https://doi.org/10.48047/AFJBS.6.7.2024.2704-2724

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Research Article

IN VITRO ASSESSMENT OF ANTIOXIDANTS AND HEPATOPROTECTIVE ACTIVITY OF *OPILIA CELTIDIFOLIA*

ANUBHAV DUBEY[1*], SAMRA[1], VIKRAM KUMAR SAHU[1] AMIT MISHRA[2]

1.Maharana Pratap College of Pharmacy, Kothi, Mandhana, Kanpur, Uttar Pradesh,India.

2.Maharana Pratap College of Pharmaceutical Sciences, Kanpur Uttar Pradesh, India.

***Corresponding author**

ANUBHAV DUBEY[1*]

E-mail: anubhavdwivedi803@gmail.com

Article History

Volume6,Issue7,April2024
Received:3 June 2024

Accepted: 20 June 2024

Published:27June2024

doi:10.48047/AFJBS.6.7.2024.2704-2724

Abstract

Opilia celtidifolia is a long-used herb with intriguing biological activity that has been employed in many different medicinal applications. This research aims to evaluate the hepatoprotective and antioxidant properties of the aqueous and ethanolic extracts of *Opilia celtidifolia* in vitro.

Using the DPPH and ABTS tests, the extracts' antioxidant properties were evaluated. The hepatoprotective properties were assessed in *HepG2 cell lines* subjected to hydrogen peroxide (H_2O_2)-induced oxidative stress. The hepatoprotective effects were assessed using cell viability, lipid peroxidation, and the activity of the enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

The ethanolic extract demonstrated exceptional antioxidant qualities in each test and performed better than the aqueous extract in scavenging free radicals. In the hepatoprotective test, pre-treating *HepG2 cells* exposed to H_2O_2 led to a significant increase in cell survival and a reduction in lipid peroxidation. Additionally, both extracts were able to effectively reduce the elevated levels of AST and ALT, with the ethanolic extract exhibiting a somewhat higher protective effect.

The findings show that *Opilia celtidifolia's* ethanolic extract has potent antioxidant and hepatoprotective properties.These results underscore the plant's historic usage in liver-related ailments and its potential as a natural antioxidant and hepatoprotective medication source.

Keywords: *Opilia celtidifolia*, Antioxidant activity, Hepatoprotective activity, Aqueous extract, Ethanolic extract.

INTRODUCTION

The liver is a vital organ that performs a variety of vital functions necessary to maintain overall health. The liver engages in several life-supporting processes, including the metabolism of nutrients, the production of vital proteins, and the detoxification of toxic compounds. However, a group of illnesses, collectively known as hepatoprotective diseases, may have an effect on this important organ.

Opilia celtidifolia Many countries have long used Linn, a member of the Opiliaceae family, for its therapeutic qualities in traditional medicine. People have used the roots of *Opilia celtidifolia*, mostly found in tropical and subtropical areas, to treat oxidative stress-related illnesses, inflammation, and liver problems. Despite millennia of use, science has yet to thoroughly investigate the therapeutic qualities of this plant, particularly its hepatoprotective and antioxidant properties. To back up the traditional use of *Opilia celtidifolia* roots and look for possible medical uses, this study looks at the liver-protecting and antioxidant properties of the roots' aqueous and ethanolic extracts.

The aims are to compare how well the two extracts work, see how they protect liver cells from chemicals that are harmful to the liver, check for antioxidant activity using standard methods, and look for possible bioactive components through phytochemical analysis. According to the hypothesis, these extracts show notable hepatoprotective and antioxidant properties, with the ethanolic extract perhaps having more efficacy since the bioactive components were better extracted. This study will support the traditional use of *Opilia celtidifolia* and look into its potential as a natural liver health and oxidative stress management therapy. The goal is to learn more about the plant's pharmacological potential[1].

Materials and Methods

Plant collection and extractions:

We collected a small amount of *Opilia celtidifolia* roots from the herbal garden of the Maharana Pratap College of Pharmacy in Kanpur, India. We placed Vocher specimen **J/Bot/OC-R/13** at the herbarium house of A.P.S. University, Rewa, M.P., India's Janta Postgraduate College, and confirmed its legitimacy there. We removed the plant's roots and dried them at 450 °C in a separate oven. We used a machine to grind the dried roots into a powder. The maceration process used 500 milliliters of ethanol and water to extract 52 grams of powdered roots. We then evaporated the filtrate in a water bath to concentrate it. The formula was used to weigh the drained extracts and determine the yield of the soluble components.

$$\text{Yield \%} = \frac{\text{Weight of dry extract} \times 100}{\text{Weight of dry roots powder}}$$

We kept the dried extract at 40 °C for further research.

Chemicals

Vikas Sales Corporation, India, provided all the AR-grade components.

Qualitative phytochemical screening

We performed a standard qualitative investigation of the aqueous and ethanolic extracts to confirm the existence of different bioactive components.

Test for alkaloids.

- **Dragendorff's Test:** Add a few drops of Dragendorff's reagent to the filtrate; the formation of a reddish-orange precipitate indicates the presence of alkaloids.
- **Mayer's Test:** We added a few drops of Mayer's reagent to the filtrate; the formation of a white or cream-colored precipitate indicates the presence of alkaloids.
- **Wagner's Test:** We added a few drops of Wagner's reagent to the filtrate; a reddish-brown ppt formation indicates the presence of alkaloids.
- **Hager's Test:** We added a few drops of Hager's reagent to the filtrate; the formation of a yellow precipitate indicates the presence of alkaloids.

Test for Tannins

- **Test for ferric chloride:** Add a few drops of 5% ferric chloride solution to the sample; a dark blue or greenish black color indicates the presence of tannins.

Test for Phenols

The sodium nitrate test involves adding a small amount of powdered root material to the test tube, adding 1 ml of phenol solution, gently heating the mixture, allowing it to cool, and then adding 1 ml of concentrated sulfuric acid to the cooled mixture. The presence of a deep blue color indicates the presence of phenol.

Test for Glycoside

The Keller-Killani test involved taking a 2 ml extract of Opilia celtidifolia root, adding glacial acetic acid to it, and then adding one drop of a 5% ferric chloride solution to the test tube. Finally, we added concentrated sulfuric acid to the test tube, causing a blue solution to appear, indicating the presence of glycosides.

Test for Steroids

We mixed a crude extract of a portion with 2 ml of chloroform, then added concentrated sulfuric acid to the test tube, allowing it to flow down the sides. A red color in the lower chloroform layer indicates the presence of steroids.

Test for saponins

Add about 0.5 g of root extract to 5 ml of distilled water, shake well, and gently warm the mixture. Persistent frothing even after warming indicates the presence of saponins.

Test for Terpenoids

- **Salkowski Test:** We took a portion of crude extract and added concentrated sulfuric acid to the test tube, allowing it to flow down the slides. A reddish brown color in the lower chloroform layer indicates the presence of terpenoids.
- **Keto-Sugar Test:** Add a crude extract, 2 ml of chloroform, and a few drops of Wagner's reagent to the test tube along the sides. A reddish-brown color in the lower chloroform layer indicates the presence of keto sugars[2]**.**

Determination of Anti-oxidant activity

1, 1 Diphenyl-2-picrylhydrazyl radical (DPPH) scavenging activity:

We combined a 0.1 mM DPPH solution with 5 l of a different stock of the test medication in a 96-well plate. The reaction setup was duplicated three times, and five microliters of different chemical dosages and 0.2 milliliters of DMSO/methanol were used to create blanks [3].We referred to the untreated wells as control wells. We kept the plate in the dark for thirty minutes. We used a microplate reader (iMark, BioRad) to find the decolorization at 517 nm at the end of the incubation. The control was a reaction mixture containing 20 µl of deionized water. In comparison to the control, the expression for scavenging activity was "% inhibition." We used Graph Pad Prism 6 software to calculate the IC_{50}. On graph , we plotted the sample concentration (X axis) and control percentage (Y axis) against each other [4].

DPPH Scavenging has a value of = ((Abs Control-Abs Sample)/Abs Control)×100.

ABTS (2,2'-azino-bis 3-Ethyl benzo thiazoline-6-sulfonic Acid) Radical Cation Decolorization Assay:

We mixed and diluted APS (2.45 mM) and ABTS (7 mM) solutions 100 times to create ABTS (SRL-Chem-Cat no. 28042) radicals. We then utilized these radicals to create the ABTS free radical reagent. 200Ascorbic Acid-SD Fine-F13A/0413/1106/62 was used as an alternative standard stock, along with 200µl of the ABTS free radical reagent and the samples. These were then put on a 96-well platWe then left the dish to stand at room temperature in the dark for 10 minutes. We referred to the untreated wells as control wells. Using a microplate reader (iMark, BioRad), determine the decolorization's absorbance at 750 nm after incubation. We displayed the results for the negative control. We determined the IC_{50} using Software Graph Pad Prism 9.5.1. We displayed the control percentage (Y axis) and sample concentration (X axis) against each other on a graph[4].

MTT Assay for *in-vitro* cytotoxicity study of Aqueous and Ethanol extract of *Opilia Celtidifolia* root extract

We used the MTT assay to assess the cytotoxicity of the substances given to the *HepG2 cell line* (acquired from NCCS Pune). We cultured 10,000 cells per well in a 96-well plate for 24 hours at 37°C and 5% CO_2 in DMEM medium (Dulbecco's Modified Eagle media-AT149-1L) with 1% antibiotic solution and 10% FBS (Fetal Bovine Serum; HIMEDIA-RM 10432). The next day, we treated different cell concentrations. The cells in the control group received no attention at all. We treated the cell culture with MTT solution and incubated it for an extra two hours after the first 24-hour incubation period Dimethyl sulfoxide (DMSO-SRL-Cat no.-67685) was used to break up the cell layer matrix and get rid of the culture supernatant after the experiment. . We used the Elisa plate reader (iMark, Biorad, USA) to measure the data at 540 and 660 nm. We calculated the IC_{50} using GraphPad Prism 6. We used an AmScope digital camera (10 MP Aptima CMOS) and an inverted microscope (Olympus eK2) to take the photos[4].

Investigation of *In vitro* Hepatoprotective activity of (Ethanolic and Water) plant *Opilia Celtidifolia*.

Cell Culture

We received the *HepG2 cell line* from NCCS Pune. We removed 90% confluency cells from the flask and evenly distributed them in 96-well plates using DMEM medium (Dulbecco's Modified Eagle Media-AT149-1L), supplemented with 10% FBS (Fetal Bovine Serum-HHIMEDIA-RM 10432) and 1% antibiotic solution at 37°C with 5% CO_2. The following day, we removed the medium and replaced it with fresh culture media on each plate. A total of 10% (5 to 50µl) of the medium was added to the wells that were set aside for the treatment dilutions, which came in different concentrations. The treated plates were then put in a Heal Force-Smart cell CO_2 Incubator-Hf-90 for 24 hours [5].

Scavenging of Superoxide Anions.

We combined standard concentrations of the riboflavin solution with a variety of extracts, then incubated the combination at room temperature for 30 minutes on a 96-well plate. We added the reaction mixture to the previously incubated mixture and thoroughly stirred it after incubation. We referred to the untreated wells as "control wells." Next, we measured the absorbance at 560 nm using an Elisa plate reader (iMark, Biorad, USA). GraWe calculated the IC_{50} using GraphPad Prism 6. displayed the control percentage (Y axis) and sample concentration (X axis) against each other on a graph [16].

The scavenging activity (%) was calculated as follows:

[1-(sample at −A550 nm / control at ΔA550 nm)] x the scavenging rate (%) equals 100%.

As opposed to

At a distance of 70 seconds, the absorbance change for the sample is represented by ΔA550 nm, sample, while the absorbance change for the control is represented by ΔA550 nm, control.

Estimation of Reduced Glutathione Activity

There were 140µl of NADPH (SRL Chem-99197) solution,20µl of DTNB (HiMedia-GRM1677) solution, 35µl of water, and 5µl of sample (the IC_{50} concentration of the sample). Put them in a 96-well plate. We developed controls (those not receiving therapy) and reference standards (glutathione solution, SRL Chem-074011). The plates were read using an iMark Biorad USA microplate reader set to 415 nm [11].

Activities of Aminotransferases Activity Assay

We used the SGOT Kit (ADX226) to measure the activity of aminotransferases. The cuvette contained 100 µl of material. 200 µl of Reagent 2 and 800 µl of Reagent 1 were added to distilled water, and the combination was then incubated at 37 °C for three minutes. Once a minute has passed, repeat the absorbance measurements for the first and second minutes. The Selectra Pro-S instrument was used to measure the absorbance at 340 nm [7].

Determination of Lipid Peroxidation

TBARS Assay KIT (Cat. No. OPKA003) was used in this investigation. Following the addition of 100µl of emulsion to 1800µl of solution B (TBA Reagent - TBARS Assay KIT; Cat. No. OPKA003), the mixture was incubated at 80°C for 15 minutes. The absorbance was measured at 532 nm relative to a blank, which consisted of all the compounds except the samples.1.56 x 105 M-1cm-1 was the estimated extinction coefficient for malondialdehyde concentration[6].

Malondialdehyde concentration (M) = (Absorbance $_{532\ nm}$ / 1.56 x 10^5) ×dilution factor

Activity of Alkaline Phosphatase (ALP)

Activity of Alkaline Phosphate We performed the test using the Alkaline Phosphate Test Kit (Cat. No. KEA017). Each well of a 96-well plate received 10 milliliters of both the sample and reference. Fill each well in the designated set with 50 µl of AMP buffer containing 50 µl of PNP substrate. Incubate for fifteen minutes at 37°C. The process was halted by adding 10 µl of NaOH. We measured the absorbance at 405 nm in a microplate reader [13].

The ALP activity U/mg of the protein is calculated using the following formula:

The ALP activity in the test (mol/min) is equal to Test absorbance × standard PNP amount)/Standard absorbance ×15 B. [The total protein amount (mg) of the cellular lysate = Standard absorbance × 10 × 1000) × Test absorbance × Standard BSA quantity (g) × 210}]

Estimation of Lactate Dehydrogenase(LDH)

We filled a 96-well plate with ten microliters of substrate and then added the reaction mixture. We then incubated the plate for 30 seconds at 25 °C without light. We used a microplate reader to measure the absorbance at 570 nm. After 10 µl of enzyme/lysate was added to start the reaction, the plate was shaken nonstop and incubated in the dark for varying lengths of time. We provided an example blank to obtain the most current value.

The quantity of NADH generated from the standard curve was calculated using the equation ΔA 570nm = (A Final 570nm - A Initial 570nm).

[NADH](nmol)/{[Tfinal – Tinitial (min)] ×V(ml)} equals LDH Activity (mU/ml). =nmol/(min·ml)

GC-MS Analysis

We used a separating funnel to combine 10 milliliters (50 mg/ml) of the sample with 10 milliliters of water and ethyl acetate in a 1:4 ratio (mix 2.5 milliliters of water with 7.5 milliliters of ethyl acetate). Finally, we collected the ayer and reduced it to one milliliter using a rotating evaporator.

We added 50 microliters of N, O-Bis(trimethylsilyl)trifluoroacetamide and trimethylchlorosilane (BSTFA+TMCS) and ten microliters of pyridine after that. To make a 100µl solution for BSTFA+TMCS, combine 1µl TMCS with 99µl BSTFA. After placing the samples into GC vials, we dried them using nitrogen gas. We finally dissolved the samples in methanol prior to GC-MS analysis [17].

Results

Phytochemical screening

Once phytochemical screening identifies bioactive chemicals, it may be possible to identify drugs and medicinal formulations. Table 1 displays the results of a phytochemical study on Opilia celtidifolia extracts, both ethanolic and aqueous.

Table-1: *Opilia Celtidifolia* root plant extract in ethanol and water for phytochemical analysis

Compound	Test	Ethanolic Roots Plant extract	Aqueous Roots Plant extract
Alkaloids	Dragendroff's test	+	+
	Mayer's test	+	−
	Wagner's test	+	+
	Hager's test	+	−
Tannins	Ferric chloride test	+	+
Phenols	Sodium nitrate test	+	+
Glycosides	Keller-kiliani test	+	+
Steroids		+	+
Saponins		+	+
Terpenoids	Salkowski's test	+	+
	Keto-sugar test	+	+

\+ = **Detected,** **- = Not Detected**.

Antioxidant activity

DPPH and ABTS free radical scavenging activity

Table 2 and Figure 1 show that, when compared to both the ethanolic and aqueous extracts of AR seeds, water extract has higher antioxidant activity.We estimated the antioxidant activity in the samples using the DPPH assay, and Table 2 lists the 50% inhibitory concentrations. Both samples were found to have very low DPPH scavenging properties (IC_{50} = Above Dose Limit, i.e., above 1000 µg/ml) with respect to ascorbic acid.We estimated the antioxidant activity in the samples using the ABTS Radical Scavenging Assay, and Table 2 lists the 50% inhibitory concentrations. We found both samples to be active, with Sample OCE-01 being the most active among all the samples. 110 g of samples (OCE-01 and OCE-01) were found to be equivalent to 8.38 g of standard ascorbic acid [14].

Table-2: Antioxidant activities of AR root extract of DPPH and ABTS extracts

Herbal Extracts	Anti – oxidant Activities	
	DPPH (IC_{50}; µg/mL)	ABTS (IC_{50};µg/mL)
Water extract	Above Maximum Dose Limit	578.9 ± 0.043
Ethanol extract	Above Maximum Dose Limit	110.4 ± 0.051
Ascorbic Acid	9.207 ± 0.022	8.385 ± 0.031

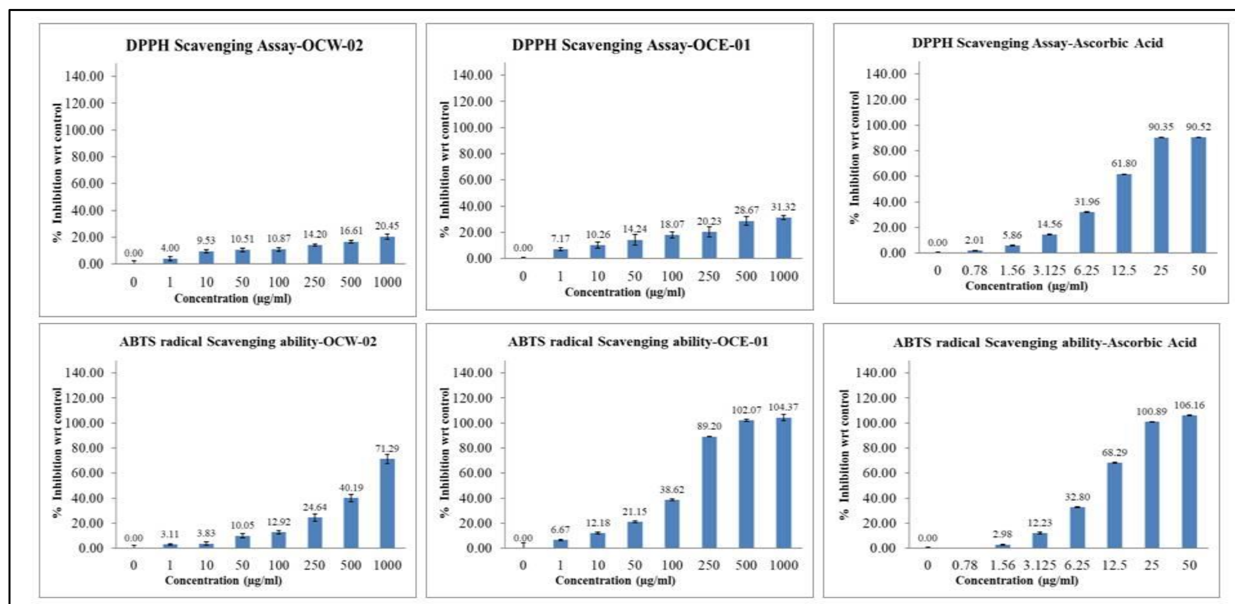

Figure-2: DPPH and ABTS free radical scavenging activity of Aqueous and Ethanolic AR roots extracts of plant *Opilia Celtidifolia*

In-vitro cytotoxicity study

We estimated the cytotoxic activity for samples OCE-01 and OCW-02 at 50% inhibitory concentration when we exposed the *HepG2 cell line* to different concentrations of the sample, as

mentioned in Table 3. We found that sample OCE-01 was more cytotoxic than sample OCW-02. The IC50 represents the concentration at which an inhibitor, sample, or formulation reduces viable cells by half [5].

Table-3: IC_{50} value *in vitro* cytotoxic study on SH-SY5Y cell line in water and ethanol extract of plant *Opilia Celtidifolia*

Herbal extracts	IC_{50} value (µg/mL)
OCE- 01	162.7 ± 0.09
OCW-02	301.6 ± 0.13

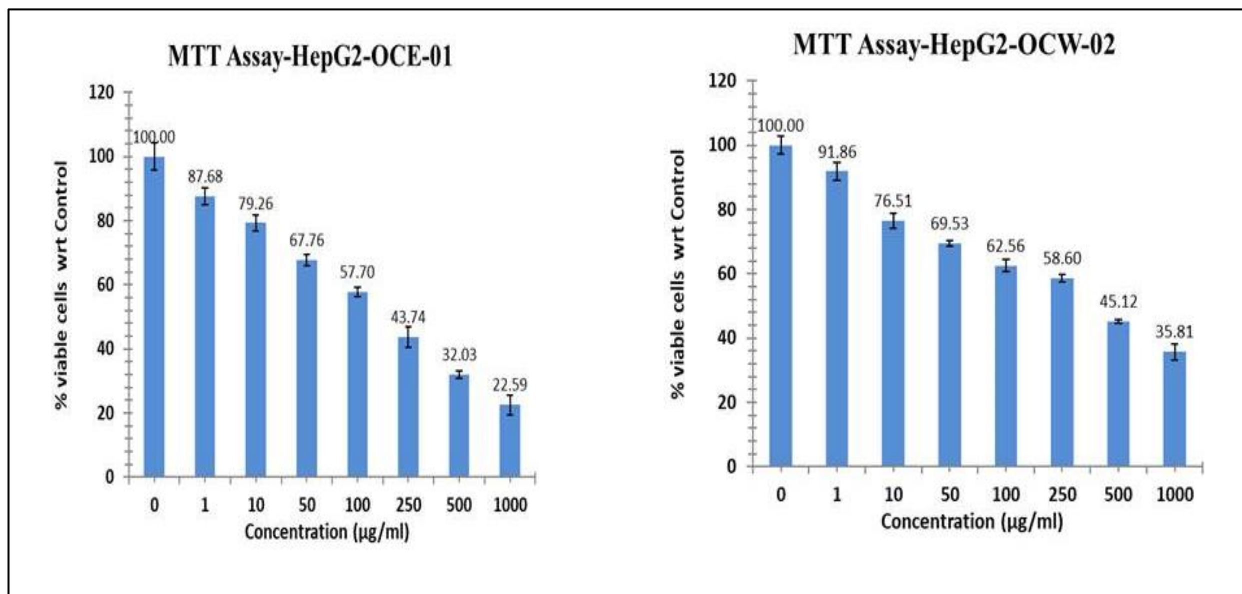

Figure-4: MTT Assay – HepG2 of Aqueous and Ethanolic AR roots extracts of plant *Opilia Celtidifolia*

MTT Cell Viability Assay

After treatment, the sample control showed 93.56% viable cells and 6.44% dead cells. The treated sample OCE-01 showed 85.53 % viable cells and 14.47 % dead cells, while sample OCW-02 showed 90.10 % viable cells and 9.90 % dead cells with respect to the control [4].

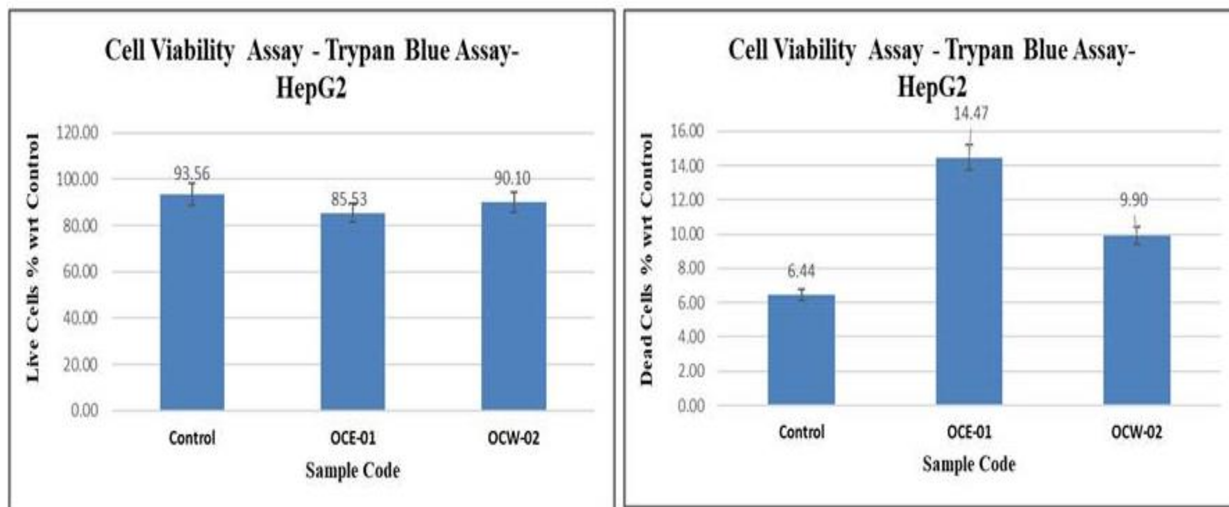

Figure-5: MTT Cell Viability Assay of Aqueous and Ethanolic root extracts of plant *Opilia Celtidifolia*

Scavenging of Superoxide Anions

Antioxidant (Super Oxide Anion Radical Scavenging) activity was estimated in Samples and 50% inhibitory concentration were mentioned in table 3 as compared to standard Gallic Acid.Sample OCW-02 was found more active than sample A OCE-01. 50 % inhibitory concentration could not be calculated for sample OCE-01 due to very low activity. 444.6 µg of the sample OCW-02 was found equivalent to 11.23 µg of the standard Gallic Acid [16] .

Table-3: Scavenging of superoxide anions activities of AR root extract of plant *Opilia Celtidifolia*

Herbal Extracts	IC_{50} value (µg/mL)
Gallic Acid	11.23 ± 0.05
OCE-01	Above Dose Limit
OCW-02	444.60 ± 0.11

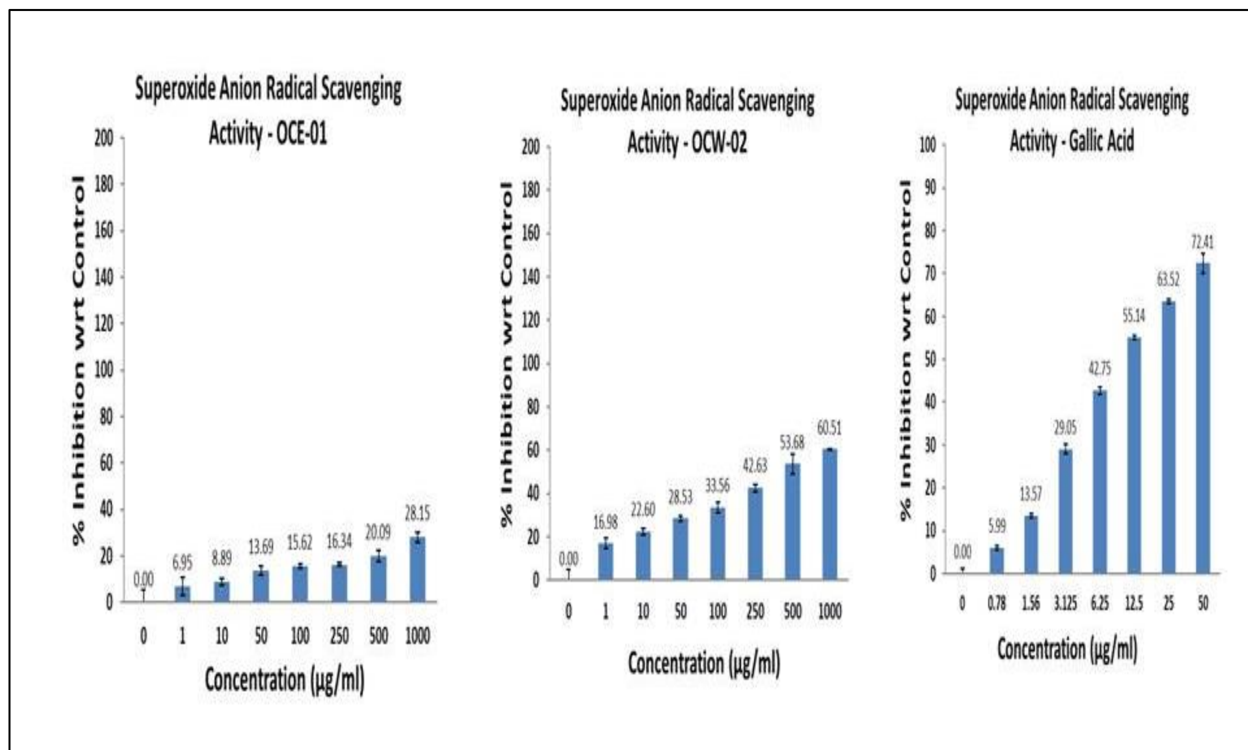

Figure-6: Effect of aqueous and ethanolic root extract of *Opilia Celtidifolia*

Estimation of Reduced Glutathione Activity

Reduced glutathione was estimated insample OCE-01 as 22.42µM and in sample OCW-02 23.92µM *wrt* control which was estimated as 16.42µM [15].

Table-4: Reduced Glutathione Activities of aqueous and ethanolic root extract of *Opilia Celtidifolia*

Herbal Extracts	Mean	SD	SEM
Control	16.42	1.89	0.95
OCE-01	22.42	1.89	0.95
OCW-02	23.92	1.89	0.95

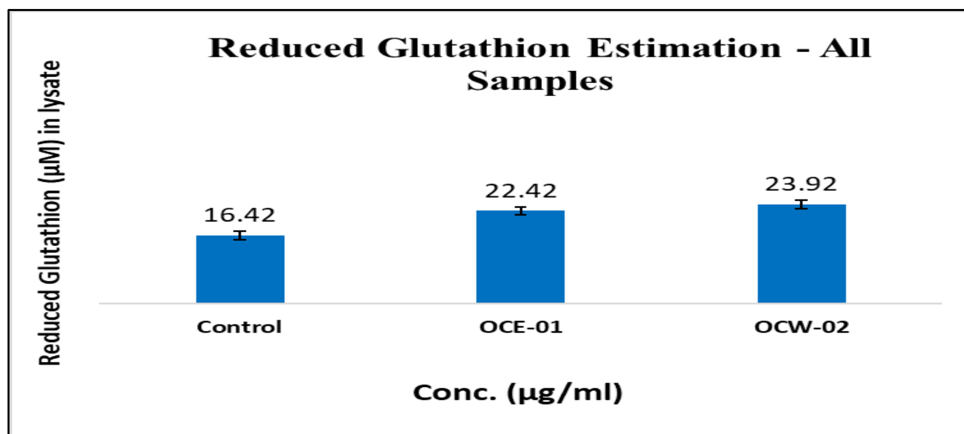

Figure-7: Effect of aqueous and ethanolic root extract of plant *Opilia Celtidifolia*

Aminotransferases

Based on the result obtained from the study Aminotransferases Activity was observed in samples. Aminotransferases Activity (Aspartate Aminotransferase) was found in Cells treated with sample- OCE-01 (2220 U/ml) and in sample- OCW-02 (400 U/ml) with respect to Control (2990 U/ml)[7] .

Table-5: Aminotransferases activity of plant *Opilia Celtidifolia*

Herbal Extracts	Amino Acid (U/ml)
Control	2990
OCE-01	2220
OCW-02	400

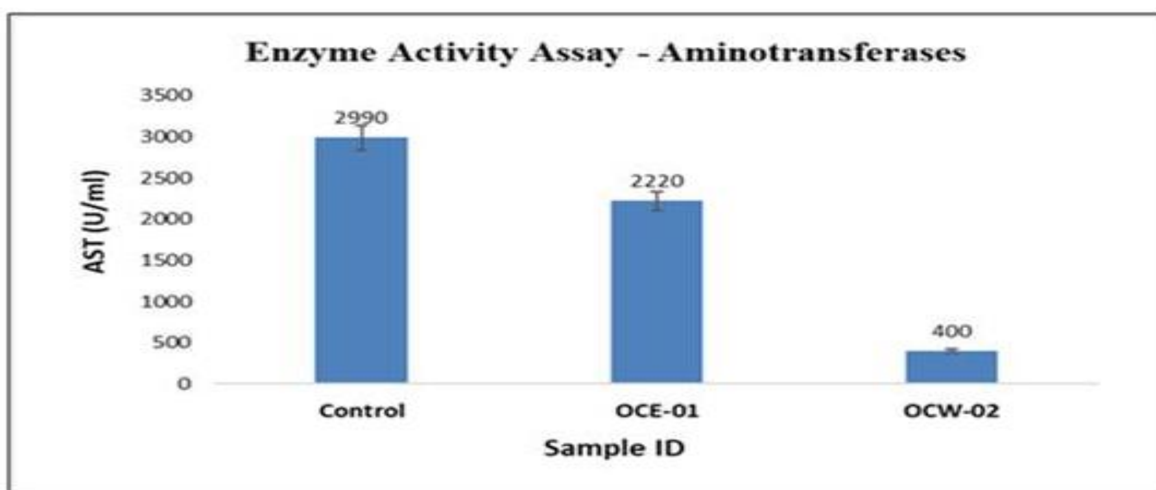

Figure-8: Effect of aqueous and ethanolic root extract *of Opilia Celtidifolia*

Lipid peroxidation

Lipid Peroxidation Assay (TBARS Assay), MDA Content was found decreasing in dose dependent manner. Sample-OCE-01was found to be highly active as compared to the sample OCW-02[6] .

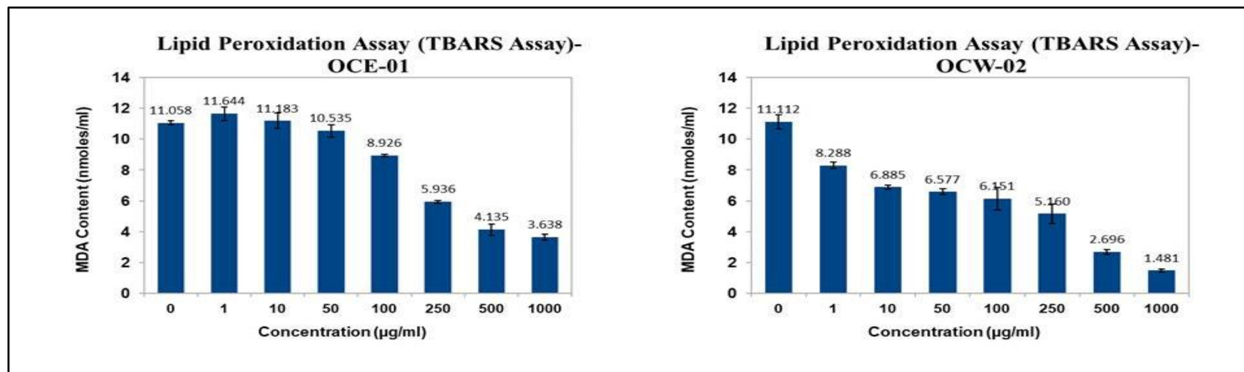

Figure-9: Effect of aqueous and ethanolic root extract of *Opilia Celtidifolia*

Alkaline Phospate

Alkaline Phosphate Activity was observed in samples OCE -01 (14383.03 U/ml) and OCW -02 (31471.78 U/ml) *wrt* control (35601.56 U/ml).

Table-6: Alkaline Phosphate activities of AR root extract of plant *Opilia Celtidifolia*

Herbal extracts	S Mean	SD	SEM
Control	35601.56	239942.18	119971.09
OCE-01	14383.03	96933.92	48466.96
OCW-02	31471.78	210703.48	105351.74

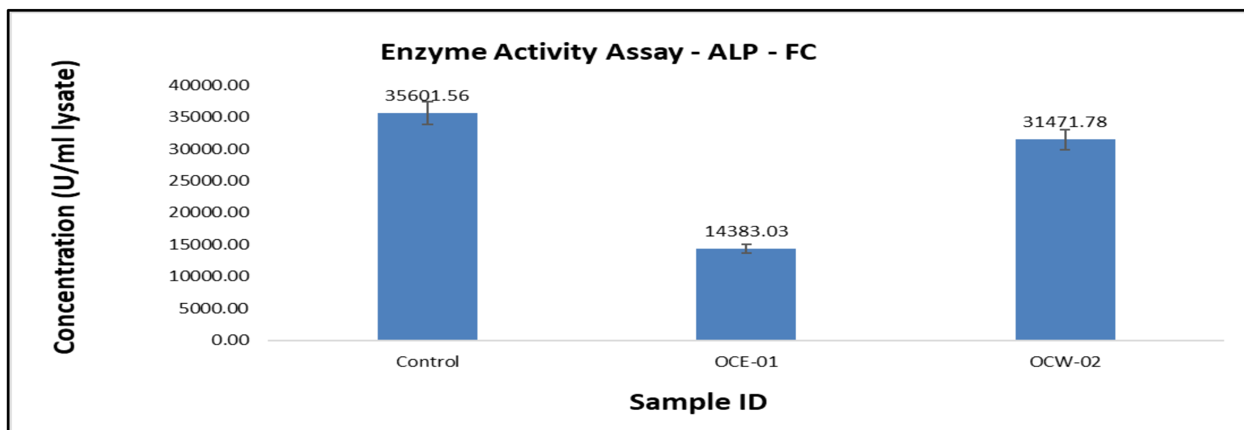

Figure-10: Effect of aqueous and ethanolic root extract of plant *Opilia Celtidifolia*

Lactate Dehydrgenase

Lactate Dehydrogenase Activity was observed in both samples. In sample OCE-01 Lactate Dehydrogenase Activity was found (0.09 mU/ml) and in sample OCW-02 Lactate Dehydrogenase Activity was found (0.13 mU/ml) with respect to Control (0.28 mU/ml).

Table-7: LDH activity of aqueous and ethanolic root extract of *Opilia Celtidifolia*

Herbal Extracts	LDH Activity(U/ml)
Control	0.28
OCE-01	0.09
OCW-02	0.13
PC	0.18

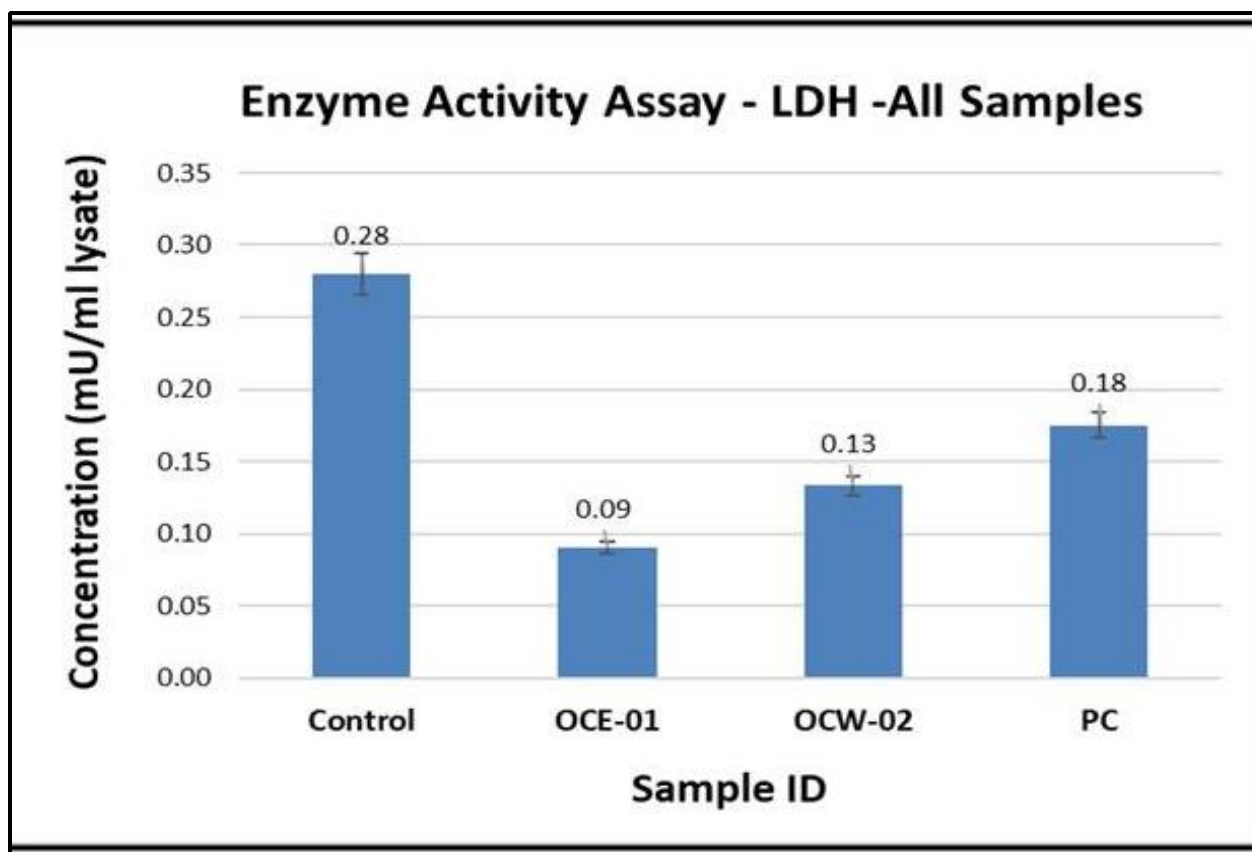

Figure-11: Effect of aqueous and ethanolic root extract of *Opilia Celtidifolia*

GC-MS Analysis

Identification of components

The computed fragments, molecular mass and molecular structure were used for identification. The GC-MS spectra were interpreted using the NIST database and the Willy8-library which has over 62,000 pattern entries. The test material's constituents were identified along with their molecular weights and chemical structures. Component spectra from the NIST library and the Willy8 collection were compared to the percentage amounts found in the sample. This is done to see whether this plant species has any chemical or combination of components that might back up its commercial and traditional medicinal uses. It also aids in identifying the best strategies for extracting these substances. The components of the test materials were identified (Table 7,8 and Figure 7,8) together with their molecular weights and structures [1].

Peak Report AR-E-01

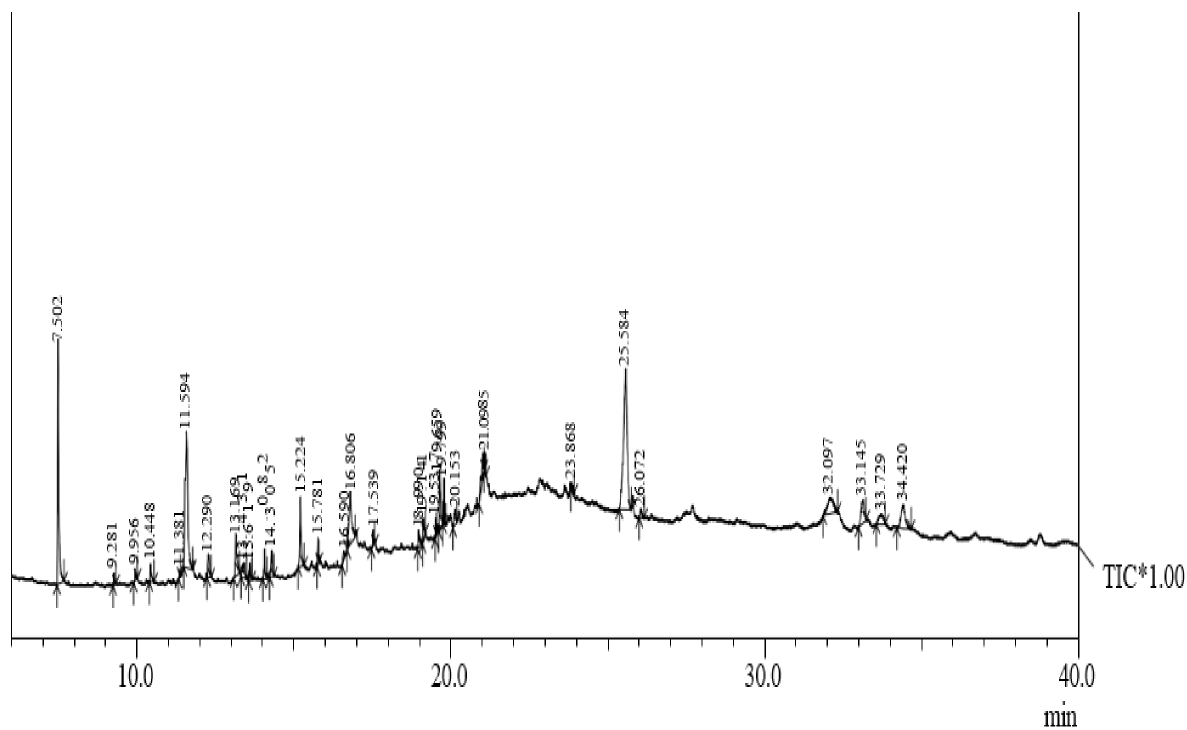

Figure-12: GC-MS chromatogram of Ethanolic AR roots extracts

Table-8: The GC-MS analysis of ethanolic AR root extract and the elements found there and their biological activity

Peak Report TIC-Sample- OCE-01

Peak	R.Time	Area	Area %	Name	Biological activity
1	7.502	3062824	11.78	Pyridine,3-(1-Methyl-2-Pyrrolidinyl)-,(S)	Antioxidant Activity, Hepatoprotective Activity.
2	10.448	264783	1.02	1,2-BenzenedicarboxylicAcid,Diethyl Ester	Anti-inflammatory Activity.
3	13.169	771755	2.97	Neophytadiene	Anti-oxidant Activity,Anti-inflammatory Activity.
4	13.619	225316	0.87	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol	Anti-inflammatory Activity, Hepatoprotective Activity, Anti-oxidant Activity.
5	14.082	356578	1.37	HexadecanoicAcid,MethylEster	Anti-inflammatory Activity, Hepatoprotective Activity, Anti-oxidantActivity.
6	14.305	426780	1.64	D-(+)-Talofuranose,Pentakis(Trimethylsilyl)Ether(Isomer1)	Hepatoprotective Activity.
7	15.224	1018303	3.92	PalmiticAcid,TMSDerivative	Anti-inflammatory Activity, Hepatoprotective Activity, Anti-oxidant Activity.
8	15.781	229925	0.88	9-Octadecenoic Acid (Z)-,MethylEster	Anti-inflammatory Activity, Hepatoprotective activity, Anti-oxidant Activity.
9	16.590	141359	0.54	Nonanamide	Anti-inflammatory Activity.
10	16.806	1535647	5.91	5,8,11-EicosatrienoicAcid, (Z)-,TMSDerivative	Anti-inflammatoryActivity.
11	17.539	165155	0.64	GlycidylPalmitate	Hepatoprotective Activity.
12	19.659	895068	3.44	1,5,9-Trioxacyclododecane,3,3'-[1,6-Hexanediyl]Bis	Hepatoprotective Activity.
13	19.799	396289	1.52	1-Monopalmitin,2TMSDerivative	Hepatoprotective Activity.
14	21.038	676239	2.60	2-Linoleoylglycerol,2TMSDerivative	Anti-inflammatory Activity, Hepatoprotective Activity, Anti-oxidant Activity.
15	21.095	150276	0.58	1-Linolenoylglycerol,2TMSDerivative	Anti-inflammatory Activity, Hepatoprotective Activity, Anti-oxidant Activity.
16	26.072	162324	0.62	Spirost-5-En-3-YlAcetate	Anti-inflammatory Activity, Hepatoprotective Activity, Anti-oxidant Activity.
17	33.145	825737	3.18	2-Linoleoylglycerol,2TMSDerivative	Anti-inflammatory Activity, Hepatoprotective Activity, Anti-oxidant Activity.

18	33.729	495162	1.91	2-Linoleoylglycerol,2TMSDerivative	Anti-inflammatory Activity, Hepatoprotective Activity, Anti-oxidant Activity.
19	34.420	1146120	4.41	2-Linoleoylglycerol,2TMSDerivative	Anti-inflammatory Activity, Hepatoprotective Activity, Anti-oxidant Activity.
		25990930	100.00		

Peak Report-AR-W-02

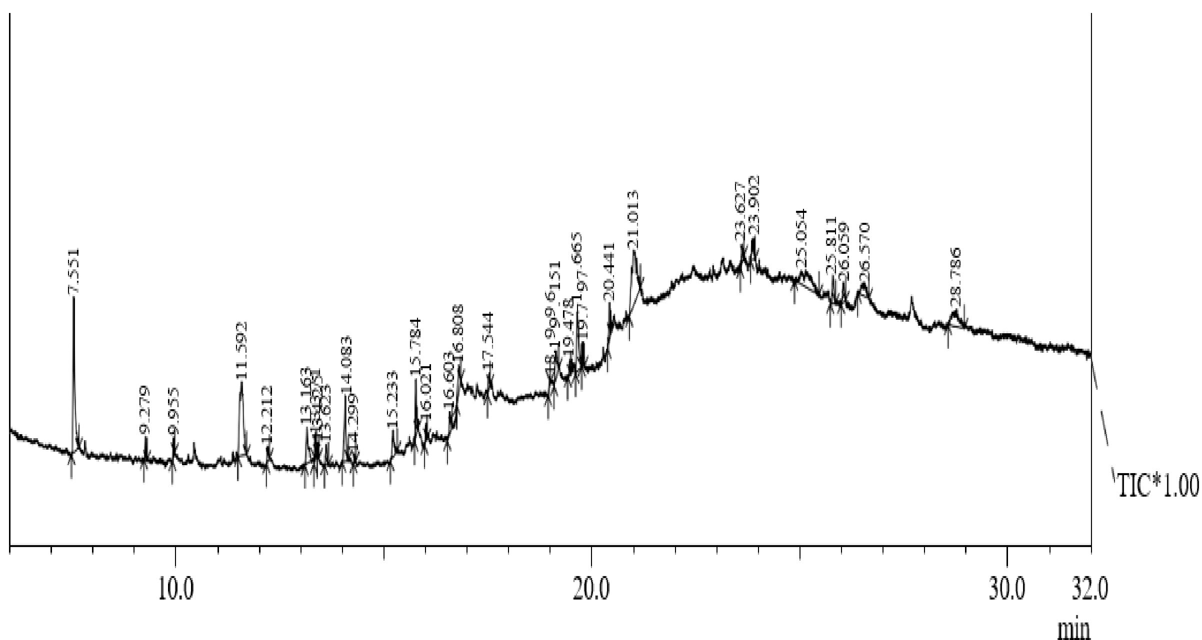

Figure-13: GC-MS chromatogram of AR Aqueous roots extracts

Table-8: The GC-MS analysis of an AR aqueous extracted root and the results of its biological examinations

Peak Report TIC-Sample- OCW-02					
Peak	**R.Time**	**Area**	**Area %**	**Name**	**Biological activities**
1	7.551	819822	12.35	Pyridine,3-(1-Methyl-2-Pyrrolidinyl)-,(S)	Antioxidant Activity, Hepatoprotective Activity.

2	11.592	876611	13.20	Mannofuranoside,Methyl2,3,5,6-Tetrakis-O- (Trimethylsilyl)-, .Alpha.-D	Anti-inflammatory Activity Hepatoprotective Activity Anti-oxidant Activity.
3	14.083	340712	5.13	HexadecanoicAcid,MethylEster	Anti-oxidant Activity, Anti-inflammatory Activity.
4	14.299	23905	0.36	2-Palmitoylglycerol,2TMSDerivative	Anti-inflammatory Activity, Hepatoprotective Activity, Anti-oxidant Activity.
5	15.784	184707	2.78	9,12-OctadecadienoylChloride,(Z,Z)	Anti-inflammatory Activity, Hepatoprotective Activity, Anti-oxidant Activity.
6	16.808	166875	2.51	9,12-OctadecadienoicAcid(Z,Z)-,2-[(TrimethyLsilyl)Oxy]-1-[[(Trimethylsilyl)Oxy]Methyl]Ethyl Ester	Anti-inflammatory Activity Hepatoprotective Activity Anti-oxidant Activity.
7	17.544	61340	0.92	GlycidylPalmitate	Hepatoprotective Activity.
8	21.013	884096	13.31	5,8,11-EicosatrienoicAcid,(Z)-,TMSDerivative	Anti-inflammatory Activity, Anti-oxidant Activity.
9	23.627	57145	0.86	Spirost-5-En-3-Ol,Acetate,(3.Beta.,25R)	Anti-inflammatory Activity, Anti-oxidant Activity.
10	23.902	136412	2.05	Stigmast-5-En-3-Ol,(3.Beta.)	Anti-inflammatory Activity, Hepatoprotective Activity, Anti-oxidant Activity.
11	26.059	102183	1.54	Diosgenin	Anti-inflammatory Activity, Hepatoprotective Activity
12	26.570	193037	2.91	HexadecanoicAcid,4-[(Trimethylsilyl)Oxy] Butyl Ester	Anti-inflammatory Activity, Hepatoprotective Activity, Anti-oxidant Activity.
13	28.786	346944	5.23	HexadecanoicAcid,2-[(Trimethylsilyl)Oxy] -1,3-Propanediyl Ester	Anti-inflammatory Activity, Hepatoprotective Activity, Anti-oxidant Activity.
		6639875	100.00		

Conclusion

Positive outcomes from studies examining the ethanol and water root extracts' in vitro hepatoprotective effects show that *Opilia celtidifolia* may protect and improve liver health. The increased survival of *HepG2 liver cells* exposed to ethanol-induced damage is proof of the study's noteworthy hepatoprotective qualities for both extracts. Compared to the water extract, the ethanol

extract had a marginally greater protective effect, indicating that ethanol is a more effective solvent for eliminating bioactive compounds.

Significant bioactive components, including flavonoids, phenolics, and alkaloids—all of which are recognized for their antioxidant qualities—were found, according to phytochemical analysis. These substances most likely lessen oxidative stress and cell damage, which contributes to the hepatoprotective benefits that have been shown. The extracts' strong antioxidant activity was further corroborated by antioxidant experiments, which included DPPH and ABTS radical scavenging activities.

The results of this research provide *Opilia celtidifolia's* traditional usage in managing liver health scientific validation. Research has shown the hepatoprotective and antioxidant properties of root extracts from *Opilia celtidifolia,* indicating that these plant extracts may be useful as natural treatments for liver conditions linked to oxidative stress and toxicity.

It is imperative that future research concentrate on in vivo study to validate these in vitro findings and investigate the mechanisms behind the hepatoprotective benefits. Furthermore, it may be possible to identify and define the specific bioactive molecules producing these effects, which might result in the creation of novel hepatoprotective medications. All things considered, this study adds to the increasing amount of research demonstrating the therapeutic potential of medicinal herbs in the management and prevention of liver disease.

Acknowledgement

The authors express gratitude to Aakaar Biotechnologies Private Limited Lucknow for providing GCMS, MTT Assay, antioxidant activity study, and in vitro hepatoprotective activity, as well as to Maharana Pratap College of Pharmacy, Kanpur, Uttar Pradesh, India, for their technical and administrative assistance.

Conflict of interest

None

Funding

None

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