PHYTOCHEMICAL INVESTIGATION AND PHARMACOLOGICAL EVALUATION OF INDIAN MEDICINAL PLANT EXTRACT, AND CHARACTERIZATION OF BIOACTIVE PHYTOCONSTITUENTS FOR ANTI-OXIDANT ACTIVITY

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Abstract:
A study was conducted to determine the antioxidant potential of methanolic extract Clerodendrum infortunatum Linn. (MECI), which is widely used for many purposes in the Indian indigenous medical system. A variety of well-established in vitro antioxidant tests were used to assess the antioxidant potential, including the reductive power assay, nitric oxide scavenging, superoxide anion radical scavenging, hydroxyl radical scavenging, and total amount of polyphenolic compounds. In every test, MECI was shown to have a high concentration of polyphenolics and strong free radical scavenging action. The presence of the greatest concentration of flavonoids and polyphenolics in it may have contributed to its increased activity.

Keywords: Anti-oxidant Activity, Clerodendrum infortunatum Linn., Polyphenolics, etc.

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Introduction

The partially reduced metabolites of oxygen, known as free radicals, are extremely reactive and poisonous. Most diseases, including ageing, atherosclerosis, cancer, diabetes, liver cirrhosis, cardiovascular problems, etc., are linked to free radicals (Gutteridgde, 1995; Aruoma, 1998). Superoxide anion, hydrogen peroxide, peroxyl radical, and reactive hydroxyl radical are the most prevalent reactive oxygen species. Nitric oxide and peroxynitrite anion are the free radicals produced from nitrogen. One of the most significant processes that produces free radicals in food, medications, and even biological systems is oxidation. Antioxidants are compounds that, at low concentrations, considerably slow down or prevent the substrate from oxidizing (Halliwell, 2000). By reacting with free radicals and other reactive oxygen species within the body and impeding the process of oxidation, antioxidants shield the organism from detrimental oxidation reactions.

Consequently, antioxidant therapy has become extremely important in the prevention of diseases associated with free radicals. The focus of current study is primarily on plants to identify naturally occurring antioxidants. Synthetic antioxidants that are now on the market, such as gallic acid esters, tertiary butylated hydroquinone, butylated hydroxy anisole (BHA), and butylated hydroxy toluene (BHT), may have harmful impacts on health. As a result, there are severe limitations on their use, and replacing them with antioxidants that occur naturally is becoming more popular. Additionally, these artificial antioxidants have moderate antioxidant activity and poor solubility (Branen, 1975). Carcinogenesis and liver damage are thought to be caused by BHA and BHT (Wichi, 1986; Grice, 1988).

Natural antioxidants that have been traditionally used from tea, wine, fruits, vegetables, and spices are already being used commercially as nutritional supplements or antioxidant additions (Schuler, 1990). Examples of such natural antioxidants include sage and rosemary. In the hunt for novel antioxidants, numerous other plant species have also been studied (Koleva et al., 2002; Auddy et al., 2003; Parejo et al., 2003). Nevertheless, there is still a great need to learn more about the antioxidant potential of plant species. It has been suggested that plants' phenolic chemicals may be the cause of their antioxidant action (Cook and Samman, 1996). Flavonoids are a class of polyphenolic chemicals that have been shown to have anti-inflammatory, hydrolytic and oxidative enzyme inhibition, and free radical scavenging effects (Frautchy et al., 2001; Wang et al., 2006; Clavin et al., 2007). Traditional medicine is still widely used, and plants continue to be a rich source of naturally occurring antioxidants that could lead to the creation of new medications.

According to Kirtikar and Basu (2001), Clerodendrum infortunatum Linn. (Family: Verbanaceae) is a hairy, terrestrial shrub with a square, blackish stem and simple, opposite, decussate, petiolate, exstipulate, coriaceous, hairy leaves that have an unpleasant odour. Many Clerodendrum species have been used for millennia in traditional medicine, and research has shown that they have promising therapeutic and antioxidant potential (Rajlakshmi et al., 2003; Chae et al., 2004; Chae et al., 2005; Gopal and Sengottuvelu, 2008). Clerodendrum infortunatum is a widespread plant found in West Bengal and the Indian plains. Tribes employ different portions of the plant to treat colic, snakebite and scorpion stings, tumour, and some skin conditions (Nadkarni and Nadkarni, 2002). According to Chopra et al. (1992), the leaves have a faint bitter taste, can treat skin conditions, inflammation, and smallpox. It was discovered that the plant contained flavonoids, steroids, and triterpenes (Joshi et al., 1978; Sinha et al., 1981; Akihisa et al., 1989). The plant's antimicrobial (Rajakaruna et al., 2002) and anti-malarial (Goswami et al., 1998) qualities have also spurred more research on it. Thus, the goal of this work was to quantify total polyphenolic components and assess the antioxidant activity of C. infortunatum's methanolic extract (MECI) in vitro using several free radical scavenging assays.

Material and Methods

Plant Material

Collection and Drying

Mature leaves were gathered from the Nanded district and then cleaned, dried, and stored out of direct sunlight at room temperature. In a mixer grinder, the dried leaves were reduced in size to a coarse texture. The powdered material was sieved through 60–120 mesh to remove fines and bigger particles, and the powder was used for further evaluation. The particle size difference of crude drug increases the extraction time, and fine particles can form bed and increase the extraction time.

Authentication

The Botanical Survey of India, Pune, employed Dr. C.R. Jadhav, a botanist, to authenticate the plant. upon verification of the distinguishing characteristics. The herbarium sample was stored for future use.

Macroscopy

Size and Shape	The mature leaves long and 8-17 x 6-12 cm
Color	Dark-Green-Light Green.
Odour	Characteristic
Taste	Characteristic

Standardization of Leaf

Identity, quality, and purity of the crude medication are confirmed using physical means. Purity is the absence of any extraneous material, either organic or inorganic, and quality is the essential concentration of the active ingredients in the crude medicine, which elevates it to the status of a significant drug. Using the following standardization parameters, the quality and purity of Clerodendrum Infortunatum L. were determined. The procedures outlined in I.P. 1996 were used to determine the ash value, alcohol and water-soluble extractive value, sulphated ash value, acid-insoluble and water-soluble extractive value, calculation of loss on drying, and foreign organic matter.

Determination of ash value

When determining the quality and purity of a leaf of Clerodendrum infortunatum L., the ash value is crucial. It is mostly made up of various inorganic variables and radicals, such as phosphates, carbonates, and silica, as well as silicates of calcium, magnesium, sodium, and potassium. These inorganic variables might alter the "Total Ash Value." Acid insoluble ash, a concentrated acid treatment, is a method for eliminating these factors.

Total Ash Value

Method

After weighing and adding 2 grammes of fully dried Clerodendrum Infortunatum L. leaf powder to a pre-weighed silica crucible, the mixture was burned at room temperature for no more than 450 degrees until it was carbon-free. By allowing the silica dish to cool in a desiccator, it was discovered. This procedure was carried out repeatedly until a fixed weight was obtained. The percentage of total ash was calculated by taking into account the weight of the first powdered Clerodendrum Infortunatum L leaves.

$$\%\ \boldsymbol{Total\ ash\ value} = \boldsymbol{Wt.of\ total\ ash} \div \boldsymbol{Wt.of\ crude\ drug\ taken} \times \mathbf{100}$$

Water soluble Ash

Method

Powdered Clerodendrum Infortunatum L. was taken, and 2 grammes of it was placed to a silica crucible that had been previously weighed. It was then held at a high temperature—no more than 450 degrees—until it was carbon-free. It was ascertained by weighing and cooling the silica crucible in a desiccator. Up until a constant weight was found, the same procedure was repeated. The resulting ash was then heated for a further five minutes in 25 milliliters of water. The ash that was not soluble in the water was then collected by filtering it through a silica plate and cleaned. A few minutes were spent burning the stuff at a high temperature of no more than 450 degrees. To determine the weight of water-soluble ash, the weight of insoluble materials was subtracted from the total ash. The percentage was calculated by taking the starting weight into account.

$$\%\ \boldsymbol{Water\ soluble\ ash\ value} = \boldsymbol{Wt.of\ total\ ash} - \boldsymbol{Wt.of\ water\ insoluble\ ash} \div \boldsymbol{Wt.of\ crude\ drug\ taken} \times \mathbf{100}$$

Acid-insoluble Ash
Method
In a pre-weighed silica crucible, 2 grammes of dried Clerodendrum Infortunatum L. powder was added. The mixture was then burned at a high temperature—less than 450 degrees—until carbon was removed. By weighing and chilling the silica dish in a desiccator, it was ascertained. Until a steady weight was reached, the same procedure was repeated. After mixing the ash with 25 milliliters of 2M HCL, it was heated for five minutes. Then, an insoluble substance was put to a crucible of silica gel. Once more, hot water was poured, filtered, burned, and allowed to cool in a desiccator before weight was recorded. Considering the starting weight of Clerodendrum Infortunatum L leaves, the percentage was calculated.

$$\%\ Acid\ insoluble\ ash\ value = Wt.of\ acid\ insoluble\ ash \div Wt.of\ crude\ drug\ taken \times 100$$

Sulphated ash value
Method
In the crucible that had been previously weighed, 2 gramme of powdered Clerodendrum Infortunatum L. leaves were added. It was raised to a high temperature of no more than 450 degrees until all carbon was removed. It was ascertained by weighing and cooling the silica crucible in desiccators. Up until the identification of constant weight, the same procedure was repeated. The resulting ash was combined with 1 millilitre of H2SO4 and heated until the white vapors were released. Continued to burn at 800 oC ± 250 C until all the black particles vanished. Direct air was removed from the heating system. It cooled the silica crucible. Once more, a small amount of H2SO4 was added and lit. Repeatedly going through this technique resulted in consistent weight.

Extractive Value Determination
The Indian Pharmacopoeia (1996) was followed in the process of determining extracting value.

Water-soluble extractive value
Method
Five grammes of powdered Clerodendrum Infortunatum L. leaves were weighed, placed to a closed flask, and macerated in 100 milliliters of chloroform water for a day. The leaves were shaken in frequently for the first six hours, after which they were set aside and filtered for eighteen hours. 25 milliliters of the filtrate were taken and evaporated till dry in a preweighed silica dish. By considering the starting weight of Clerodendrum Infortunatum L. leaves, the percentage was ascertained.

Calculations
If 25 ml of aqueous filtrate gives X g of residue,
Then 100 ml of filtrate will give 4X g of residue,
So 5gm of powdered *Clerodendrum Infortunatum L.* contains 4X g of water-soluble residue

Therefore, percentage of water-soluble extractive value will be 80X.

Alcohol-soluble extractive value
Method
In a closed vessel, precisely weigh 5g of powdered Clerodendrum Infortunatum L. leaves and combine it with 100ml of 95% ethanol. For the first six hours, it was occasionally shaken while it was macerated for 24 hours. held back for eighteen hours and gently filtered to prevent ethanol evaporation. A pre-weighed porcelain dish was used to evaporate the filtrate (25 ml), and the weight was determined. The original weight of powdered Clerodendrum Infortunatum L. leaves was taken into consideration in order to calculate the percentage of alcohol soluble extractive value.

Calculations
25 ml of alcohol filtrate possess about A g of residue,
So, 100 ml of filtrate contains 4A gm of residue.
Then this 100ml filtrate was prepared from 5gm of powdered of *Clerodendrum Infortunatum L.*
So 5gm of powdered *Clerodendrum Infortunatum L.* contains 4A gm of residue.
And percentage of extractive value will be 80A gm of alcohol (90%) soluble residue.

Foreign organic matter Determination
Crude medications mostly contain either plant or product parts as foreign materials. It may occasionally include any kind of organism. It also shows the presence of any minerals, such as dust, stones, and dirt, that are not components of medicinal plants. A thin coating of sterile paper was covered with an adequate amount of crude medication. The stuff present other than the crude drug was gathered and weighed with the use of a magnifying lens or by visual inspection.

Determination of Loss on Drying or Moisture content

We dried and weighed the shallow glass-stoppered weighing bottle. After adding 2 grammes of crude medication to the bottle and closing it, the weight was measured and the drug was equally distributed up to a maximum height of 10 mm. After that, the bottle was left open without a stopper in the oven to dry. After drying, the sample maintained its weight. After cooling to room temperature in the desiccator once more, the percentage of weight loss during drying was computed using the Indian Pharmacopoeia (1996).

$$\% \, Loss \, on \, Drying = Loss \, in \, weight \, of \, the \, sample \div Weight \, of \, the \, sample \times 100$$

Table: Physico-chemical Parameters of leaves of *Clerodendrum Infortunatum L.*

Sr. No	Physico-chemical Parameter	*Clerodendrum Infortunatum L.*
1	Foreign Organic Matter	**<2**
2	Total Ash	**9.95% (w/w)**
3	Acid Insoluble Ash	**0.70% (w/w)**
4	Water Soluble Ash	**2.15% (w/w)**
5	Sulphated Ash Value	**0.985%**
6	Moisture Content	**3.65**
7	Extractive Values	**Water=13.50%(w/w) Ethanol=7.50%(w/w) Ether=3.65%(w/w)**
8	Foaming index	**<100**

Extraction Method

A solid from solid separation is represented by the drug's solid extraction. One type of extraction is liquid-liquid, where any one of the two immiscible liquids can be employed (Solvent extraction).

When the concentration gradient between the drug residue and miscella becomes zero, or when the distribution of the extractive ingredient between the two reaches the value "K," the extraction process is stopped. (Mukherjee, 2002).

K=Concentration of extracted substances in the miscella / Concentration of extractive substance in the drug residue

Hot continuous extraction – Soxhlation

The easiest method for making extracts from crude medications is using a Soxhlet extractor. This method uses a pure solvent. In the center of the soxhlet device is a "thimble" composed of cotton or cellulose that holds the crude medication used for extraction. A bottom section is attached to a side arm and a syphon tube. A condenser is attached above the middle compartment, and the extraction solvent is stored in the lower section.

After adding the solvent and heating it to a boil, vapors are formed in the round-bottom flask. The vapors enter the reflux condenser through the side arm. There, the vapor cools and lands on the thimble holding the raw medication that is being extracted. Extraction occurs when the heated solvent passes through the raw medication. The extract is placed in the middle compartment's lower section. The extract is placed in the middle section of the syphon tube, travels through it, and enters the lower container—the round-bottom flask—as the height of the extract reaches the top of the tube. Up till the crude medication was completely extracted, the same procedure was repeated.

Using this extraction method, the extract gathers at the lower RBF and steadily gains concentration. When using a particular solvent, the soxhlet extraction method is quite beneficial for the complete extraction of the crude medication. For the continuous complete extraction, a variety of solvents with increasing polarity can be utilized, such as water, methanol, ethanol, pet ether, benzene, and hexane. When the crude medication was extracted using a different solvent, it was dried. The powder needs to be fully dried and the prior solvent should be eliminated. It stops the prior solvent from combining with another solvent. (Harbone, 1998; Mukherjee, 2002).

Extraction Procedure

To separate the fine and course powder, the dried powder of leaves utilized in the extraction process was sieved through a 60–120 mesh screen. We used this course powder for additional extraction. The extraction was carried out via a soxhlet device and continuous hot extraction until all contents were removed. A drop of extract from the side tube exit was taken, dried, and exposed to iodine vapors on TLC to verify full extraction. If the extraction process is finished, the TLC plate will show no colored spots. Following the extraction process, the solvent was removed using a rotary evaporator and the extracted material was quantified using 95% alcoholic solvent. A desiccator was used to store the extract. (Mukherjee, 2002).

Percentage yield of Extract

A precise 500gm of powdered stem was extracted using the prescribed methods, and the yield % was computed.

Preliminary Phytochemical screening for alcoholic extract of *Clerodendrum Infortunatum L.*

To identify various chemical ingredients, a qualitative phyto-chemical examination of the alcoholic extract of Clerodendrum Infortunatum L. was conducted. Different secondary metabolites were found using different reagents and procedures.

Tests for Carbohydrates: (Peach and Tracey, 1965; Driver,1955)

General test for Carbohydrates (Molisch's test), Benedict's test, Fehling's test, Barfoed's test has been performed.

Tests for Non-Reducing Sugars, Tests for Proteins (Hawk et al.,1954), Test Solution Preparation, Biuret Test (General Test), Million's Test also performed.

Report (HRLCMS)

The report contained a compound table with the following list of compounds (active ingredients) found in the sample:

Sr. No	Rt	Mass	Abund	Name	Formula	Hits (DB)
1	1.027	169.0378	32978	2-Furoylglycine	C7 H7 N O4	10
2	7.351	476.0915		Diosmetin 7-O-beta-D-glucuronopyranoside	C22 H20 O12	7
3	7.985	460.097		Irilone 4'-glucoside	C22 H20 O11	10
4	10.418	284.0662		Prunetin	C16 H12 O5	10
5	10.885	954.4333	51277	Deltorphin A	C44 H62 N10 O10 S2	3
6	10.99	438.2724		LPA (0:0/18:0)	C21 H43 O7 P	4
7	11.345	438.2721		LPA (0:0/18:0)	C21 H43 O7 P	4
8	12.34	381.9678		Fluopicolide	C14 H8 Cl3 F3 N2 O	1
9	12.696	410.2416		LPA (0:0/16:0)	C19 H39 O7 P	9
10	12.756	536.1568		1,4-beta-D-Glucan	C18 H32 O18	8
11	13.04	410.2417		LPA (0:0/16:0)	C19 H39 O7 P	9
12	13.114	482.1737	48648	Melleolide D	C24 H31 Cl O8	6
13	14.872	568.0962	36769	Gadodiamide	C16 H26 Gd N5 O8	2
14	15.064	632.1083	33802	UDP-2,4-bis(acetamido)-2,4,6-trideoxy-beta-L-altropyranose	C19 H30 N4 O16 P2	3
15	15.614	760.1309	30631	Prodelphinidin A2 3'-gallate	C37 H28 O18	3
16	15.637	696.1183	40443	Maclurin 3-C-(2"-p-hydroxybenzoyl-6"-galloyl-glucoside)	C33 H28 O17	2
17	15.947	760.1321	70176	Prodelphinidin A2 3'-gallate	C37 H28 O18	3
18	15.96	696.1194	40761	Maclurin 3-C-(2"-p-hydroxybenzoyl-6"-galloyl-glucoside)	C33 H28 O17	2
19	16.123	478.2953		25-Acetylvulgaroside	C27 H42 O7	5
20	16.292	838.1608	52659	Beta-Alanyl-CoA	C24 H41 N8 O17 P3 S	1
21	16.368	760.1313	55138	Prodelphinidin A2 3'-gallate	C37 H28 O18	3
22	18.536	562.3759	52127	Rhodoxanthin	C40 H50 O2	5

Looking at the above table, it was confirmed that it should be isolated because it included rhodoxanthin and LPA (lysophosphatidic acid).

The following are the reasons why LPA was chosen:

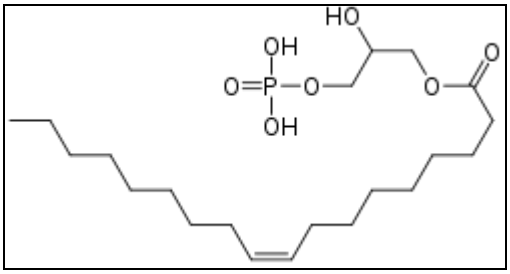
Our current study aims to investigate its anti-asthmatic activity, which has been previously reported.

2. There is a larger rate of hits of constituents, or the amount of active constituent in the sample, which raises the success rate of the isolation procedure.

Characterization by Chromatographic Studies

Lysophosphatidic Acid (LPA)

Since it was discovered that lysophosphatidic acid (LPA), a water-soluble phospholipid, functions as a strong signaling molecule with a wide range of effects on many distinct target tissues, it has attracted a lot of attention. Several factors have connected abnormal LPA-signaling to cancer, including its capacity to promote cell proliferation.

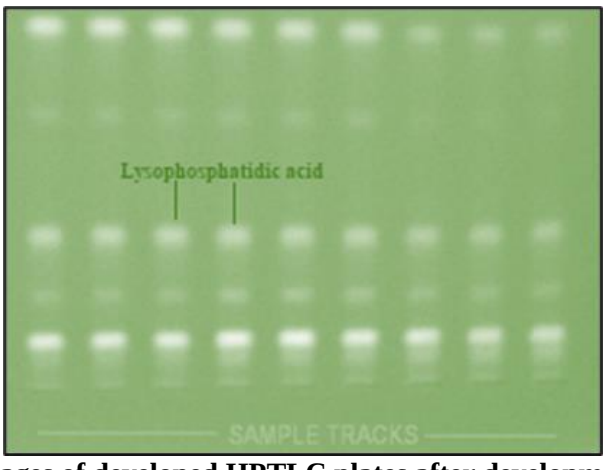


Structure of Lysophosphatidic acid (LPA)

High Performance Thin Layer Chromatography Method (HPTLC) was used to isolate and purify the material in preparation for more research. It was discovered how to isolate LPA (lysophosphatidic acid).

Method

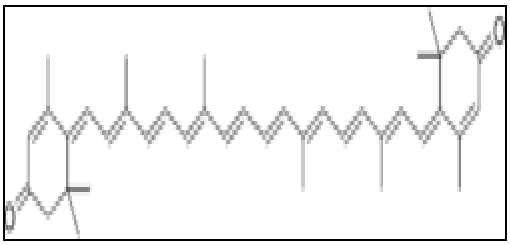
1. A mixture of 65 ml of chloroform, 32 ml of methanol, and 5.5 ml of ammonium hydroxide was utilized as the mobile phase in the development.
2. Using a micropipette, the sample was transferred to the HPTLC plates that had already been coated. It was then allowed to air dry for five minutes at room temperature.
3. The plates were left in the mobile phase-containing container for ninety minutes.
4. The plates were taken out of the chamber and allowed to dry for three minutes at room temperature following HPTLC separation.
5. 0.1% 8-anilino-1-napthalene-sulfonic acid was sprayed at the plate's marking lane to track the flow of the purified chemical.
6. The sample was removed from the plates using the marker lane as a guide.
7. The recovered powder was mixed with 2 milliliters of 2:1 (v/v) methanol-chloroform.
8. Rotted, placed on ice for an hour, then centrifuged for ten minutes at 3000 x g at 10 °C.
9. The top layer was placed inside the organic phase-containing tube and nitrogen-dried.
10. For the second extraction, 1.5 milliliters of 2:1 (v/v) methanol-chloroform was added to the powder once more.
11. After vortexing the mixture, it was centrifuged for ten minutes at 3000xg and 10 degrees.
12. The top layer was transferred to an organic phase-containing tube and nitrogen-dried.
13. The refined substance was divided and kept for additional examination.



Images of developed HPTLC plates after development

Rhodoxanthin

Purple-colored xanthophyll pigment rhodoxanthin is present in trace amounts in many different plants, such as Taxus baccata and Lonicera morrowii. Certain birds' feathers also contain it. It is used as a food colouring under the E number E161f as an additive. It is permitted for usage in Australia and New Zealand but not in the US or the EU.



Structure of Rhodoxanthin

Thin Layer Chromatography Method (TLC) was used to isolate and purify the material in preparation for more research. It was discovered how to isolate rhodoxanthin.

Method

1. Polygram Sil-G (Macherey-Nagel) precoated silica gel layers were subjected to partition TLC.
2. Adsorption TLC over a mixed layer of CaCO3, MgO, and Ca (OH)2 (30:6:5) allowed for additional purification.
3. Petroleum benzene (100–1400C) and iso-propanol mixtures were utilized as solvents in both systems; the ratio was adjusted (from 100:5 to 100:16; v/v) to achieve the best separations.
4. The carotenoids were ultimately run on pre-coated, methanol-prewashed layers of ultrapure silica gel type G-25 HR for mass spectrometry.
5. The colored bands were scraped off following TLC, and acetone was used to elute the pigments.

Observations

Under 486 nm in three fractions of rhodoxanthin isolated from Clerodendrum infortunatum using TLC.

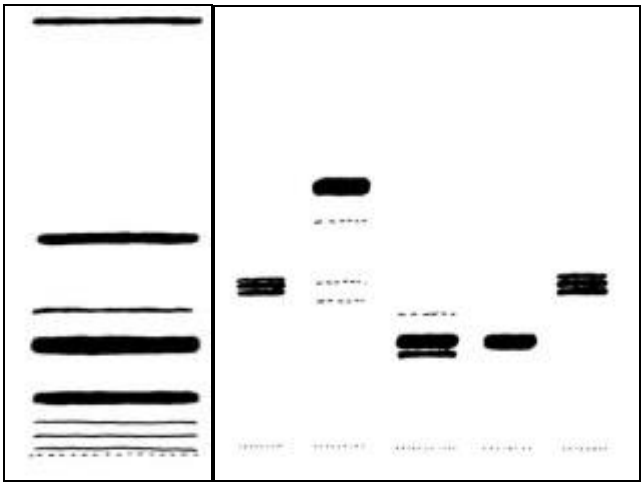


Image of TLC plate

Antioxidant activity: Following listed activities have been listed

-Determination total polyphenol compounds
-DPPH radical scavenging activity
-Nitric oxide scavenging activity
-Superoxide anion scavenging assay
-Hydroxy radical scavenging activity
-Reducing power assay

Result and Discussion

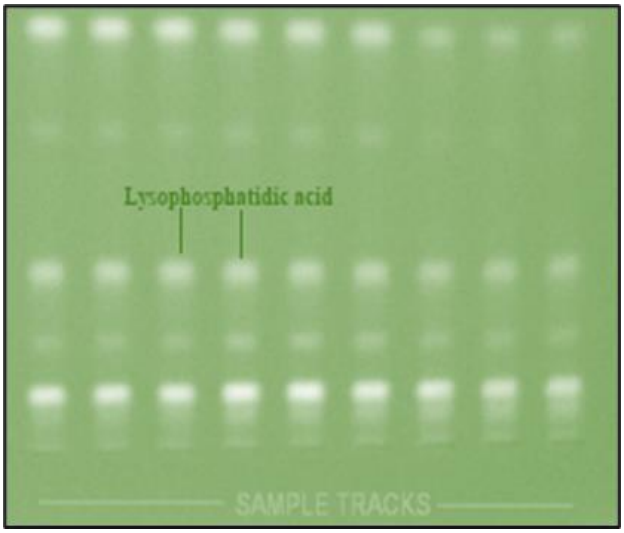
Table: Physico-chemical Parameters of leaves of *Clerodendrum Infortunatum L.*

Sr. No	Physico-chemical Parameter	*Clerodendrum Infortunatum L.*
1	Foreign Organic Matter	**<2**
2	Total Ash	**10.96 % (w/w)**
3	Acid Insoluble Ash	**1.71 % (w/w)**
4	Water Soluble Ash	**3.16 % (w/w)**
5	Sulphated Ash Value	**1.986 %**
6	Moisture Content	**4.66**
7	Extractive Values	**Water=14.5%(w/w)** **Ethanol=8.51%(w/w)** **Ether=4.66%(w/w)**
8	Foaming index	**<100**

Result: The % yield of 95% alcoholic extract of *Clerodendrum Infortunatum L.* = %w/w.

Table: Preliminary Phyto-chemical screening of *Clerodendrum Infortunatum L.* extract.

Sr. No	Name of test	*Clerodendrum Infortunatum L. (Present/Absent)*
1	Test for Carbohydrates	**Present**
2	Test for Proteins	**Absent**
3	Test for Phenolics	**Present**
4	Flavonoids Test	**Present**
5	Test for Glycosides	**Present**
5a	Cyanogenic Glycosides	**Absent**
5b	Tests for Anthraquinone Glycosides	**Absent**
5c	Saponin Glycosides	**Present**
5d	Cardiac Glycosides	**Absent**
6	Alkaloids Test	**Absent**
7	Test for Tannins	**Present**
8	Test for Coumarins	**Present**
9	Test for Saponins	**Present**
10	Test for Steroids	**Absent**



Images of developed HPTLC plates after development

Observations: Under 486 nm in three rhodoxanthin fractions obtained by TLC from *Clerodendrum Infortunatum*.

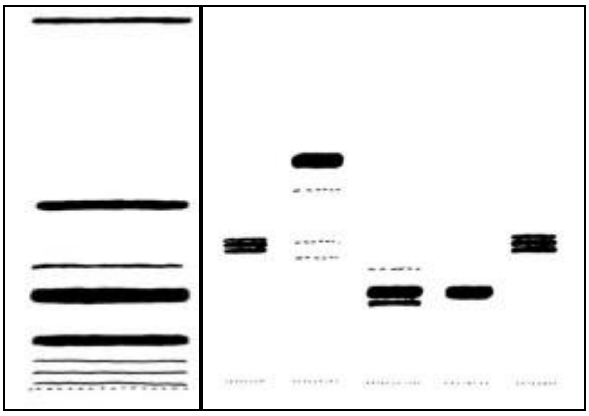


Image of TLC plate

Mass Spectra

Lysophosphatidic Acid

A molecular ion peak at m/z 409.2344 (M-1) is visible in the mass spectrum, and it agrees well with the structure of known lysophosphatidic acid (LPA), as proposed.The figure displays the mass spectra.

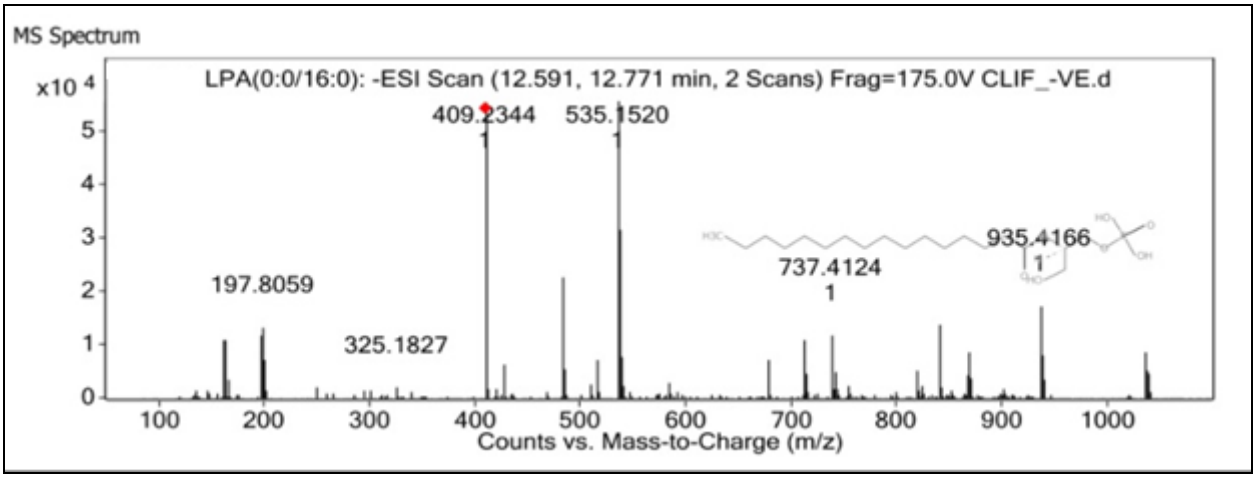
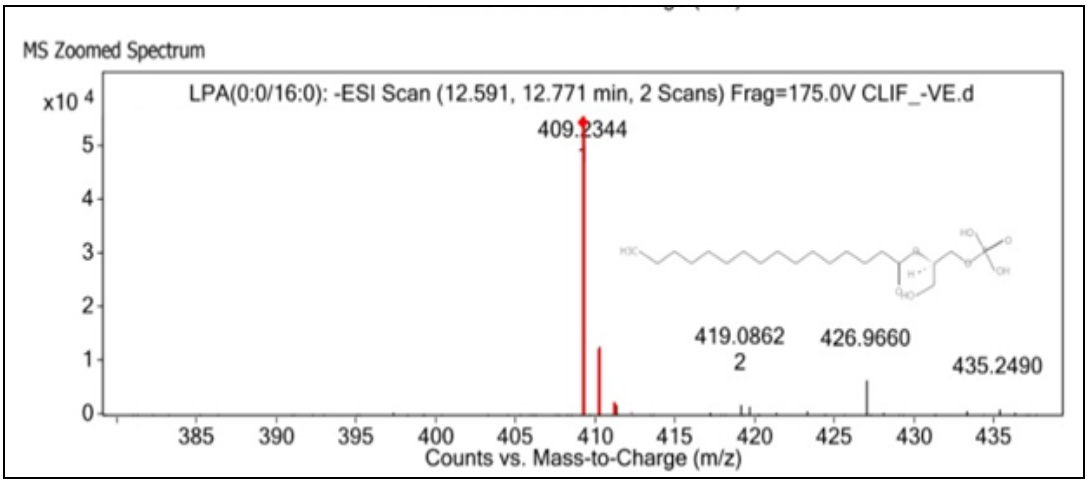
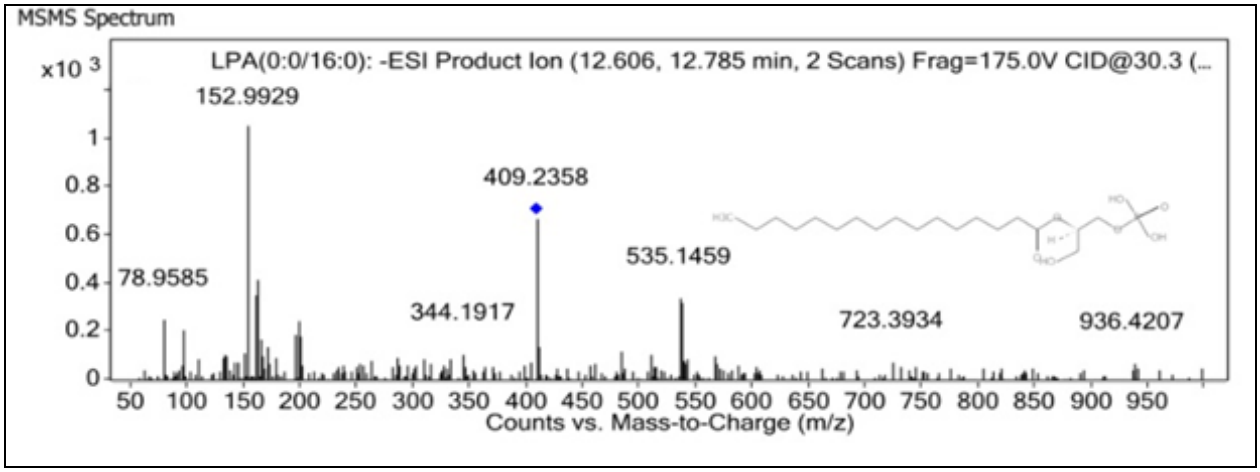


Figure: The mass spectra of Compound 1 (LPA)



MS Spectrum Peak List

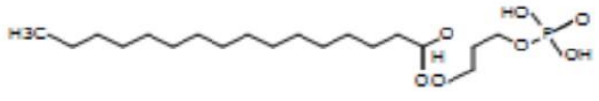
m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
197.8059				13526.64		
409.2344	409.2361	4.12	1	55418.56	C19 H39 O7 P	(M-H)-
410.2373	410.2395	5.43	1	12797.54	C19 H39 O7 P	(M-H)-
411.24	411.2419	4.57	1	1932.16	C19 H39 O7 P	(M-H)-
483.271			1	22958.2		
535.152			1	83451.6		
536.1548			1	22340.9		
537.1494			1	31638.47		
839.1786			1	14056.59		
935.4166			1	17502.39		



MS/MS Spectrum Peak List

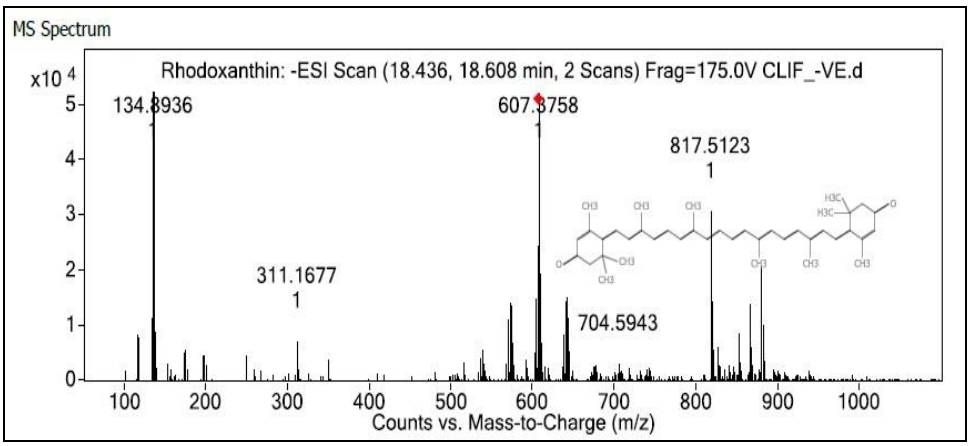
m/z	z	Abund
78.9585		250.4
96.9691		208.65
152.9929		1055.51
160.8403		351.85
162.8379		414.9
195.8098		190.4
197.8038		246.87
409.2358	1	668.27
535.1459	1	340.7
537.15	1	320.61

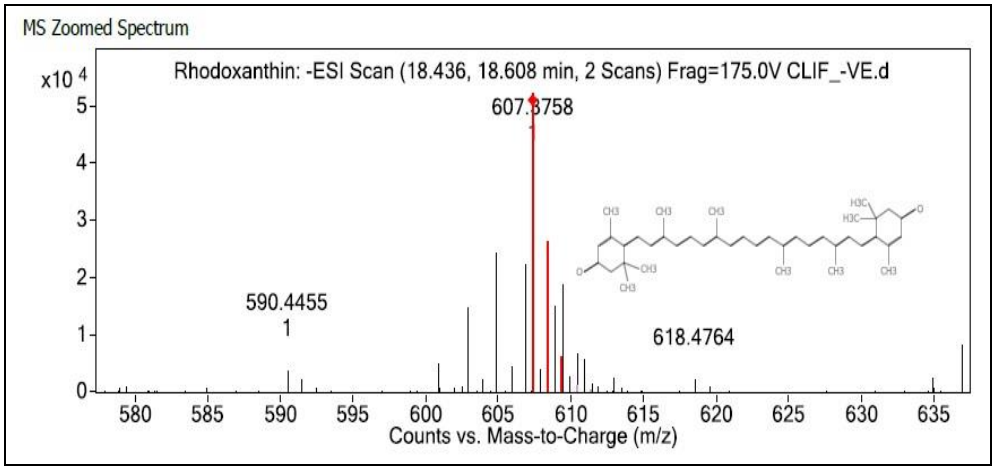
Compound Structure

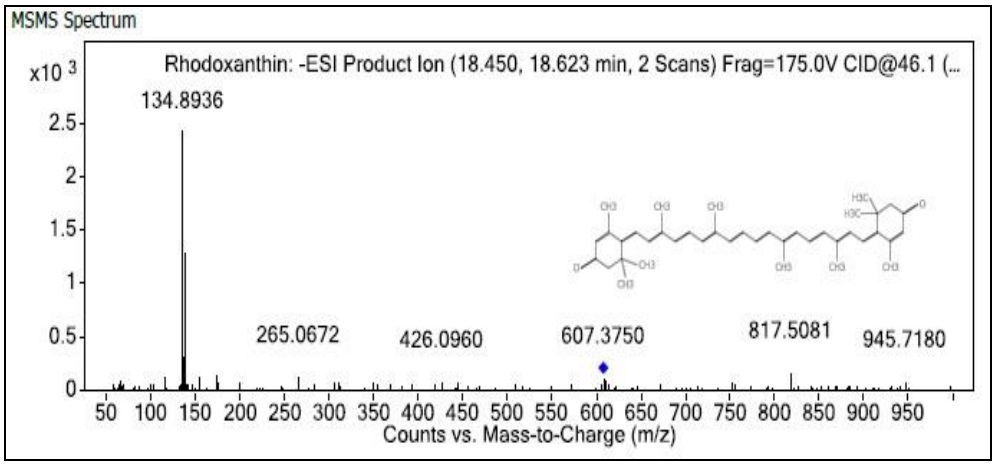


Rhodoxanthin

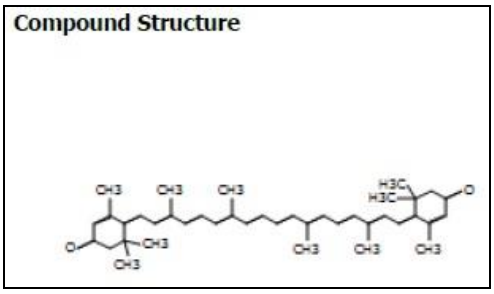
A molecular ion peak at m/z 607.3758 (M-1) is visible in the mass spectrum, and this peak is in good accord with the known rhodoxanthin's suggested structure. The figure displays the mass spectra.







m/z	z	Abund
115.9177		135.28
134.8936	1	2440.76
135.8914	1	318.32
136.891	1	1285.52
153.8631		138.56
172.8431		142.07
265.0672		124.39
607.375	1	115.7
608.7686		102.03
817.5081		165.94



NMR Analysis

Lysophosphatidic acid

The mass [M+H] + of 409 was ascertained by 13-C NMR analysis and LC-MS. Based on a study of NMR data, the compound was identified as lysophosphatidic acid.

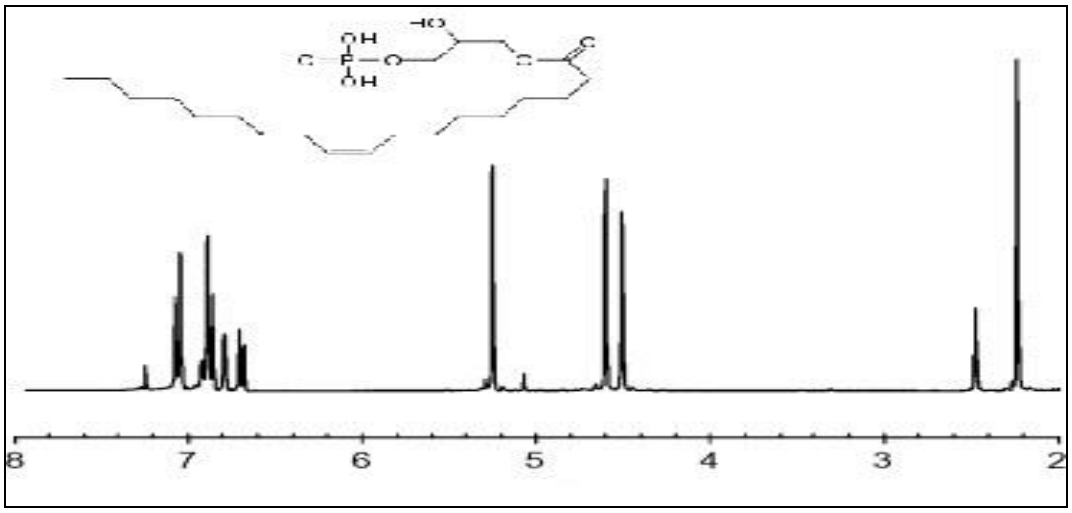


Figure: Zoomed NMR spectrum of Compound LPA

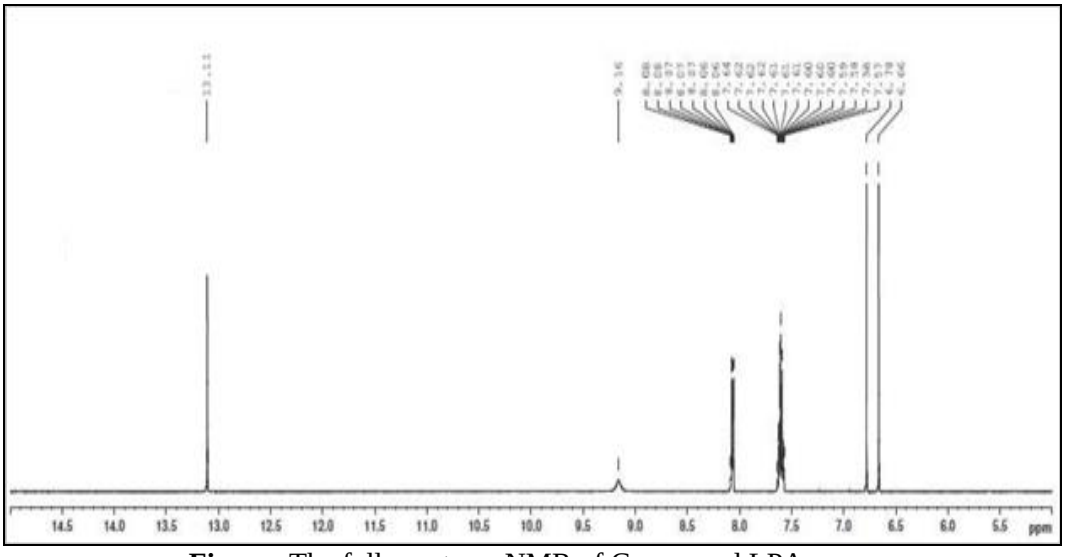


Figure: The full spectrum NMR of Compound LPA

Rhodoxanthin

The mass [M+H] + of 607.3758 was ascertained by 13-C NMR analysis and LC-MS. Rhodoxanthin was the compound identified via NMR data analysis.

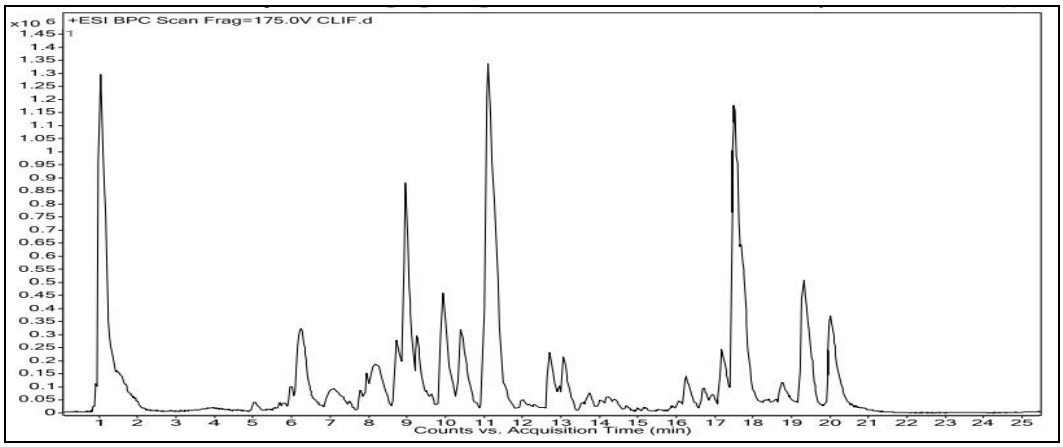
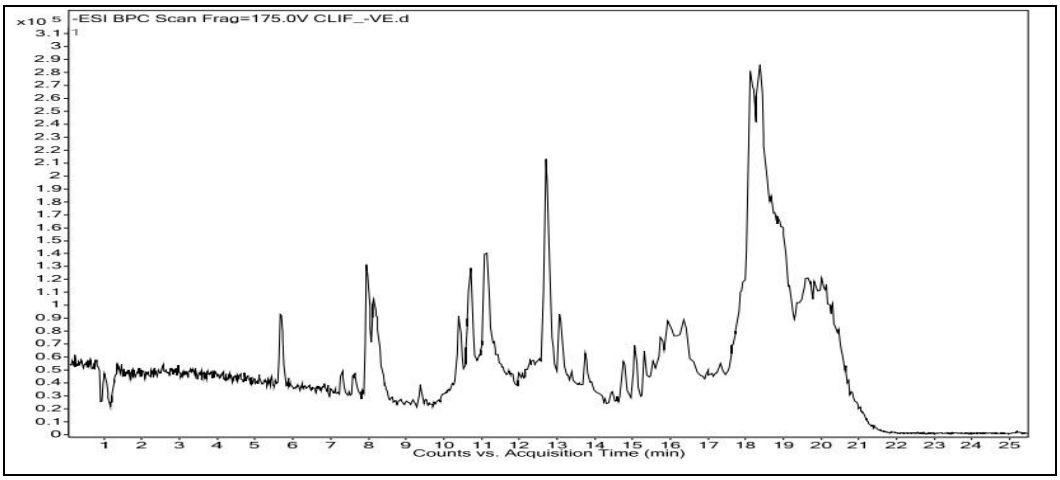


Figure: Full spectrum of Rhodoxanthin



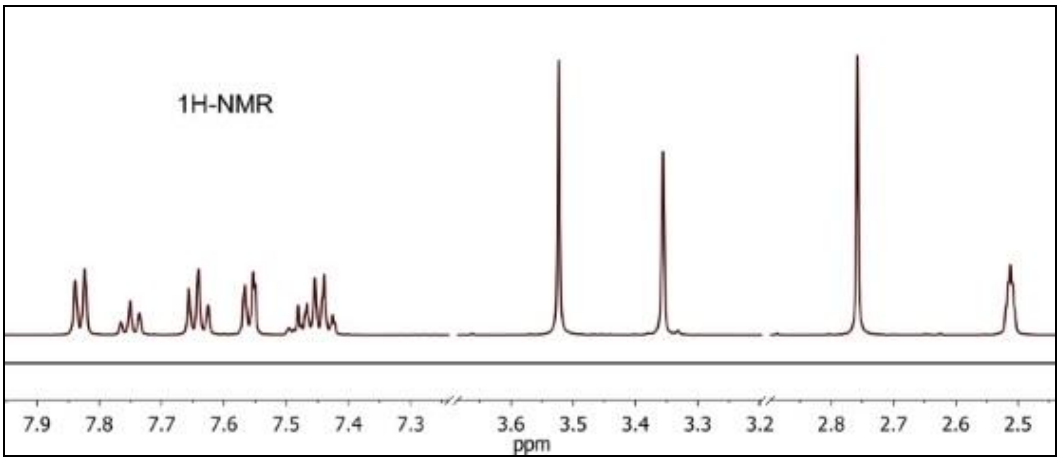


Figure: Zoomed spectrum of Rhodoxanthin

Antioxidant Activity

It was determined that the total amount of phenolic compounds in MECI was 55.6 ± 9.24 µg mg-1 pyrocatechol equivalent. Following table shows all the outcomes of MECI's free radical scavenging potentials. Table lists the IC50 values for some common antioxidants and MECI. In the hydroxyl radical scavenging assay, the methanol extract's IC50 value was discovered to be significantly lower—66.9 µg/ml. A concentration-dependent reductive effect was likewise demonstrated by the C. infortunatum methanol extract (Figure).

Table: Radical scavenging activities of methanol extract of Clerodendrum infortunatum Linn. at different concentrations

MECI concentration (µg/ml)	**% inhibition**			
	DPPH radical scavenging	**Nitric oxide scavenging**	**Superoxide scavenging**	**Hydroxy radical scavenging**
10	23.19 ± 3.3	19.57 ± 2.2	16.36 ± 0.98	32.24 ± 1.3
25	31.34 ± 3.9	29.43 ± 3.4	23.36 ± 2.22	37.66 ± 4.6
50	35.26 ± 3.0	40.26 ± 4.3	33.33 ± 1.99	46.64 ± 6.6
75	43.07 ± 3.6	50.37 ± 5.4	46.97 ± 5.22	53.37 ± 4.7
100	59.86 ± 4.6	60.15 ± 6.0	53.37 ± 4.45	64.66 ± 7.0
IC50 (µg/ml)	87.8	77.2	91.2	66.9

*Values are expressed in mean ± S.D., n = 3.

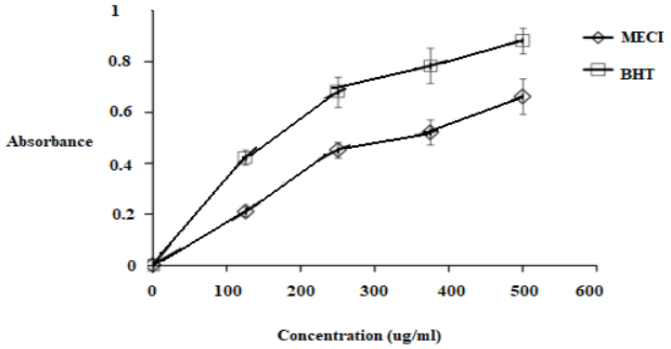


Fig. Reducing power of methanol extract of Clerodendrum infortunatum Linn. and BHT by spectrophotometric detection of the Fe3+-Fe2+ transformations

Conclusion

A variety of models are available for assessing antioxidant activity. Depending on the test used, the chemical complexity of the many fractions and compound mixtures present could produce inconsistent results. Thus, a multi-assay method would be more instructive, although not necessary, when assessing the antioxidant capacity of extracts. The study's findings, which provide the first details on C. infortunatum Linn.'s antioxidant activity, unequivocally show that the MECI is capable of efficiently scavenging a range of free radicals and reactive oxygen species in vitro. The methanol extract's ability to scavenge free radicals may be attributed to its high concentration of polyphenolics. The presence of hydroxyl groups in flavonoids and polyphenolics may be the cause of the methanol extract's ability to reduce FCR. According to certain reports, phenolic compounds play a crucial function in scavenging free radicals. Because of their hydroxyl groups, they have been shown to remove radicals. They also directly contribute to the system's antioxidant function and play a significant part in stabilizing lipid oxidation. The process of scavenging direct radicals is exemplified by its effect on superoxide and DPPH radicals. Potent pleiotropic mediator of physiological processes, nitric oxide (NO) regulates cell-mediated toxicity, inhibits platelet aggregation, smooth muscle relaxation, and neuronal signaling. As an effector molecule in a variety of biological systems, such as neuronal messenger, vasodilation, and antibacterial and anticancer actions, it is a diffusible free radical. Research conducted on animal models has indicated that NO plays a part in the pathophysiology of pain and inflammation. Additionally, studies have demonstrated that NOS inhibitors can alleviate some of the tissue alterations and inflammation observed in inflammatory bowel disease models. A powerful oxidant, the hydroxy radical interacts with nearly every biomolecule present in living cells. In this experiment, deoxyribose is incubated with a combination of FeCl3–EDTA and H2O2 in phosphate buffer (pH 7.4). Malondialdehyde (MDA) is synthesized during the breakdown of deoxyribose by OH· produced by Fenton systems. MDA can be identified by its capacity to react with TBA to form a pink chromogen. Its possible antioxidant activity can be significantly indicated by the reducing power assay. However, other mechanisms for their antioxidant activity have been hypothesized, including reductive capacity, radical scavenging, chain initiation prevention,

binding of transition-metal ion catalysts, peroxide breakdown, and prevention of continuing hydrogen abstraction. The existence of various Reductones is typically linked to the reducing properties. Reductones work as antioxidants by donating a hydrogen atom to break the chain of free radicals. Additionally, Reductones react with several peroxide precursors to stop the creation of peroxide. The antioxidant activity of certain fractions may be attributed to their reductive power. MECI scavenges free radicals like DPPH, nitric oxide, superoxide, and hydroxyl radicals, demonstrating strong in vitro antioxidant activity. The wide spectrum of action exhibited by MECI implies that the antioxidant activity is mediated by a variety of pathways. The link between free radicals and several diseases is widely established. The current findings support the plant's ability to scavenge free radicals, which explains why it has been traditionally used to treat a variety of illnesses. However, more research is required to clarify the various antioxidant mechanisms and potential synergies between the isolated compounds.

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