extracts of M. rhamnifolius confirmed that it became an excellent supply of a couple of phytoconstituents. The consequences of phytochemical screening explicated the presence of glycosides, flavonoids, steroids, anthraquinones, alkaloids, saponins, phenols, tannins and oils in maximum of the examined extracts. The consequences discovered that the presence of those phytoconstituents withinside the examined extracts various like plentiful, slight and moderate presences. The qualitative phytochemical evaluation of M. rhamnifolius is summarized.

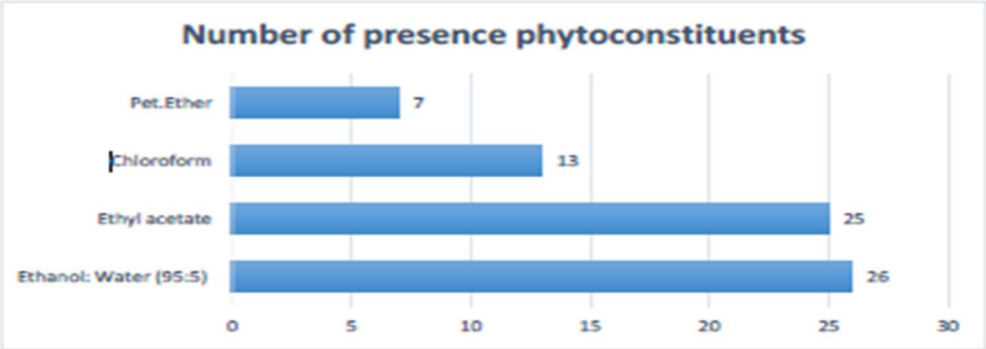


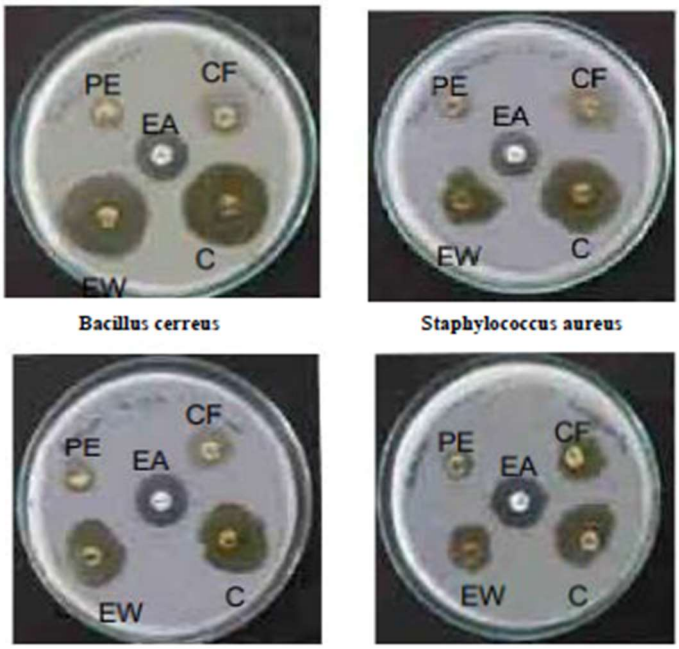
Figure-2: Numbers of phytoconstituents present in each extracts.

4.3 *In vitro* antimicrobial activity of *M. rhamnifolius*

In the existing investigation, exclusive solvent extracts of Mallotus rhamnifolius have been evaluated for exploration in their antimicrobial interest in opposition to cereus are Gram-nice; Escherichia coli and Pseudomonas aeruginosa are Gram-bad micro organism. Fusarium oxysporum.

Table-3: Antimicrobial activity of different solvent extracts of leaf of *Mallotus rhamnifolius*

Microorganisms	Control	Pet. ether	Chloroform	Ethyl acetate	Ethanol: water
Bacillus cereus	**22**	**10**	**14**	**14**	**16**
Staphylococcus aureus	**26**	**11**	**16**	**17**	**17**
Escherichia coli	**29**	**14**	**18**	**18**	**19**
Pseudomonas aeruginosa	**28**	**10**	**14**	**16**	**21**
Fusarium oxysporum	**32**	**12**	**15**	**16**	**18**
Aspergillus niger	**32**	**14**	**16**	**17**	**20**



Figure–4(a) : The Anti-bacterial analysis of *M. rhamnifolius*

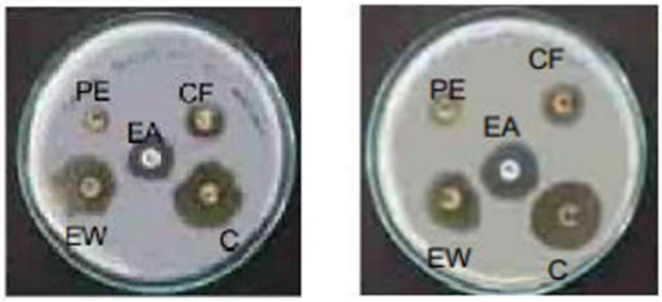


Figure-4(b) : The Anti-fungal analysis plates of *M.rhmnifolius*.

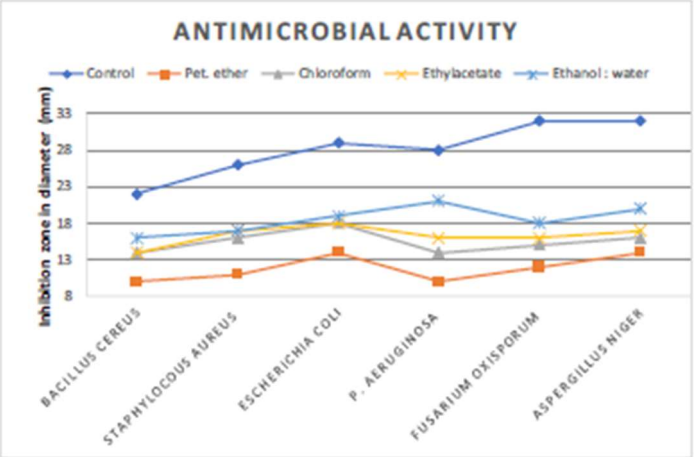


Figure-5 : Antimicrobial activity of different solvent extracts of leaf of *Mallotus rhamnifolius*

The zone of inhibitions are as follows: *Escherichia coli* (19 mm), *Pseudomonas aeruginosa* (21 mm), *Staphylococcus aureus* (17 mm), *Bacillus cereus* (16 mm) and *Aspergillus niger* (20 mm).

4.4 *In vitro* antioxidant assay of *M. rhamnifolius*

4.4.1 Nitric acid radical scavenging activity of *M. rhamnifolius*

The consequences of the unfastened radical scavenging capacity of the exclusive extracts examined through nitric acid radical scavenging interest assay are given withinside the Table-five and Figure-five. Ethanol: water extract of the leaf became discovered to have the maximum mighty antioxidant belongings amongst all of the different extracts. The excessive interest of ethanol: water extracts are typically attributed to the presence of alkaloids and phenols, as the bulk of energetic antioxidant compounds are located in those lessons of phytochemical compounds.

4.4.2 ABTS assay of *M. rhamnifolius*

The ethanol: water extracts of the leaf of Mallotus rhamnifolius have been discovered to be extra powerful scavengers of the ABTS radical as in comparison to the alternative extracts (Table-five, Figure-five). The petroleum ether extracts of the leaf confirmed decrease capacity in scavenging the ABTS radical. Proton radical scavenging is an vital feature of antioxidants. ABTS, a protonated radical, has absorbance maxima at 734 nm, which decreases with the scavenging of the proton radicals .

Table-4: Percentage of scavenging activity of different solvent extracts of leaf of the *Mallotus rhamnifolius*

Extraction on medium	**Nitric acid radical scavenging activity**	**ABTS**	**FRAP**
Pet. ether	**19.44**	33.73±.062	49.9±0.4

	± 0.93		
Chloroform	**27.78** ±1.85	46.23±1.04	55.8±0.5
Ethyl acetate	**33.04** ±1.07	58.16±1.16	62.3±2.1
Ethanol: water	**41.98** ±1.41	64.88±0.44	64.2±0.7
Rutin	**81.25** ±1.56	98.88±2.53	84.23±1.86

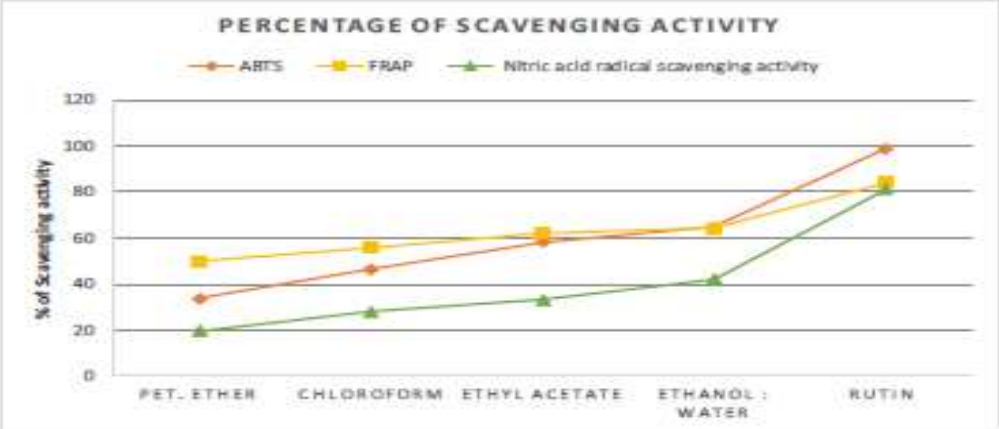


Figure-6: Percentage of scavenging activity of different solvent extracts of leaf of *Mallotus rhamnifolius*

4.4.3 FRAP assay

The decreasing capacity of the extracts is tabulated in Table-five and Figure-five. The ethanol: water extract of leaf of Mallotus rhamnifolius confirmed most interest (64.2±zero.7) observed through the ethyl acetate extract of the leaf (62.three±2.1). The petroleum ether extract of leaf confirmed decrease interest than the chloroform extract, 49.nine±zero.four and 55.eight±zero.five respectively. In the species of Mallotus apelta [303], the overall percent of scavenging interest withinside the FRAP approach became handiest 22.four±1.99.

The crude extracts have been used for trying out their antimicrobial interest and antioxidant interest the use of ABTS, FRAP and nitric acid radical scavenging interest assays. Ethanol: water extract of the leaves became discovered to show off most antimicrobial interest and antioxidant interest

amongst all of the extracts confirmed the opportunity for isolation and characterization of precise molecules from the crude extract, which can be purified and used for similarly research.

4.5 In vitro anti-diabetic assay

The results of inhibitory potential of ethanol: water leaf extract of *M. rhamnifolius* on wheat α-amylase are shown in Table-6 and Figure-6.

Table-6 : Wheat α-amylase inhibitory effects of *M. rhamnifolius* leaf extract in comparison with standard drug acarbose.

Sample	Concentration (ug/ml)	Percentage of α amylase inhabitation
Ethanol: water	100	38.11 ± 0.37
	200	53.80 ± 0.31
	300	59.66 ± 0.64
	400	66.59 ± 0.52
	500	72.10 ± 0.07
Acarbose	100	93.61 ± 0.56

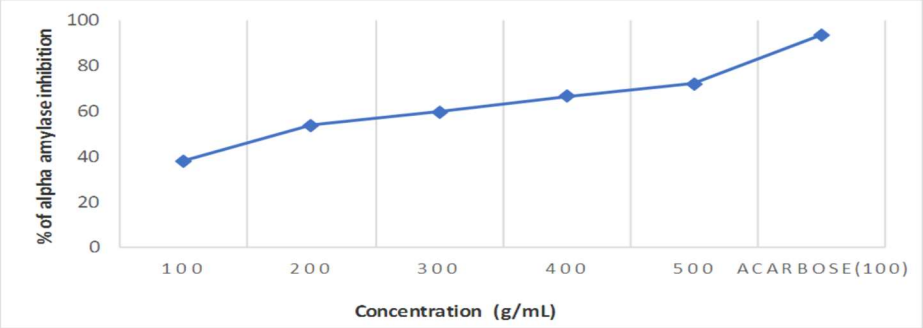


Figure-7: Wheat α-amylase inhibitory effects of *M. rhamnifolius* leaf extract in comparison with standard drug acarbose

Table-7: Yeast α-glucosidase inhibitor activity effects of *M. rhamnifolius* leaf extract in comparison with standard drug acarbose

Sample	Concentration (ug/ml)	Percentage of α amylase inhabitation
Ethanol: water	100	16.97±0.27
	200	25.03±0.27
	300	38.31±0.97
	400	47.19±0.22
	500	65.98±0.17
Acarbose	100	70.32±0.28

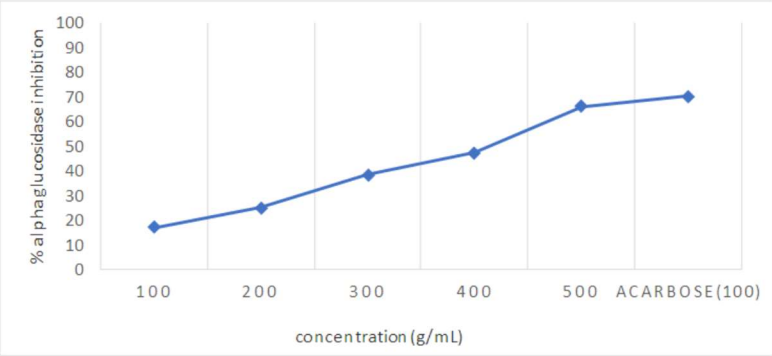


Figure-8: Yeast α-glucosidase inhibitor activity effects of *M. rhamnifolius* leaf extract in comparison with standard drug acarbose

Many natural extracts are utilized in ayurveda for the remedy of diabetes and were stated to have antidiabetic interest withinside the inhibition capacity in the direction of α-amylase and glucosidase interest. In this examine, an in vitro inhibitory impact of M. rhamnifolius on α-amylase and α-glucosidase sports became evaluated.

Our consequences are according with the preceding examine wherein, a nice dating among the overall phenol and flavonoid content material and the capacity to inhibit intestinal α-glucosidase and pancreatic α-amylase became established. In our examine, acarbose became used because the nice control, it inhibited the α-amylase interest with 500 µg/mL of the extract to 72.10%. Free radicals are shaped disproportionately throughout diabetes because of glucose oxidation and the

following oxidative degradation of glycated proteins.

The examiner end result suggests that M. rhamnifolius show off mighty α-amylase inhibitory interest and this healing potentiality can be exploited withinside the control of submit prandial hyperglycemia in remedy of kind 2 diabetes mellitus.

4.6 *In vitro* anti-inflammatory activity

Literatures at the moment are complete of medical documentation nowadays concerning medicinal vegetation and that they have capacity to therapy numerous human diseases. Thus, this remarkable destiny similarly encourages to fabricate pharmaceutical merchandise acquired from medicinal vegetation as they may be secure and reliable compared to artificial drugs, that aren't handiest high-priced however additionally have destructive consequences.

This is the primary record at the in vitro membrane stabilization capacity of M. rhamnifolius leaf extracts. In this examine, we validated that M. rhamnifolius leaf extracts inhibit heat-brought about hemolysis of erythrocytes derived from wholesome individuals. This suggests that M. rhamnifolius leaf extracts own organic membrane stabilization homes stopping strain-brought about destruction of the plasma membrane.

Table-8 : Effect of ethanol: water extract on heat induced haemolysis of erythrocyte

Sample	Concentration (ug/ml)	Percentage of Hemolysis
Control		-
	100	21
	200	30
	300	36
	400	43
Acarbose	500	51
	100	71

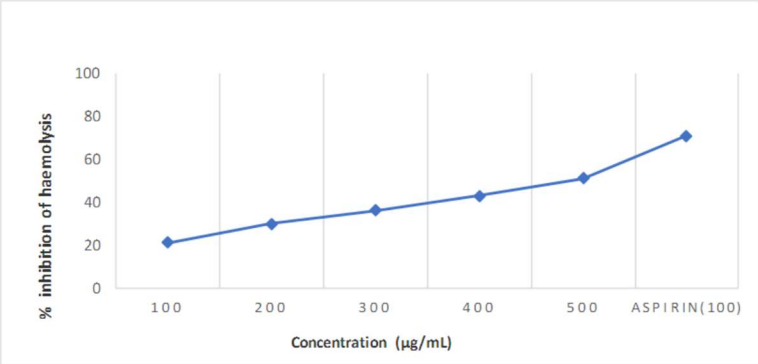


Figure-9: Effect of ethanol: water extract on heat induced haemolysis of erythrocyte

As part of the investigation on the mechanism of the anti-inflammation activity, the ability of plant extract to inhibit protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation. The ethanol: water extracts of *M. rhamnifolius* have significant anti-inflammatory activity at the concentration of 500 µg/mL, which is comparable to the standard drug aspirin. The maximum % inhibition of protein denaturation was observed as 61% at 500 µg/mL (shown in Table-9 and Figure-9).

Table-9: Percentage inhibition of protein denaturation of *M. rhamnifolius*

Sample	Concentration (ug/ml)	**% Inhibition of protein denaturation**
Control	-	-
	100	32
	200	38
Ethanol: Water	300	42
	400	55
	500	61
Asprin	100	81

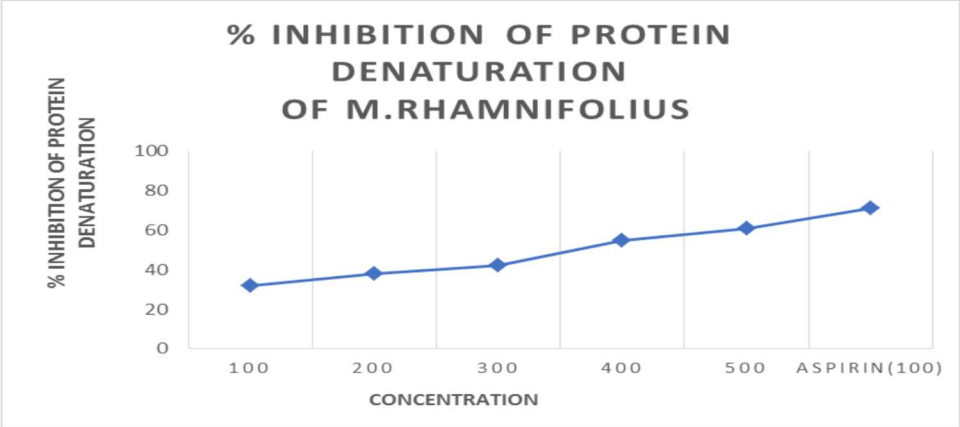


Figure-10: Percentage inhibition of protein denaturation of *M. rhamnifolius*

Sample	Concentration (ug/ml)	**% inhibition of proteinase activity**
Control	-	-
	100	21
	200	27
Ethanol: Water	300	36
	400	42
	500	53
Asprin	100	56

Table-10: Percentage inhibition of albumin denaturation of *M. rhamnifolius*

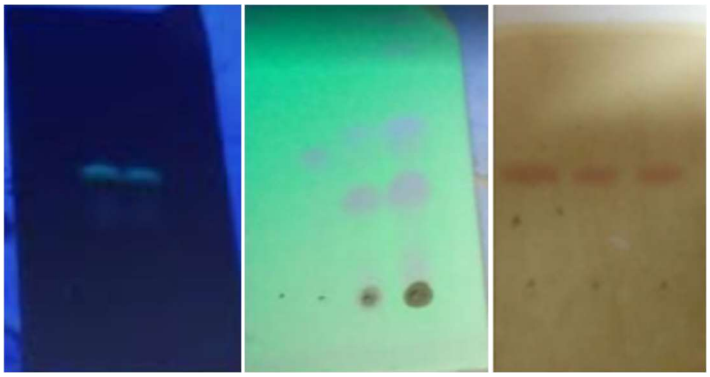
The precise underlying mechanism for the membrane stabilizing impact of M. rhamnifolius leaf extracts and the chemical constituent(s) answerable for this impact isn't always acknowledged. However, some of research have proven that flavonoids and a number of different plant compounds.

4.7 Isolation of bioactive compounds from *M. rhamnifolius*

4.7.1 Column chromatography

Ethanol: water extract (8 g) became subjected to column chromatography on silica gel (one

hundred-2 hundred mesh) (Merck) eluted with combos of chloroform, ethyl acetate, ethanol and methanol of growing polarity. About 163 fractions have been eluted with exclusive solvents with growing polarity. Column fractions from forty one to sixty nine with hexane: ethyl acetate (80:20 v/v) withinside the TLC cellular segment solvent ratio of hexane: ethyl acetate (60:forty v/v) confirmed Rf fee of zero.72 (proven in Figure-eleven).



b a c

Figure: 11 a) The crude ethanol: water extracts on short UV (240 nm) light
b) The crude ethanol: water extracts on long UV (520 nm) light
c) The crude ethanol: water extracts on DNPH stain after heating.

4.7.2 Compound - 1

4.7.2.1 Preparative thin layer chromatography (PTLC)

The above purified compound-1 (three hundred mg) became dissolved in five mL DCM solvent and loaded in 20 cm × 20 cm, 1,000 µm layer thickness glass assist silica gel Sigma-aldrich preparative TLC plates for similarly purification. The chromatogram became advanced with ethyl acetate: hexane (60: forty v/v).

4.8 Liquid chromatography/mass spectrometry (LC/MS identification)

The remoted plant compound-1 became analyzed through LC-MS. It has been effectively implemented for a fast separation and identity of the remoted compound-1 from ethanol: water extracts of leaves of Mallotus rhamnifolius. The LC-MS consequences explicated the purity (96.90%) of compound-1 (Figure-12a)

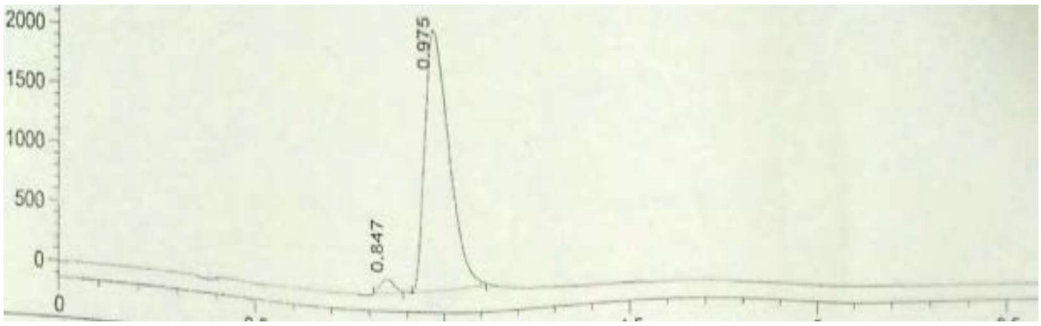


Figure-12(a) : LC-MS chromatogram of compound-1

Compound Name	Area	Meas. R	Area %
	329.472	0.847	3.094
	1.032e4	0.975	96.906

Figure-13(b) : LC-MS purity of the compound – 1.

The chromatogram of the isolated compound-1 is shown in figure 12(c, d). Its molecular weight is found to be 302 based on LC-MS with fragment pattern m/z 303.0 in positive mode (figure- 12 (c)) and 301.0 in negative mode was found in its first order mass spectrum(figure-12 (d))

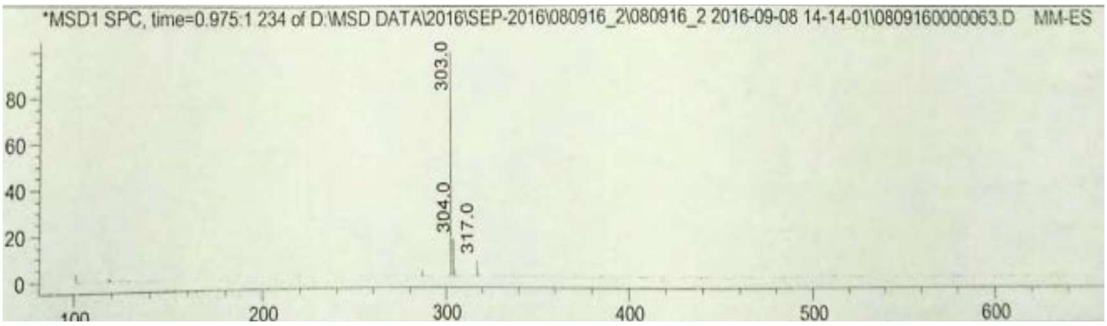
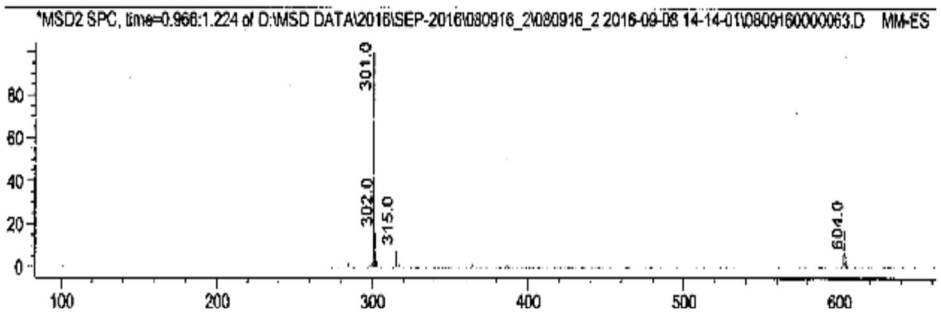
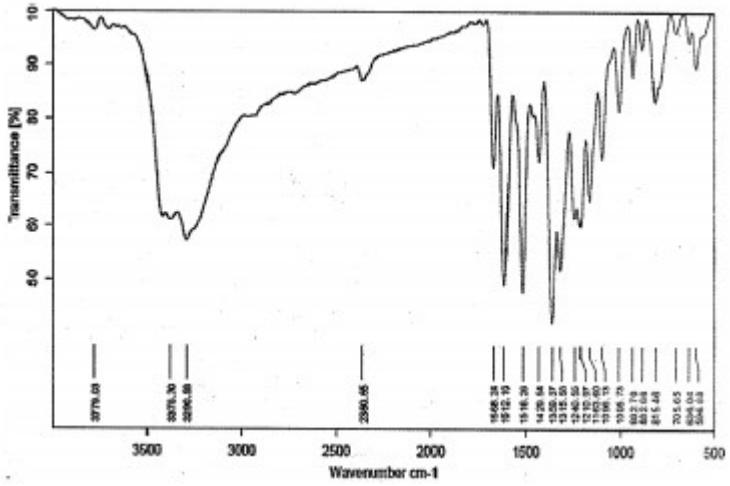


Figure-14 (c) : LC-MS mass fragmentation of compound-1 (positive mass)



4.8.1 Fourier transforms infrared (FT-IR) spectrum analysis of compound-1

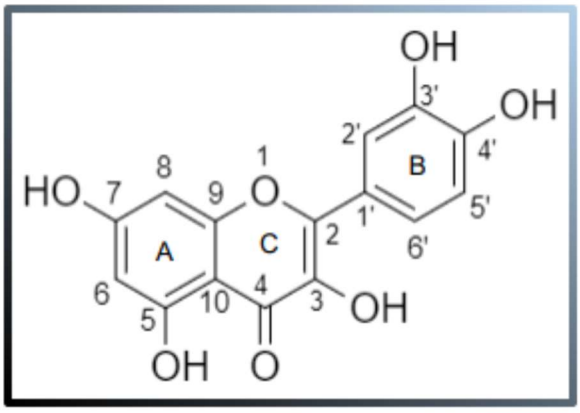
The FT-IR (K.Br) spectrum of isolated compound-1is shown in Figuer-13 and their corresponding characteristic peak positions and assignments are as follows. The broad absorption peak at around 3290.58 cm-1 was assigned to the OH stretching vibration of phenol.



Figuer-15 FT-IR spectrum of isolated compound-1

4.8.2 NMR spectrum of isolated compound-1

NMR studies were carried out to confirm the positions of proton and carbon binding sites. The isolated compound-1 displayed a better resolved 1H-NMR spectrum. The 1H-NMR spectrum of the isolated compound-1 showed aromatic hydrogen groups from 6.18-7.66 ppm and phenolic-OH groups from 9.36-12.48 ppm respectively (Figure-14 and Figure-14a).



1H-NMR spectrum (Figure-14 and 14a) of the isolated compound-1 showed the presence of 10 protons. Five aromatic protons of δ values 6.166 (d), 6.340 (d), 6.892 (dd), 7.5 (dd) and 7.655 (d) ppm. The 6th proton coupled with the 8th proton and thus 6th proton appears as a double. Like that, the 8th proton coupled with 6th proton and thus 8th proton appears as a double.

D2O exchange spectrum of *M. rhamnifolius*

D2O was added to the above proton NMR sample and the analysis was done again. Different NMR spectrum showing only five peaks at δ values 6.172, 6.404,
7.617, 6.866 and 7.509 was obtained (Figure-15 and Figure-15a). So, it is understood that the disappeared protons are three –OH protons. Because of the five –OH protons are exchanged with deuterium of D2O, it becomes –OD.

13C-NMR spectrum of *M. rhamnifolius*

The 13C-NMR spectrum showed totally fifteen carbon signals including the carbonyl group at 175.75 ppm and aromatic carbons from 93.35-163.83 ppm in (Table-11).

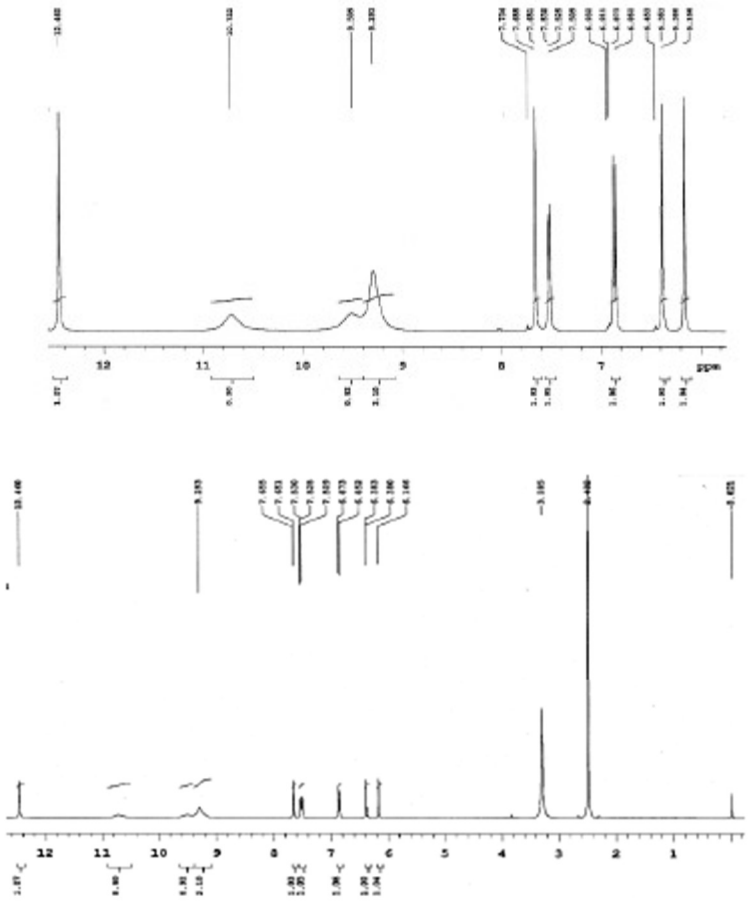


Figure-16 : 1H-NMR of compound-1

Figure-16a : Expansion 1H-NMR of compound-1

4.8.1 Heteronuclear multiple bond correlation (HMBC) spectrum of compound-1.

HMBC spectrum helps to know about the weak proton-carbon couplings information in the compounds. The weak coupling appeared that the proton is two, three or four bonds away from the carbon. The long-range proton-carbon correlations of HMBC tells vast amount of information about the structure of compounds including protonated carbons and quaternary carbons. The assembly of carbon and hydrogen in compound-1 was mainly confirmed by the HMBC experiment.

The HMBC cross peak correlations between the hydroxyl group (3-OH) at δH 9.509 with C-3 at δC 135.66; C-4 at δC 175.75 and C-2 at δC 146.75 ppm. The hydroxyl group (5-OH) at δH 12.460 with C-5 at δC 160.64; C-10 at δC102.96; C-6 at δC 98.17 and C-7 at δC 163.83 ppm. The hydroxyl group (7-OH) at δH 10.755 with C-7 at δC 163.83, C-8 at δC 93.35 and C-6 at δC 98.17 ppm. The hydroxyl group (3'-OH) at δH 9.32 with C-3' at δC 144.98, C-4' at δC 147.61 and C-2' at δC 115.02 ppm. The hydroxyl group (4'-OH) at δH 9.293 with C-4' at δC 147.61, C-3' at δC 144.98 and C-5' at δC 115.56 ppm (Figure-17)

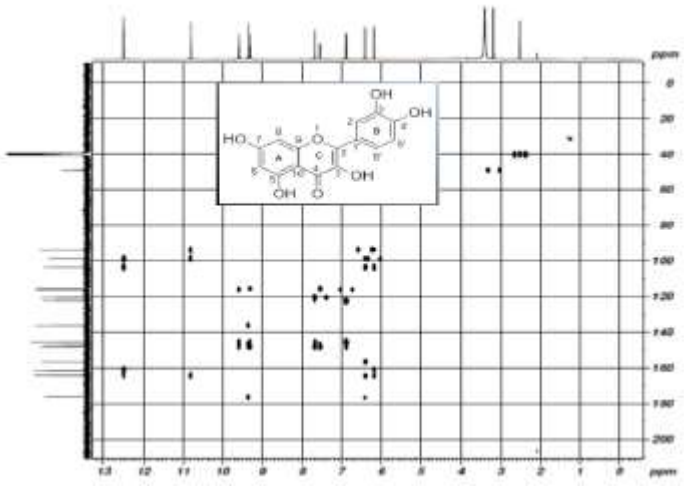


Figure -17: HMBC NMR spectrum of compound-1

The aromatic hydrogen atoms of compounds-1 is also correlated to HMBC spectral studies. The hydrogen atom of A-ring in H-6 at δH 6.166 with C-5 at δC 160.64, C-10 at δC 102.96, C-7 at δC 163.83 and C-8 at δC 93.35 ppm. The H-8 atom at δH 6.380 with C-7 at δC 163.83, C-8 at δC 93.35, C-9 at δC 156.1and C-10 at δC 102.96 ppm. The hydrogen atom of B-ring in H-2' at δH 7.655 with C-1' at δC 121.96, C-6' at δC 120.00, C-3' at δC 144.98 ppm and C-4' at δC 147.61. The H-6' at 7.553 with C-5' at δC 115.56, C-4' at δC 147.61, C-1' at δC 121.96 ppm and C-2' at δC 115.02. The H-5' at 6.87 correlated with C-4' at δC 147.61, C-3' at δC 144.98, C-6' at δC 120.00 ppm and C-1' at δC 121.96 ppm The overall HMBC correlations are shown in Figure-18.

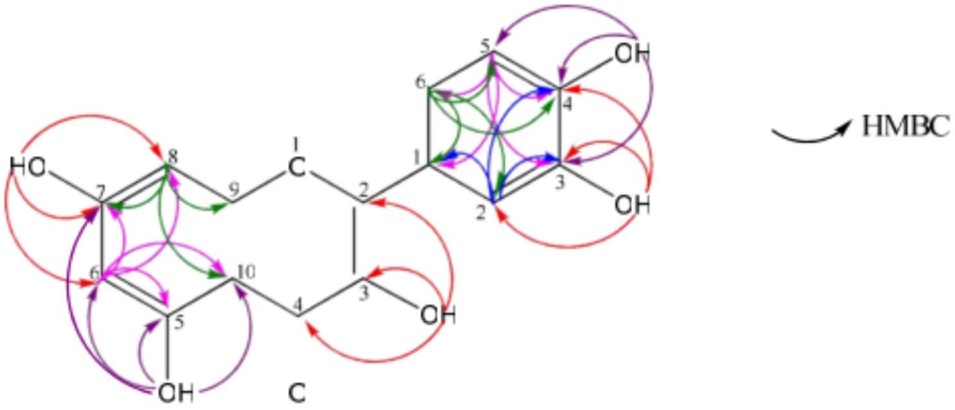


Figure-18: The overall HMBC correlations of compound-1

4.8.2 Heteronuclear single quantum coherence spectroscopy (HSQC) of compound-1

HSQC gives the information about the strong proton carbon J-couplings. HSQC confirmed that the proton is directly bonded to the carbon. This spectral technique is used for finding the direct C-H couplings. A cross peak correlation was observed between the carbons containing the hydrogen atom by HSQC.

The doublet peak at δH 6.166 (1H) ppm, which showed cross peak correlation with the carbon signal at 98.17 ppm and doublet at 6.380 (1H) ppm with the carbon signal at 93.35 ppm were assigned to H-6 and H-8 respectively of aromatic protons in ring A. Doublet at δH 7.655 (1H) ppm correlated with δC 115.02, δH 6.8792 (1H) ppm correlated with δC 115.56 ppm and doublet of doublet at δH 7.553 (1H) ppm with 120.00 ppm were assigned to aromatic protons H-2', H-5'and H-6' respectively in ring B (Figure19).

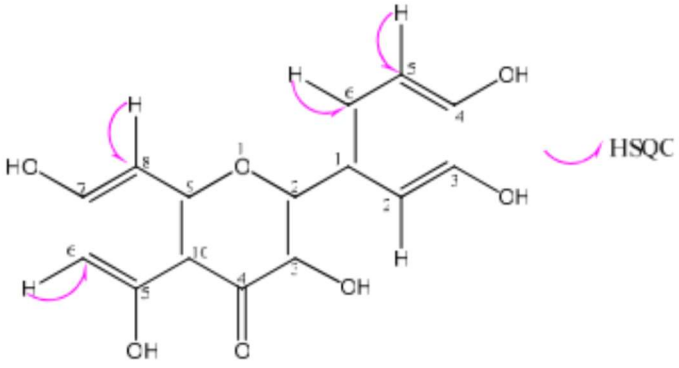


Figure-19: Thee overall HSQC correlations are shown

From all the spectral evidences, physical properties, chemical properties, LCMS, element analysis, FTIR, 1H-NMR, D2O exchange NMR, 13C-NMR, the isolated compound-2 is proved to be (2-(3,4- dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one) or quercetin.

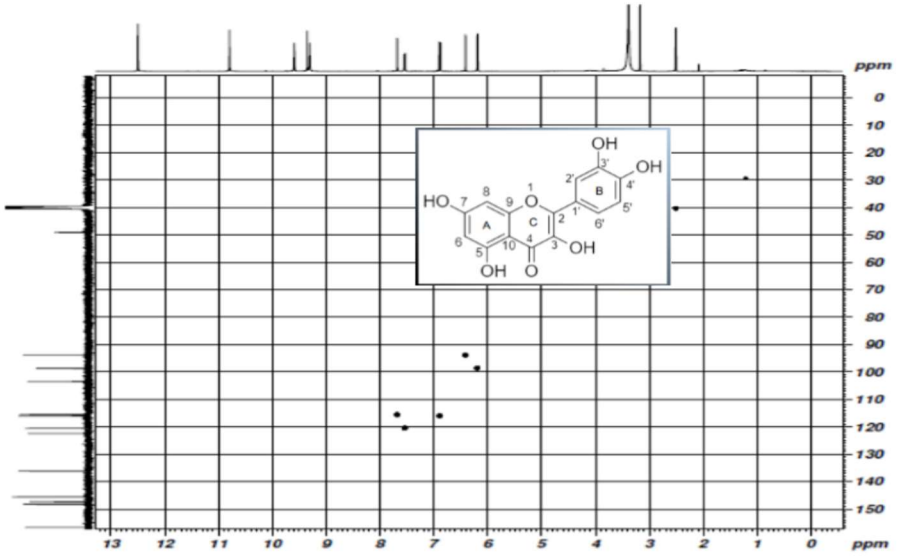


Figure-20 : HSQC NMR spectrum of compound-1

Several studies reported the isolation and structure elucidation of quercetin from various plants such as *Azolla Microphylla*, *Bauhinia championii*, rhizome of *Smilax china*, *Costus igneus*, bark of *Butea frondosa*, *Tectona grandis* and *Azadirachta indica* . Thus, it can be confirmed that the isolated compound-1 is found to be quercetin with the following structure.

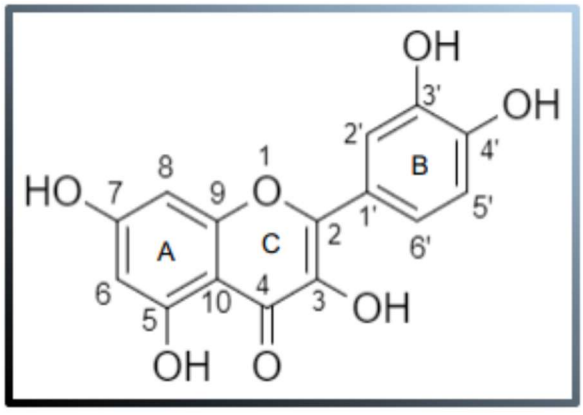


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Figure-21 : The structure of quercetin

Quercetin, a flavonols occurring in vegetables and fruit is a food component with proven high beneficial impact on health. Its biochemical activity is well proved. It is one of the highly potent antioxidants among all the polyphenols. Quercetin has been documented to display antibacterial, antifungal, antiviral, anticarcinogenic and anti-inflammatory effects. The anticarcinogenic

properties of quercetin is due to the ability to increase in the apoptosis of mutated cells, inhibition of DNA synthesis, inhibition of cancerous cell growth and decrease and modification of cellular signal transduction pathways.

4.9 Compound – 2

4.9.1 Preparative thin layer chromatography (PTLC)

The above purified compound-2 (250 mg) was dissolved in 5 mL DCM solvent and loaded in 20 cm × 20 cm, 1000 µm layer thickness glass support silica gel Sigma-aldrich preparative TLC plates for further purification. The chromatogram was developed with ethyl acetate: hexane (75:25 v/v). After elution of the plate, it was examined under UV lamp. The fluorescing spots were scraped out from the plates and extracted with chloroform.

The diluted mixture was then evaporated on a rotary evaporator under reduced pressure and the final yield was approximately 135 mg. The collected compound (compound-2) was used for further spectral (LC-MS, FT-IR, NMR) analysis.

4.9.2 Structural elucidation of the compound -2

Physical properties

Colour: Light brown

Appearance: powder

Melting point: 227-228ºC

Solubility: Dichloromethane, acetone, methanol and DMSO

Yield: 135 mg.

4.9.3 Chemical properties

NaOH test: Showed yellow colour (flavonoid nature)

Shinoda test: Pink colour appeared (flavonoid nature)

4.9.4 Thin layer chromatography

Mobile phase: Hexane: ethyl acetate (75:25 v/v)

Rf valu e: 0.56

Spraying agent: DNPH stain

4.9.5 Elemental analysis observed

C=63.57; H=4.63; N=0; S=0 and O=31.70

Hence, molecular formula of compound-2: C16 H14 O6

4.9.6 Liquid chromatography/ mass spectrometry (LC/MS identification)

The isolated plant compound-2 was analyzed by LC-MS. It has been successfully applied for a quick separation and identification of the isolated compounds from ethanol: water extract of leaves of *Mallotus rhamnifolius*. The LC-MS results explicated the purity (99.90%) of compound-2 (Figure-22 a and Figure-22 b).

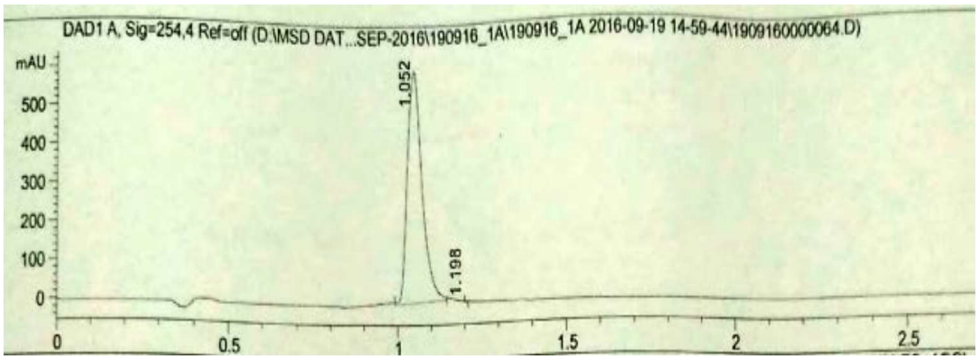


Figure-22 a : LC-MS chromatogram of compound 2

Compound Name	Area	Meas. R	Area %
	1.825e3	1.052	99.901
	1.805	1.198	0.099

Figure-22 b : LC-MS purity of compound 2

The chromatogram of the isolated compound-2 is shown in Figure-22 a. Its molecular weight 302 based on LC-MS with fragment pattern m/z 303.1 in positive mode (figure-22 c) and 301.0 in negative mode was found in its first order mass spectrum(figure-22 d)

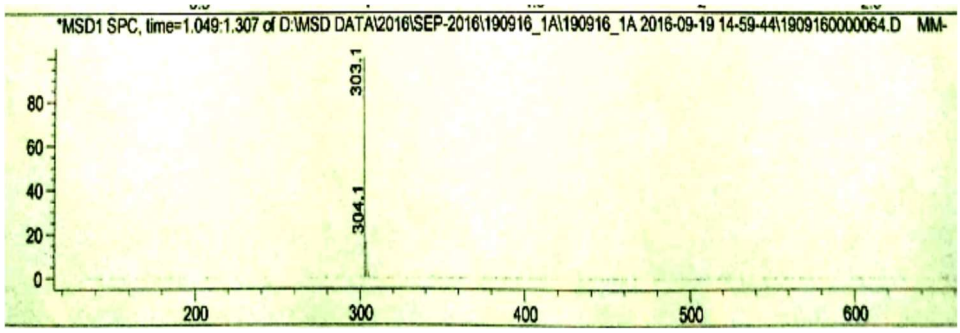


Figure-22c : LC-MS mass fragmentation of compound-2 (positive mass)

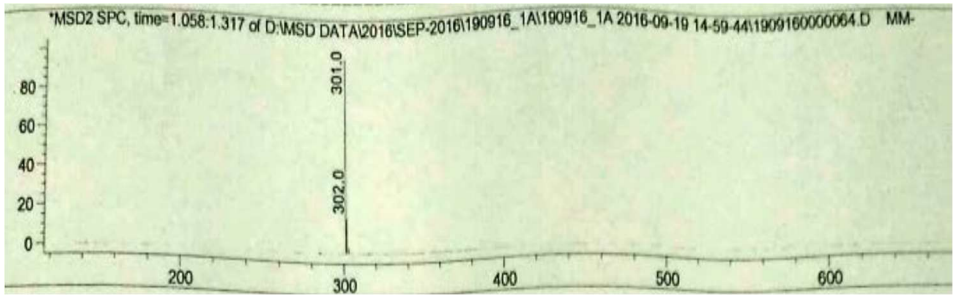


Figure-22d : LC-MS mass fragmentation of compound 2 (negative mass)

4.9.7 Fourier transforms infrared (FT-IR) spectrum analysis

The FT-IR (KBr) spectrum of isolated compound-2 is shown in Figure-23 and their corresponding characteristic peak positions and assignments are as follows. The broad absorption peak at around 3435.01 cm-1 was assigned to the OH stretching vibration of phenol. The absorption peaks positioned around 1487.40 cm-1 are assigned to the aromatic C=C stretching vibrations. The OH bending vibrations of phenols were observed at 1321.85 cm-1. The absorption peak at 1084.48 cm-1 and the peaks at the lower frequencies below 1000 cm-1 were assigned to the C-H bending vibrations of aromatic hydrocarbons.

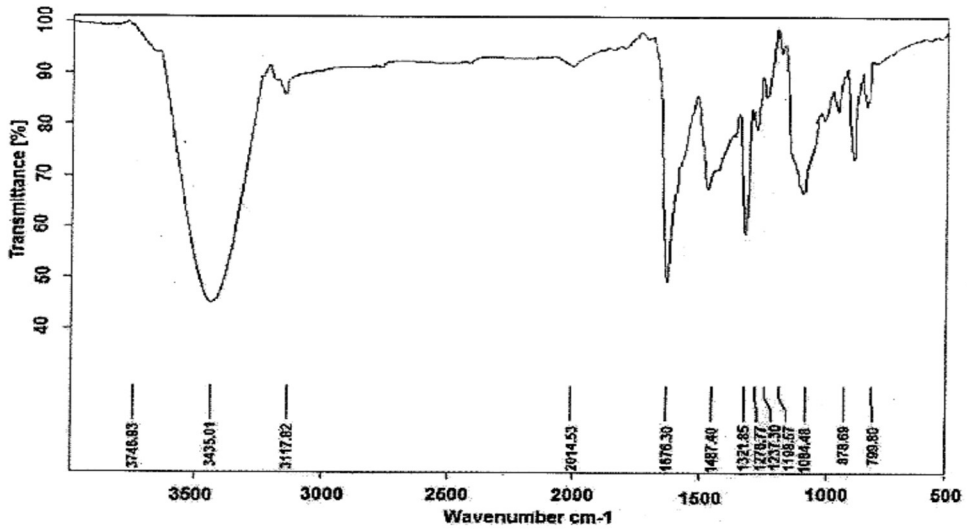


Figure-23: The FT-IR spectrum of compound-2

The C-O stretching vibrations of aryl ether and phenols were observed at 1276.77 cm-1 and 1237.30 cm-1 respectively. The C-CO-C stretching and bending vibrations of ketones were observed at 1198.57 cm-1. The presence of carbonyl group was confirmed by the characteristic band at 1676.30 cm-1. The IR spectrum results indicate the flavonoid nature of the compound-2.

4.9.8 NMR spectrum of isolated compound-2

A) 1H-NMR spectrum

NMR studies were taken to confirm the positions of proton. The isolated compound-2 displayed a better resolved 1H-NMR spectrum. The 1H-NMR spectrum of the isolated compound-2 showed the alkane protons at 2.281-3.756, the chiral proton at 5.424 and the aromatic protons from 5.860-6.926 ppm and phenolic-OH groups from 9.038-12.10 ppm respectively (Figure-24, Figure-24 a and Figure-24 b).

1HNMR (400 MHz in DMSO, δ ppm) 2.709 (1H, dd, J=14.4 Hz, H-3-eq), 3.176 (1H, m, J=4.8 Hz, H-3-ax), 3.756 (3H, s, H-OCH3), 5.400 (1H, dd, J=9.6 Hz, H-2), 5.860 (2H, m, H-6,8), 6.841-6.926 (3H, m, H-2',5',6'), -OH protons, 9.038 (1H, s, H-3'), 10.735 (1H, s, H-7), 12.100 (1H, s, H-5).

B) D2O exchange spectrum of *M. rhamnifolius*

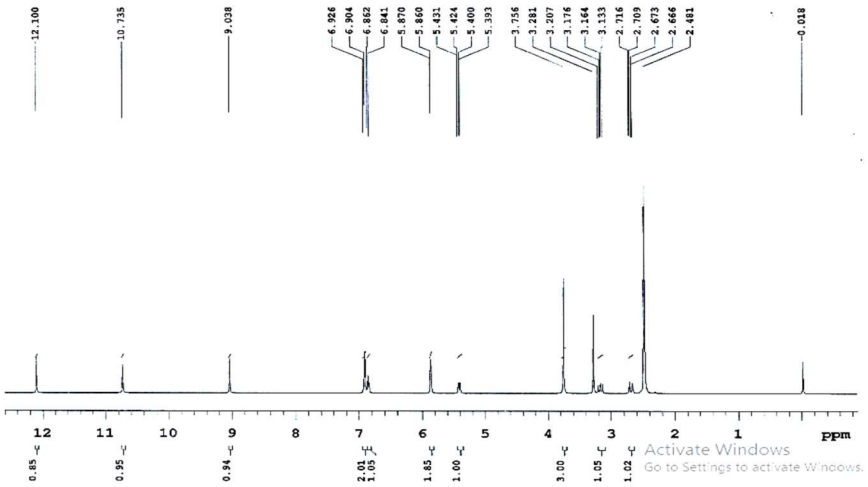
D2O was added to the above proton NMR sample and the analysis was done again. Different NMR spectrum showing peaks only at δ values 2.709 to 6.909, totally 11 protons was obtained (Figure-25, Figure-25a and Figure-25b). So, it is understood that the disappeared protons are three –OH protons. Because of the five –OH protons are exchanged with deuterium of D2O, it becomes –OD.

C) 13C-NMR spectrum.

The 13C-NMR spectrum showed totally fifteen carbon signals including the carbonyl group at 196.08 ppm and aromatic carbons from 55.59-166.61 ppm (Figure-26).

13C-NMR (400 MHz in DMSO, δ ppm). 196.08(C-4), 166.61(C-7), 163.45(C-5), 162.74(C-9), 147.85(C-3'), 146.41(C-4'), 131.10(C-1'), 117.65(C-6'), 114.01(C-5'), 111.89(C-2'), 101.77(C-10), 95.81(C-8), 94.98(C-6), 78.19(C-4'), 55.59(C-OCH3).

From all the spectral evidences, physical properties, chemical properties, LCMS, element analysis, FT-IR, 1H-NMR, D2O exchange NMR, 13C-NMR, the isolated compound-2 is proved to be 2,3-Dihydro-5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-4H-1-benzopyran-4-one or hesperitin.



Several studies reported the isolation and structural elucidation of hesperitin from various plants [333-336]. The structure of can the isolated compound-2 (hesperitin) is shown below.

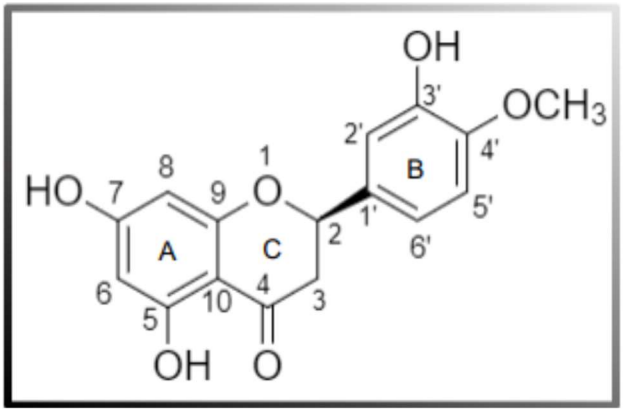


Figure-25: The structure of hesperitin

SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1 Summary of the studies The thesis entitled "Phytochemistry and in vitro organic pastime research of Mallotus rhamnifolius species" offers with physiochemical, phytochemical and pharmacological research of historically used leaves of the medicinal plant "Marai-Yirdiyam" (in Tamil).

5.1.1 Physiochemical evaluation of M. rhamnifolius In physiochemical evaluation, loss on drying, overall ash, acid insoluble ash and water-soluble ash had been done.

5.1.2 Phytochemical screening Phytochemical screening confirmed that the ethylacetate and ethanol: water extracts of M. rhamnifolius possessed an enriched phytoconstitution. The ethylacetate and ethanol: water extracts of M. rhamnifolius ethanolic confirmed the presence of alkaloids, sugars and carbohydrates, steroids, tannins, proteins and amino acids, triterpenoids, flavonoids, anthocyanins, quinones, saponin, phenol, coumarin and anthraquinone.

5.1.3 Antimicrobial pastime The antimicrobial pastime (in opposition to fungi and each Gram-fine and Gram-bad bacteria) changed into finished for all of the crude extracts of M. rhamnifolius.

5.1.4 Antioxidant pastime Antioxidant pastime of various solvent extracts of M. rhamnifolius changed into studied the use of nitric acid radical scavenging pastime, ABTS assay and FRAP technique. All the extracts confirmed antioxidant pastime while as compared with the standard, rutin.

5.1.5 In vitro anti-diabatic assay Anti-diabatic pastime of ethanol: water extract of M. rhamnifolius changed into studied the use of wheat α-amylase inhibitory and yeast α-glucosidase inhibitor technique. The extract confirmed anti-diabatic pastime while as compared with standard, acarbose.

5.1.6 In vitro anti inflammatory pastime Anti-inflammatory pastime of ethanol: water extract of M. rhamnifolius changed into studied the use of protein denaturation and albumin denaturation technique. The extract confirmed the anti inflammatory pastime while as compared with the

standard, aspirin.

5.1.7 Chromatographic separation of robust crude extracts of M. rhamnifolius Ethanol: water extract, the maximum robust extract displaying accurate antioxidant and antimicrobial sports, changed into selected for the column chromatographic separation.

5.1.8 Spectral evaluation of compounds remoted from M. rhamnifolius The remoted and purified compounds had been accumulated and subjected to the spectral research namely, IR, 1D and 2D-NMR spectra, D2O change spectrum, 13C-NMR spectrum and mass spectroscopy. The facts accumulated had been as compared with the prevailing facts to comply compound-1 (Quercetin) and compound-2 (Hesperitin).

5.2 Conclusion and recommendation

From the prevailing research, it's far glaring that the ethanol: water crude extract confirmed robust antioxidant, antimicrobial pastime, anti-diabatic and anti- inflammatory pastime. The compound-1and compound-2 had been remoted from the ethanol: water crude extract of M. rhamnifolius.

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