

Phytochemical analysis and identification of active compounds using GC-MS analysis of *Commelina benghalensis*

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ABSTRACT

Now a day's herbal medicines are taking the leading positions in the health care systems. It was the essential driving force for better treatment from the ancient times. *Commelina benghalensis* belongs to Commelinaceae family is an annual herbaceous weed commonly known as Benghal Dayflower. The phytochemical analysis of *Commelina benghalensis* plant revealed the presence of Alkaloid, Anthocyanin, Carbohydrates, Coumarin, Flavonoids, Glycosides, Phenol, Quinones, Saponins, Steroids, Tannins and Terpenoids. The silver nanoparticles were synthesized in the extracts of *Commelina benghalensis* plant. The result suggest that this plant possess anti-bacterial capability. The free radical scavenging activity of the plant extracts were shown by Anti-oxidant assays against Hydrogen peroxide. The GC-MS analysis of the root Ethanolic extract of *Commelina benghalensis* were identified the presence of 30 Bio- active compounds. Among that, the first 1 peak compounds were analyzed for the presence of anti-viral effect.

Key words: *Commelina benghalensis*, Commelinaceae, Benghal Dayflower, Phytochemical analysis, Hydrogen peroxide, Gas chromatography Mass spectrometry (GC-MS)

I. INTRODUCTION

Traditional medicine is the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures used in the maintenance of the health, prevention of diseases and improvement of physical and mental illness. In practice, traditional medicine refers to the acupuncture (China), Ayurveda (India), Unani (Arabic countries), traditional birth attendant's medicine, herbal

medicine, and various forms of indigenous medicine (K. Andrae-Marbela 2017).

Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care. They have stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects. The chemical constituents present in them are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body (Martins Ekor 2014).

Ancient literature also mentions herbal medicines for age related diseases namely memory loss, osteoporosis, diabetic wounds, immune and liver disorders, etc. for which no modern medicine or only palliative therapy is available. These drugs are made from renewable resources of raw materials by eco- friendly processes and will bring economic prosperity to the masses growing these raw materials (VP Kamboj 2000).

Indigenous cultures such as African and Native American used herbs in their healing rituals, while other developed traditional medical systems such as Ayurveda and traditional Chinese medicine in which herbal therapies were used. Medicinal plants have been used for the treatment of a large number of human dies in different parts of the world throughout the history of human kind. (SY Pan 2014).

Thus medicinal plants are used in crude or purified form in the preparation of drugs in different systems. Structural novelty and new modes of action are common features of plant drugs. Plants have been used for medicinal purposes long before recorded history. Ancient Chinese and Egyptian papyrus writings describe medicinal uses for plants as early as 3000 BC (SY Pan 2014).

In indigenous/traditional systems of

medicine, the drugs are primarily dispensed as water decoction or ethanolic extract. Fresh plant parts, juice or crude powder are a rarity rather than a rule. Thus medicinal plant parts should be authentic and free from harmful materials like pesticides, heavy metals, microbial or radioactive contamination, etc. The medicinal plant is subjected to a single solvent extraction once or repeatedly, or water decoction or as described in ancient texts (P Agarwal 1996)

They are still used in rural communities of many developing countries, where up to 80% of the of the time traditional medicines are used in health care. The past decade has witnessed a tremendous resurgence in the interest and use of medicinal plants (M Ekor 2013).

The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in them known as phytochemicals.

Phytochemicals are biologically active, naturally occurring chemical compounds synthesized in all parts of the plant body. Different types of phytochemical include flavonoids, steroids, terpenoids, carbohydrates, tannins, coumarins, alkaloids, saponins, glycoside, anthocyanin, etc. Phytochemicals are also called as Secondary metabolites.

These biological active compounds are derived from different parts of plants such as leaves, barks, seed, seed coat, flowers, roots and pulps and thereby used as sources of direct medicinal agents. They serve as a raw material base for elaboration of more complex semi-synthetic chemical compounds (A Altemimi 2017).

Now- a-days these phytochemicals become more popular due to their countless medicinal uses. Phytochemicals play a vital role against number of diseases such as asthma, arthritis, cancer etc. Unlike pharmaceutical chemicals these phytochemicals do not have any side effects. Since the phytochemicals cure diseases without causing any harm to human beings these can also be considered as “man friendly medicines”

(K. Sahira Banu, Dr.L. Cathrine 2015).

Phytochemicals are not essential nutrients and are not required by the human body for sustaining life, but have important properties to prevent or to fight some common diseases (P. Saranraj, S. Sivasakthi, M.S. Deepa). Because of this property, many studies have been undertaken to reveal the health benefits of phytochemicals. The abundance of scientific evidence indicates that such bioactive compounds have biological properties such as

antioxidant activity, antibacterial activity, analgesic activity, antiviral activity, antiinflammatory, and anticancer property. *Commelina benghalensis* also known as Benghal day flower, tropical spiderwort or wandering Jew. which is perennial medicinal plant inhabitant to tropical Asia and Africa. Around $\frac{3}{4}$ of the world population depends on plant and plant products for health care. The plant has been proved to be a potential source for pharma phytochemistry study for industrial purposes as well. This plant is being used as a part of the traditional medicinal system for different diseases.

Commelina benghalensis is used in traditional medicine system to treat various ailments. It used for the treatment of headache, fever, snake bite, and jaundice. It is also used in the treatment of mouth thrush. In Lesotho it is applied to treat infertility in women and in India it is used as diuretic and febrifuge. In Pakistan it is used as vegetable. In Nepal paste of the plant is used to treat burns and juice of roots is used to treat indigestion. *Commelina benghalensis* is often found in forest edges, road sides, cultivated fields, agricultural sites and home garden.

II. MATERIALS AND METHODS

The plant sample were collected from vandalur at Chennai. It was authenticated by Prof. P. Jayaraman, M. Sc, Ph.D., Director, Plant Anatomy Research Center (PARC), Chennai-600045

Figure1:Commelinabenghalensis



Preparation of plant (Commelina benghalensis)

The plant was brought to the laboratory washed thoroughly with fresh water and cleaned to remove all the dust particles. The plant

(Commelina benghalensis) was dried in shade for 7days. The dried plantmaterial (Root and Leaf) was crushed and powdered.



Figure 2: Powdered Commelina benghalensis (Leaf and Root)

1. For Phytochemical Analysis

The weight of dried powder equivalent to 10gram were macerated in 100ml of ethanol, water and methanol separately. The mixtures were kept for 3 days at room temperature. Then it is filtered

through muslin cloth and followed by sterile Whatmann filter paper. Then the extract was stored in Glass bottle for further phytochemical analysis.

2. For antioxidant activity

The weight of dried powder equivalent to 0.1mg, 0.2mg, 0.3mg, 0.4mg and 0.5mg in 5 different test tubes of 3 sets were macerated in 5ml of Benzene, Chloroform, Ethanol, Methanol and Water. The solvent sample for Anti – Oxidant activity was kept for 3 days at room temperature.

3. For GC-MS Analysis

The weight of dried powder equivalent to 10g were macerated in 100ml of ethanol and kept at 37°C for 5 days. Then the sample is evaporated, the weight equivalent of 1g is macerated in 10ml ethanol and used for GC-MS analysis.

III. RESEARCH METHODS

1. PHYTOCHEMICAL ANALYSIS (K. Sahira Banu, Dr.L. Cathrine, 2015)

Standard methods were used to detect the presence of secondary metabolites such as Alkaloid, Anthocyanin, Carbohydrates, Coumarins, Flavonoid, Glycosides, Phenol, Quinones, Saponin, Steroids, tannins and terpenoids.

Flavonoids (Alkaline reagent test)

To 1ml of extract, 1ml of 10% NaOH was added. Appearance of yellow fluorescence indicates the presence of flavonoids.

Alkaloid (Wagner's test)

To 1ml of extract, 1ml of Wagner's reagent was added. Reddish brown precipitate indicates the presence of Alkaloids.

Phenol (Ferric chloride test)

To 1ml of extract, 1ml of 5% ferric chloride was added. Appearance of Dark green/reddish brown/blue/violet/purple color indicates the presence of phenol.

Carbohydrates (Molisch's test)

To 2ml of extract, 2ml of Molisch and 2ml of concentrated sulphuric acid was added. Appearance of Reddish ring indicates the presence of carbohydrates.

Tannins (Ferric chloride test)

To 1ml of extract, 3ml of water, few drops of 10% ferric chloride is added and appearance of blue green precipitate indicates the presence of tannins.

Steroids (Chloroform test)

To 1ml of extract, 1ml of concentrate H₂SO₄ is added along the sides of the test tubes. The upper layer in the test tube turns red and sulphuric acid layer showed yellow with green fluorescence.

Saponin (Foam test)

To 2ml of extract, 2ml of distilled water was added and shaken vigorously for 30 seconds. Formation of stable foam persist on warming indicates the presence of saponin.

Glycosides (Keller-killiani test)

To 2ml of the extract, 2ml of Glacial Acetic acid and few drops of 5% ferric chloride and concentrated sulphuric acid were added. Appearance of Reddish brown or blue green precipitate indicates the presence of Glycosides.

Terpenoids (Sulphuric acid test)

To 2ml of extract, 2ml of chloroform and 3ml of Sulphuric acid was added. Reddish brown color indicated the presence of terpenoids.

Anthocyanin (Sulphuric acid test)

To 1ml of extract, 1ml of conc. H₂SO₄ Appearance of yellowish Orange color indicates the presence of Anthocyanin.

Quinones (Hydrochloric acid test)

To 1ml of extract, 1ml of concentrated hydrochloric acid was added. Appearance of yellow color indicates the presence of Quinones.

Coumarin (Sodium hydroxide test)

To 1ml of the extract, 1ml of 10% NaOH are added. Appearance of yellow color indicates presence of coumarin.

Tannin: Present in high amount in methanol, water extract and less in petroleum ether, benzene and acetone extract. Tannin shows antiviral and anti-bacterial activities which help to cure wound healing and burns, besides it shows anti-diabetic and anti-inflammatory activities.

Phenolic: Present high amount in methanol and water extract and less in Acetone extract of plant which shows anti-microbial, antiviral activities.

Saponins: Present in petroleum ether, benzene, chloroform and methanol extract of the plant. Leaves and stem shows anti-hypertensive, anti-oxidant, anti-cancer and immunomodulatory properties in methanol extract, which are helpful to treat tachycardia and myocardopathy.

Steroids and Terpenoids: Present in petroleum ether, benzene, chloroform and methanol extract of plant. This shows anti-diabetic and analgesic activities in animal studies.

Alkaloid: are present in successive extraction of petroleum ether, benzene, chloroform and water

extract of plant, which shows antiviral, anti-bacterial anti- diabetic and anti- inflammatory activities.

Flavonoid: Present high amount in chloroform, methanol, water extract of the plant, which shows antioxidant and anti-inflammatory activities.

Carbohydrates: Present in water extract of plant, which provide energy and regulation of blood glucose level.

2. SILVER NANOPARTICLES SYNTHESIS (Narayanaswamy Krithiga et.al., 2015)

1.0.037g of silver nitrate was dissolved in 18ml of deionized water separately in 2 conical flasks (to obtain 0.1mm of silver nitrate precursor solution).

2.2ml of plant extract was added to this solution and mixture was magnetically stirred for 30 minutes.

3. The solution was stored in dark room for 48 hrs. The synthesis of silver nanoparticles in the solution was monitored by using UV visible spectrophotometer and respective peaks were recorded at 420nm

3. ANTI OXIDANT ACTIVITY HYDROGEN PEROXIDE SCAVENGING ACTIVITY (Halliwell and Gutteridge, 1996)

Principle:

HPSA is used to find the scavenging activity of free radicals like hydrogen peroxide in the presence of different concentration of plant samples.

Working procedure:

1.1ml extracts was added to 4ml 50mm phosphate buffer (PH – 7.4) followed by the addition of 6ml H2O2 (2mm).

2. The reaction mixture was vortexed and after 10

minutes of reaction its absorbance was measured at 230 nm.

3. Ascorbic acid was used as positive control.

4. GC-MS Analysis (Radhakrishnan et.al., 2017)

1. The root sample weight equivalent of 10mg is soaked in 100 ml Ethanol. After 5 days the solvent was filtered and allowed to evaporate. The yield of 2 mg was dissolved in 20 ml ethanol used to run gas chromatography - mass spectrometry with ethanol as column solvent.

2. GC-MS analysis of root extract was studied by SHIMADZU GC- MS QP 2010 with CARBOWAX capillary column and helium as carrier gas. The Ethanolic extract *Commelina benghalensis*. Was injected in to the column.

3. The column was fused with silica 50m x 0.25mm. The study conditions were 20 minutes. At 100 ° C, 235° C for column temperature at 3 minutes and 240 ° C for injection temperature, carrier gas was helium, and split ratio was 5:4.

4. The 1µl of the sample was evaporated in a split-less injector at 300degree C and the run time was 22 minutes. The active biomolecule was identified by Gas Chromatography coupled with Mass Spectrometry and the resultant spectrum was analyzed using the NIST08 library.

IV. RESULT AND DISCUSSION PHYTO CHEMICAL ANALYSIS

Ethanolic and Aqueous extract of *Commelina benghalensis* (Root and Leaf) was subjected to qualitative chemical analysis. The various chemical tests were performed on this ethanolic and aqueous extract for the identification of phytochemicals, secondary metabolites and the results are displayed in Table:1 and 2

Table 1: Chemical constituents present in successive ethanolic extract of *Commelina benghalensis* (Root and Leaf)

S.No	Phytochemicals	Ethanolic extract of <i>Commelina benghalensis</i> (Root)	Ethanolic extract of <i>Commelina benghalensis</i> (Leaf)
1	Flavonoids	-	-
2	Phenol	-	+
3	Alkaloids	+	-

4	Carbohydrates	+	-
5	Steroids	-	-
6	Tannins	-	+
7	Saponins	+	+
8	Glycoside	+	+
9	Anthocyanin	+	-
10	Quinones	-	-
11	Coumarins	-	-

(+)-Present(-)-Absent

In the Ethanolic extract of *Commelina benghalensis*(Root) Alkaloids, Carbohydrates, Saponin, Glycoside and Anthocyanin were present.

In the Ethanolic extract of *Commelina benghalensis*(Leaf) Glycosides, Phenol, Saponins, and Tannins were present.



Figure 3: Ethanolic extract of *Commelina benghalensis* (Root and leaf)

Table 2: Chemical constituents present in successive Aqueous extract of *Commelina benghalensis* (Root and Leaf) (+)- Present (-)- Absent

S.No	Phytochemicals	AqueousextractofCommelina benghalensis(Root)	AqueousextractofCommelina benghalensis(Leaf)
1	Flavonoids	-	-
2	Phenol	-	+
3	Alkaloids	-	+

4	Carbohydrates	+	+
5	Steroids	-	+
6	Tannins	-	-
7	Saponins	-	+
8	Glycoside	-	+
9	Terpenoids	+	+
10	Anthocyanin	+	+
11	Quinones	-	-
12	Coumarins	+	-

In the Aqueous extract of *Commelina benghalensis*(Root) Carbohydrates, Terpenoids, Quinones, Coumarins and Anthocyanin were present. In the Aqueous extract of *Commelina benghalensis*(Leaf) Phenol, Alkaloids, Carbohydrates, Steroid, Saponins, Glycoside, Terpenoids and Anthocyanin were present.



Figure 3: Aqueous extract of *Commelina benghalensis* (Root and leaf)

The extract containing Alkaloid possess Anti-viral, Anti-inflammatory, Anti-bacterial, Anti-diabetic activity. Anthocyanin possess Anti-cancer, Anti-obesity, Anti-inflammatory properties, Anti-microbial. Carbohydrates were used as Energy source. Coumarins possess Anti-inflammatory, Anti-coagulant, Anti-microbial, Anti-tubercular, Anti-oxidant activity. Flavonoids possess Anti-oxidant, Anti-inflammatory activity. Glycosides and Steroids

possess Anti-inflammatory properties. Quinones possess Anti-inflammatory, Anti-oxidant, Anti-cancer activity. Saponin possess Anti-hypersensitive, Anti-oxidant, Anti-cancer, Immuno- modulatory properties. Tannins were used to treat tonsillitis, Haemorrhoids, skin eruptions. Terpenoids possess Anti-cancer, Anti-inflammatory, Anti-parasitic, Anti-hyperglycemic, Anti-microbial properties.

1. SILVER NANOPARTICLES SYNTHESIS

Silver nanoparticles synthesis was taken place in Aqueous and Ethanolic extract which has been

confirmed with the color change from pale brown to dark brown/grey color respective peaks were recorded at 420nm.

Figure 4: Graph obtained in ethanolic extract of leaf (Commelina benghalensis)

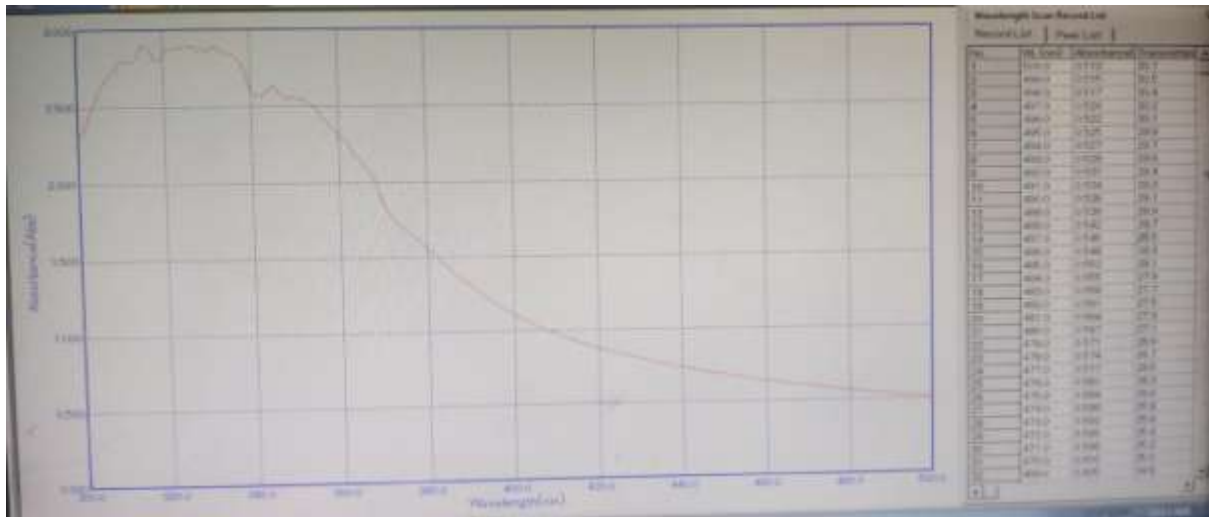
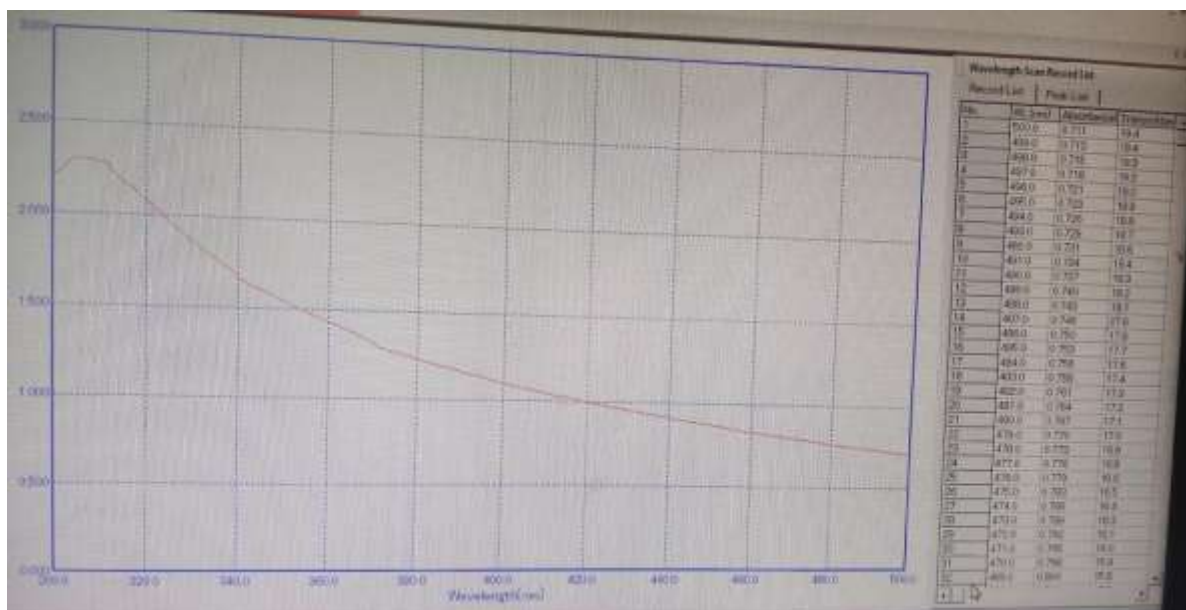


Figure 5: Graph obtained in ethanolic extract of Root (Commelina benghalensis)



**2. ANTI OXIDANT ACTIVITY
 HYDROGEN PEROXIDE SCAVENGING ACTIVITY**

The free radicals in Hydrogen peroxide were scavenged by the plant (root) extract of Commelina benghalensis. In benzene extract 47.1% in 0.1mg, 29.8% in 0.2mg, 33.6% in 0.3mg, 31.2%

in 0.4mg. In chloroform extract 8.84% in 0.1mg, 52.4% in 0.2mg, 37.9% in 0.3mg, 27.8% in 0.4mg. In ethanolic extract 81.2% in 0.1mg, 77.8% in 0.2mg, 62.5% in 0.3mg, 35.5% in 0.4mg. From the given inference ethanolic extract showed high percentage of radical scavenging activity against Hydrogen peroxide. The anti-oxidant effect of

Commelina benghalensis root is in the following order: Ethanol extract > Benzene extract >

Chloroform extract.

Table3:

S.NO	CONCENTRATIONS	BENZENEEXTRACT		CHLOROFORMEXTRACT		ETHANOLEXTRACT	
		OD	%RSA	OD	%RSA	OD	%RSA
1	0	0.208	0	0.208	0	0.208	0
2	0.1mg	0.110	47.1%	0.024	8.84%	0.039	81.2%
3	0.2mg	0.146	29.8%	0.099	52.4%	0.046	77.8%
4	0.3mg	0.138	33.6%	0.129	37.9%	0.078	62.5%
5	0.4mg	0.143	31.2%	0.150	27.8%	0.134	35.5%

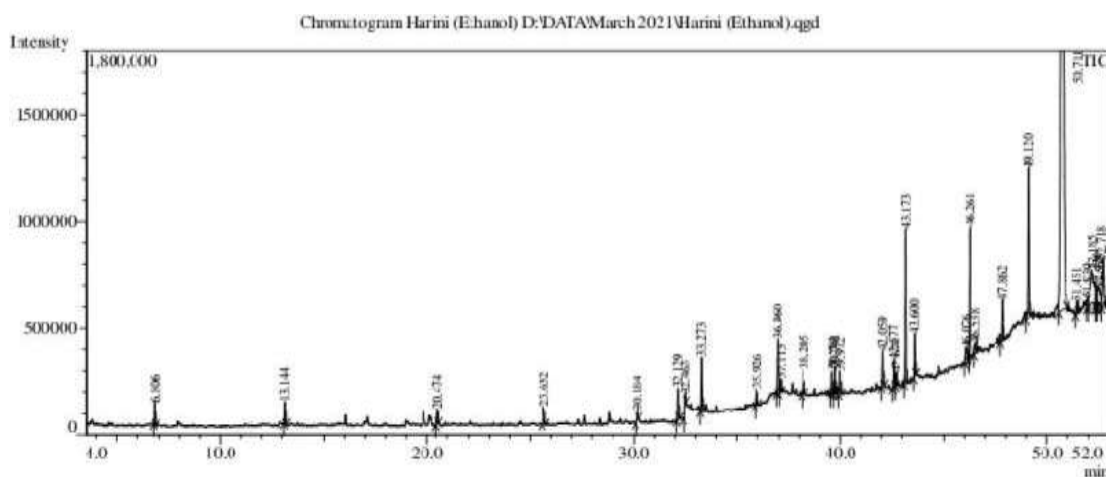
OD-OpticalDensity
 %RSA-PercentageofRadicalScavengingActivity

3. GC-MS ANALYSIS

The GC-MS analysis of Commelina benghalensis root extract revealed the presence of

30 bioactive compounds. 30 compounds among that 5 peak compounds and 1 compound were analyzed for the presence of anti-viral activity.

Figure 6: Peak Obtained Through GC-MS Analysis of Commelina benghalensis root sample



**Figure7: Bioactive Compounds Obtained Through GC-MS
Analysis of Commelina benghalensis root sample**

Peak Report TIC				
Peak#	R.Time	Area	Area%	Name
1	6.806	276367	0.61	Dodecane
2	13.144	310115	0.68	TETRADECANE
3	20.474	224245	0.49	Hexadecane
4	25.632	332043	0.73	Phenol, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-
5	30.184	223229	0.49	n-Pentadecanol
6	32.129	463440	1.02	Dibutyl phthalate
7	32.463	397730	0.87	n-Hexadecanoic acid
8	33.273	787439	1.73	Hexadecanoic acid, ethyl ester
9	35.926	255492	0.56	PHYTOL ISOMER
10	36.960	902378	1.98	4,4-DIMETHYL-5.ALPHA.-D1-ANDROST
11	37.113	267979	0.59	Ethyl Oleate
12	38.205	335707	0.74	(2,3,5,6-Tetrafluorophenyl)methyl 3-(2,2-dic
13	39.594	306694	0.67	Glycidyl palmitate
14	39.741	365384	0.80	HEXATRIACONTANE
15	39.972	334427	0.73	2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-e
16	42.059	505619	1.11	Cyclohexane, 1,3,5-triphenyl-
17	42.577	409748	0.90	n-Propyl 9,12-hexadecadienoate
18	42.678	201265	0.44	9-Octadecenoic acid (Z)-, oxiranylmethyl est
19	43.173	1998000	4.38	HEXATRIACONTANE
20	43.600	520512	1.14	Bis(2-ethylhexyl) phthalate
21	46.076	404819	0.89	28-Norolean-17-en-3-one
22	46.261	1702411	3.74	Tetrapentacontane
23	46.538	307070	0.67	STIGMASTA-5,24(28)-DIEN-3-OL, (3.BET
24	47.862	561388	1.23	2,6,10,15,19,23-Pentamethyl-2,6,18,22-tetra
25	49.120	2123548	4.66	Tetrapentacontane
26	50.711	25227776	55.35	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphit
27	51.451	278608	0.61	.gamma.-Tocopherol
28	51.930	334088	0.73	
29	52.185	2486425	5.46	METHYL COMMATE A
30	52.430	479433	1.05	
31	52.550	924229	2.03	Octadecane, 2,6,10,14-tetramethyl-
32	52.718	1330737	2.92	Cholesterol
		45578345	100.00	

Among the 30 compounds 5 peak compounds were analyzed such as Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1), Tetrapentacontane, Tetrapentacontane, Hexatriacontane, 2,6,10,15,19,23-Pentamethyl-2,6,18,22-tetracosatetraen-10,15-diol and 1 compounds possess antiviral activity STIGMASTA-5,24(28)-DIEN-3-OL, (3. BETA.)-

V. CONCLUSION

Natural medicines are well known for their constant, gradual and effective actions towards various diseases and disorders. *Commelina benghalensis* was been studied for Phytochemical analysis, Silver Nano particles, anti-oxidant activity and GC-MS analysis. The phytochemical including alkaloids, flavonoids, carbohydrates, phenol, Glycoside, Anthocyanin, Quinones, Saponins, steroids, tannin, Terpenoids. The 30 bio active compounds that present in the ethanolic extract of root was identified by GC- MS analysis.

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