RESEARCH ARTICLE

Isolation, Characterization and Determination of Structural Composition of *Ocimum Tenuiflorum* (Krishna Tulsi Plant) by IR, NMR, Mass Spectroscopy.

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Received: 2nd Aug, 2024; Revised: 10th Aug, 2024; Accepted: 19th Aug, 2024; Available Online: 31st August, 2024

ABSTRACT
Background: Known by its common name, Ocimum tenuiflorum, this leaf is a fascinating one with a long history of therapeutic and medicinal uses. This evergreen leaf, which is indigenous to the Indian subcontinent, is well known around the world for its versatility and capacity to treat a wide range of illnesses. **Objective**: The primary goal of this bioanalytical investigation is to identify and structurally clarify the principal phytoconstituents found in the Ocimum tenuiflorum ethanolic extract. **Method:** In this phyto-analysis, spectral analysis was used to clarify the structure of the phytoconstituents that were extracted from the Ocimum tenuiflorum plant using column chromatography.
Result and Conclusion: Using spectrum analysis (I.R., NMR, and mass spectroscopy), two significant isolates from Ocimum tenuiflorum, **Apigenin** and **Carnosic Acid**, were found and their structures clarified.
Key words: Antioxidants; Anti-inflammatory; Cough and cold; Anti-bacterial; Respiratory disorders.
How to cite this article: Jay Prakash Singh, Subhashish Tripathy, Saloni Srivastava, Veerendra Kumar Chaurasia. Isolation, Characterization and Determination of Structural Composition of Ocimum Tenuiflorum (Krishna Tulsi Plant) by IR, NMR, Mass Spectroscopy.International Journal of Pharmaceutical Quality Assurance. 2024;15(3):1989-1998.
DOI: 10.25258/ijpqa.15.3.133
Source of support: Nil.
Conflict of interest: None

INTRODUCTION

Since the dawn of civilisation, medicinal plants have been utilised in India and around the world to cure a wide range of illnesses. In actuality, both conventional and synthetic herbal medication may be found in natural goods. We are blessed by nature with a very rich botanical diversity, with many different kinds of plants growing throughout the nation. Thousands of species are known to have therapeutic potential in India, and using various sections of multiple medicinal plants to treat particular illnesses has long been popular. Since the beginning of human civilisation, medicinal plants have been essential to maintaining and restoring human health. Every illness or condition found in nature has a remedy, and traditionally, plants have been utilised to address them. The most revered herb among Hindus in India is called "Thulsi" in Hindi, "Tulsi" in Tamil, "Holy Basil" in English, and "Tulsi" in Sanskrit. Ocimum (Lamiaceae) is a fragrant, upright herb with silky hairs that is often grown as a holy plant in homes and temples [1]. There are 63 Ocimum species known to exist worldwide. Two varieties of Ocimum are cultivated in India: O. tenuiflorum, also known as shyamatulasi, has purple leaves, and sritulasi, ramatulasi, and lakshmitulasi (Ocimum sanctum) have green leaves. Kumartulasi, Krishnatulasi. The plant is indigenous to India and is found throughout the subcontinent, reaching as high as 1,800 meters in the Himalayas and the Andaman and Nicobar islands. In addition to being widely spread throughout Asia, it is also present in Australia, West Africa, and a few Arab nations[2].

The O. tenuiflorum plant is a 30–60 cm tall, upright, heavily branched sub-shrub with purple sub-quadrangular branches. The leaves are pubescent on both surfaces, simple, opposite, elliptic, oblong, obtuse, or acute, with whole, sub-serrate, or dentate edges. The petioles are hairy and thin. Purple blooms are produced in tight, elongated racemes.spirals. The round, flattened, shiny seeds have a reddish-yellow hue. Insect-repelling mixtures of dried Tulasi leaves and stored grains have been used for millennia. Due to its wide spectrum of medicinal applications, tulasi plays a significant part in the traditional Ayurvedic and Unani medical systems[3]. Because of its many therapeutic benefits, Ayurveda has employed it for hundreds of years. When water is combined with petals, it is given to the dying to elevate their spirits to paradise. The whole O. tenuiflorum plant is useful medicinally; the majority of the plant's leaves, as well as occasionally the stem, flower, root, and seeds, are known to have therapeutic potential[4,5,6]. Tulasi Ayurvedic treatments for common colds, headaches, stomach issues, inflammation, heart problems, different types of poisoning, and malaria use extracts. O. tenuiflorum is traditionally consumed in a variety of ways, including fresh leaf, dried powder, and herbal tea. Numerous illnesses, including bronchitis, malaria, diarrhoea, dysentery, skin conditions, rheumatoid arthritis, eye disorders, and insect stings, have been treated with them. Anti-fertility, anticancer, antidiabetic, antifungal, antibacterial, cardioprotective, analgesic, antispasmodic, and adaptogenic properties have also been proposed for

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a: *Ocimum tenuiflorum* Plant

b: Ocimum tenuiflorum Fruit

c: Ocimum tenuiflorum leaf

d:Ocimum tenuiflorum dry leaf Powder

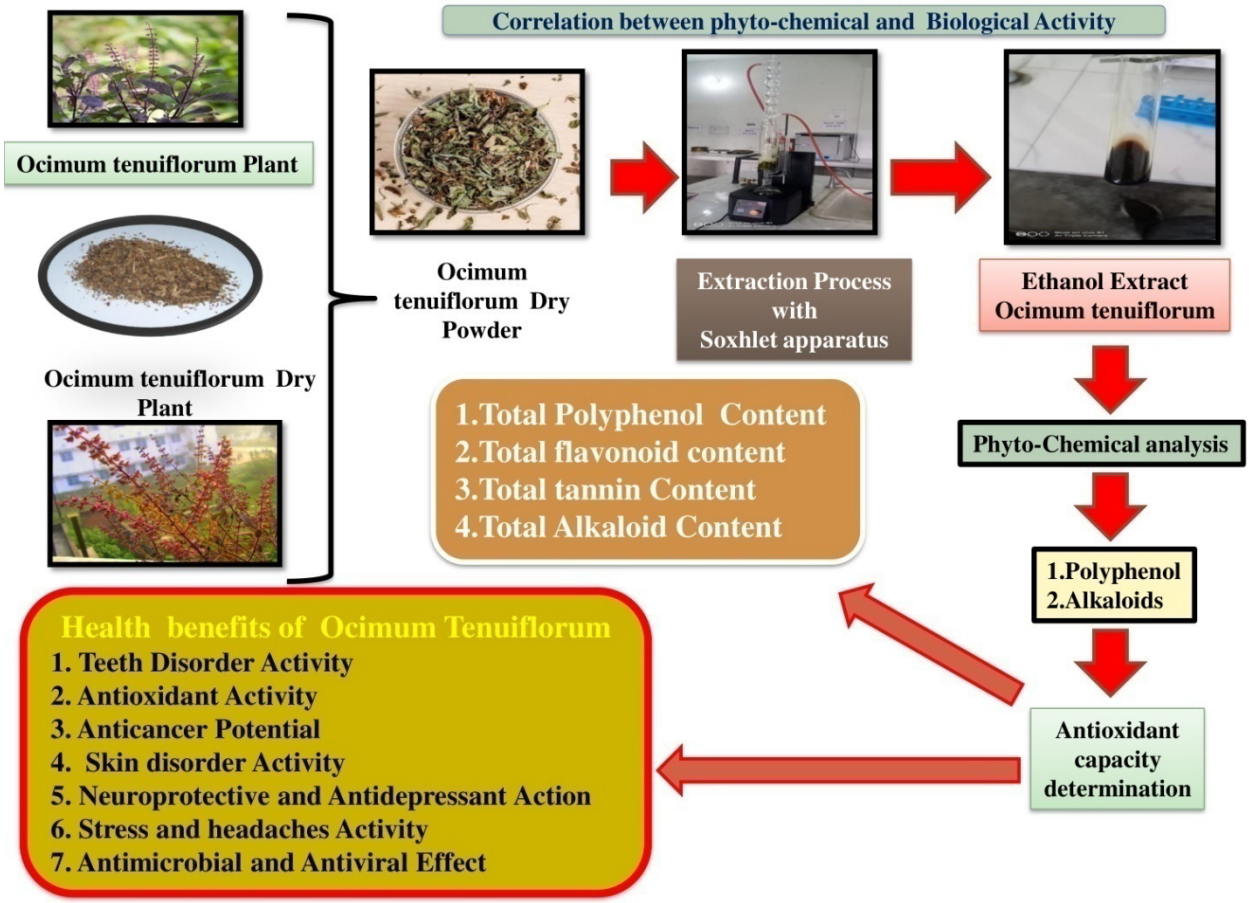

Figure 1: (a-d) Ocimum ternuiflorum in different states, 1e: Correlation between phyto-chemical and Biological Activity

the plant[7,6]. Its oil has a nice scent typical of the plant, with a noticeable clove undertone. The plant has many morphologically distinct chemotypes. Similar plants with different chemical components. In O. tenuiflorum, ethanolic rich chemotypes predominate[8,9]. There has been significant variation documented in the volatile components found in O. tenuiflorum leaves and inflorescence oil[10]. Apocynaceae family member Catharanthus roseus is a potentially therapeutic plant with a variety of pharmacological properties, including antibacterial, antioxidant, anthelmintic, antifeedant, antisterility, antidiarrheal, and antidiabetic effects[11]. Antioxidant properties are seen in the petals, seeds, and other plant components of C. roseus. attributes. It is used in a variety of pharmaceutical, cosmetic, and food sectors. More than seventy different types of chemical components, including reserpine, ajmalicine, serpentine, and indole type alkaloids, might be synthesised using it. C. roseus possesses antihypertensive and antispasmodic activities as a result of the presence of those alkaloids. Vinblastine, which is derived from C. roseus, is one of the significant classes of alkaloids because of its widespread application in medicine and anticancer activity[12]. The aforementioned chemicals are widely used in the treatment

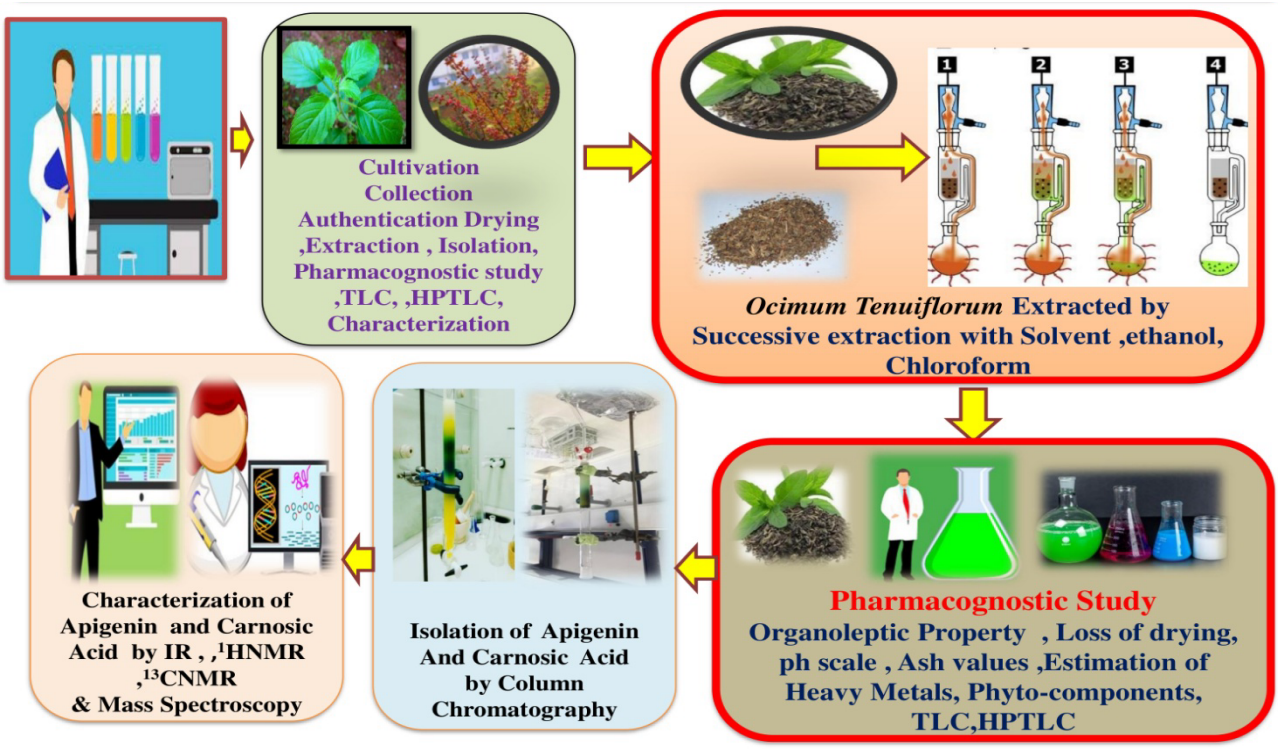

Figure 1f: Pharmacognostic and Phytochemical Analysis of Ocimum Tenuiflorum Extract

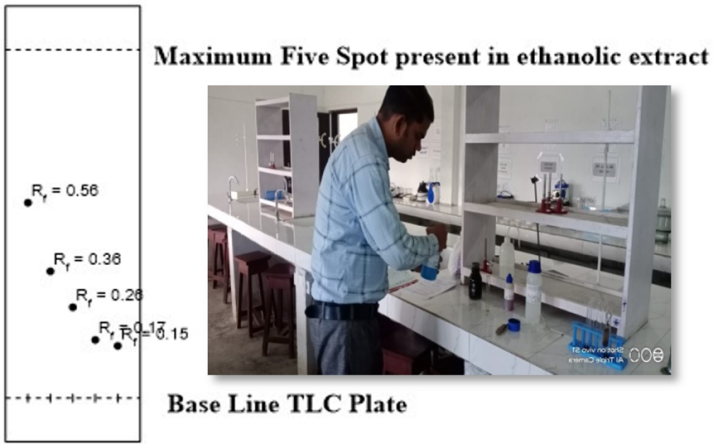

Figure 2: Graphical presentation of base line TLC plate

Figure 3: Isolation of *Ocimum tenuiflorum* TLC of Ethanolic extract of *Ocimum tenuiflorum* by Column chromatography

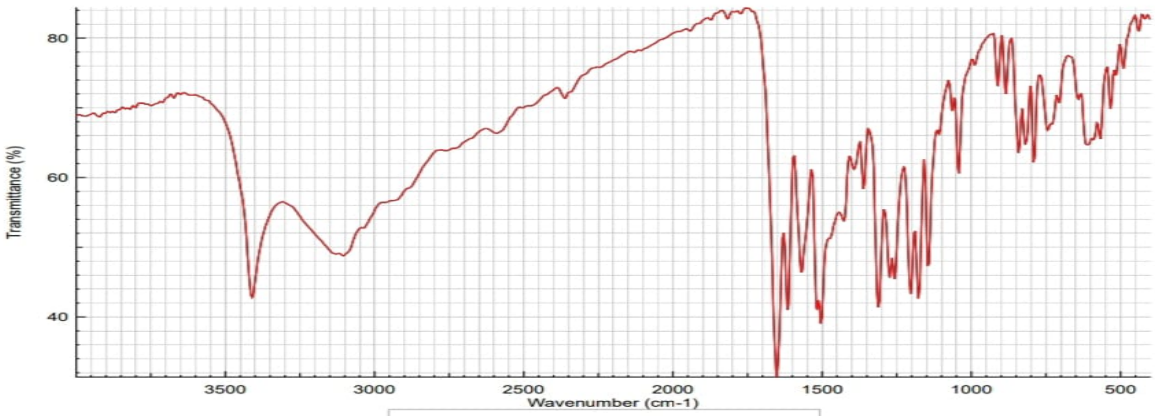

Figure.4: I.R. of D-1

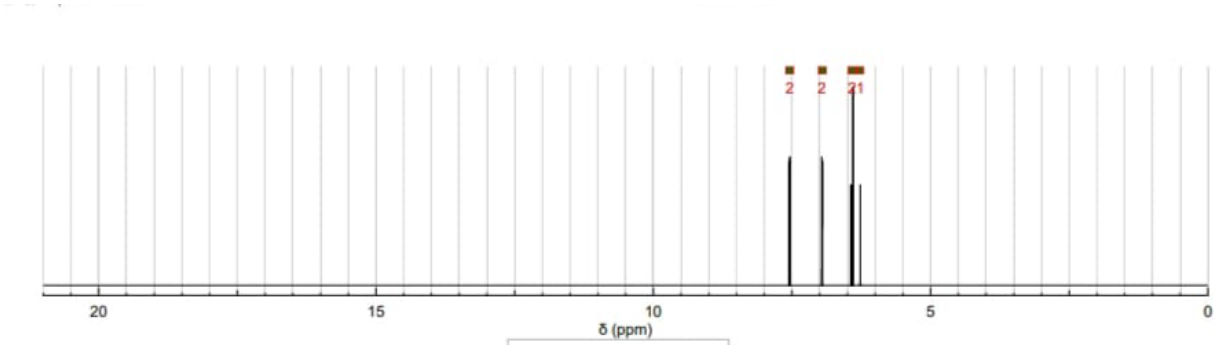

Figure.5 :[1]H NMR of D-1

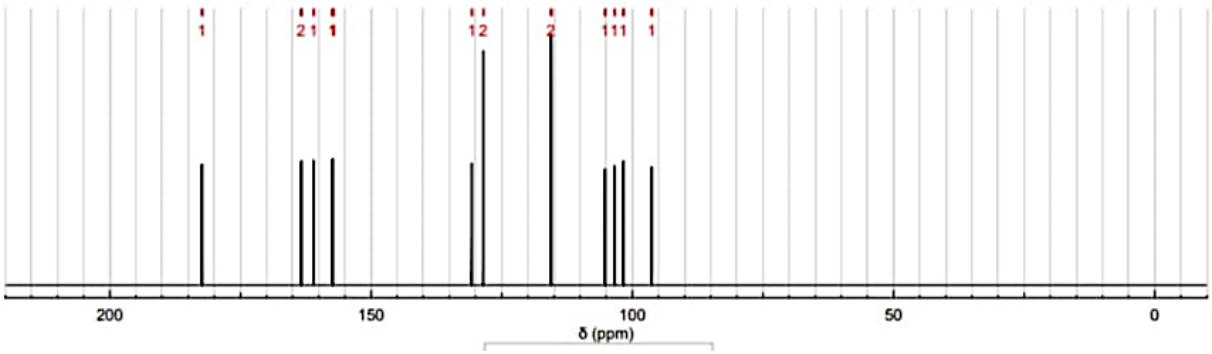

Figure.6:13 C NMR of D-1

of several cancers, including lymphocytic cancer, Wilkins's cancer, neuroblastoma and reticulum cell tumours, andillness other than choriocarcinoma and lymphosarcoma[13]. It has notable concentrations of phenolic and volatile substances, such as flavanol glycosides and caffeoylquinic acids, which are known to

Hydrogen	Chemical Shift(ppm)
21	6.27
22	7.58
23	6.92
25	6.93
26	7.58
27	6.26
29	6.19

Figure.7: Chemical shift of [1]H NMR and [13]C NMR of D-1

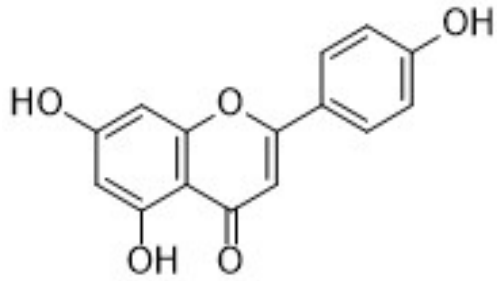

Figure.9: Structure of D-1, 5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one

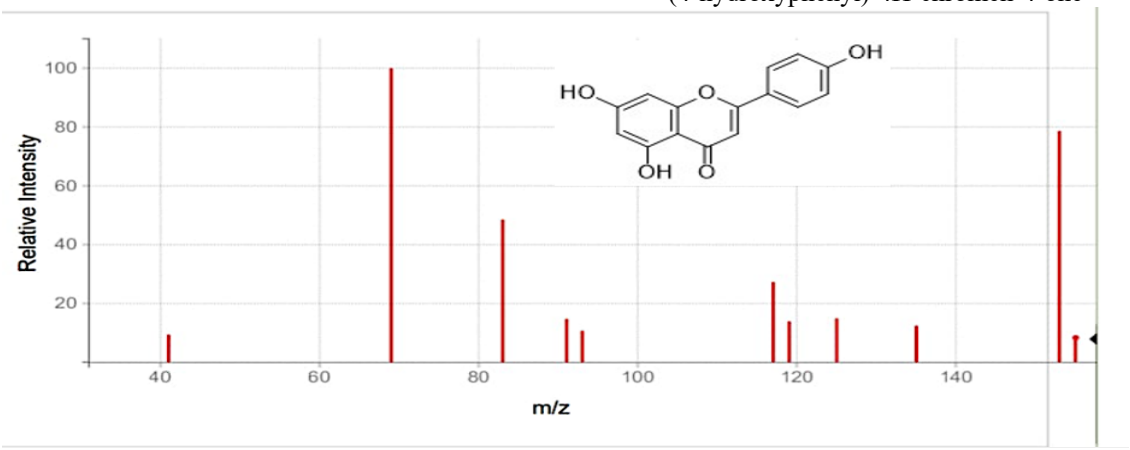

Figure 8: **Mass spectrum of D-1**

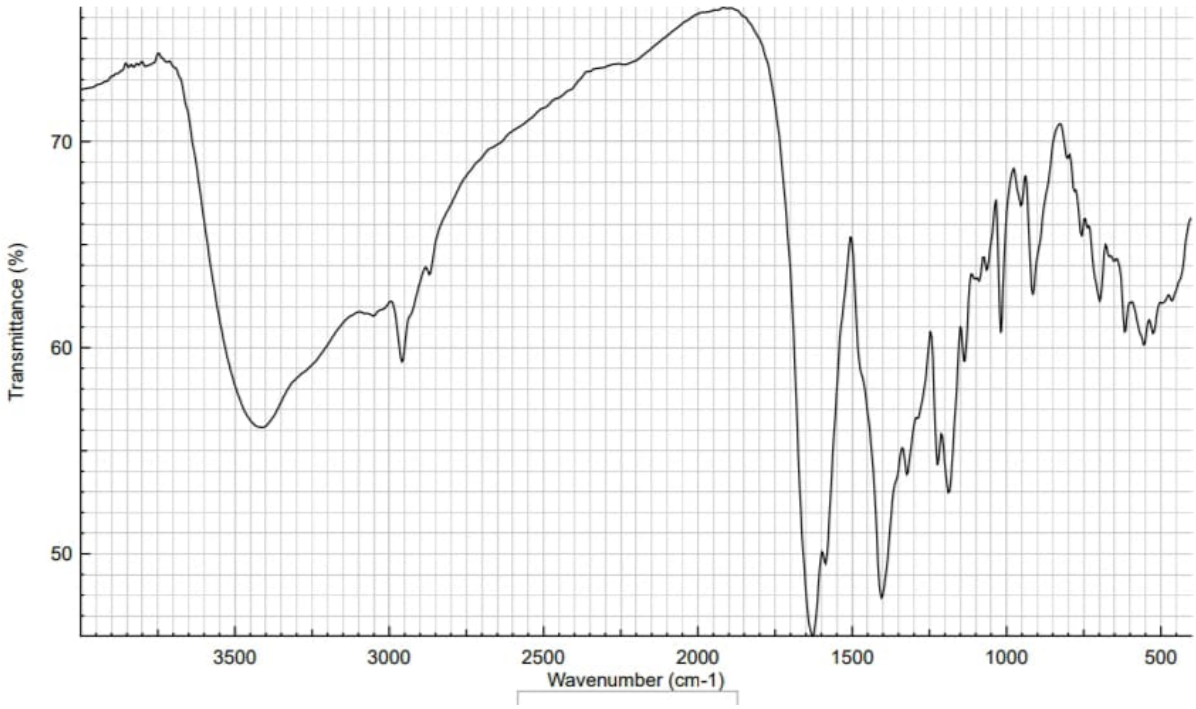

Figure.10.IR of D-2

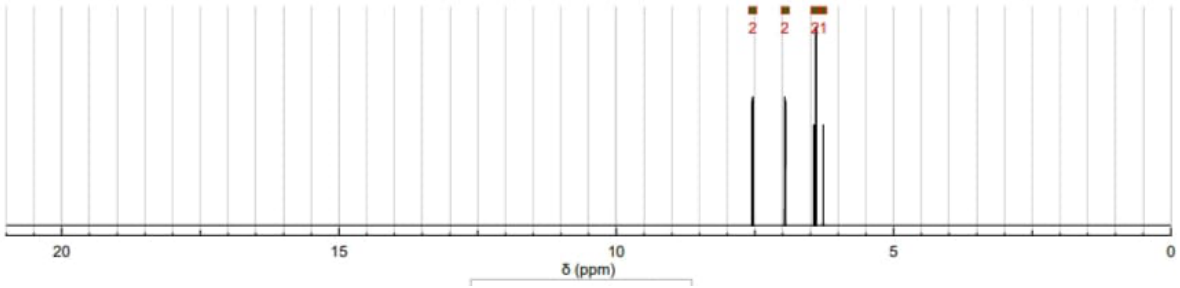

Figure.11.[1]H NMR of D-2

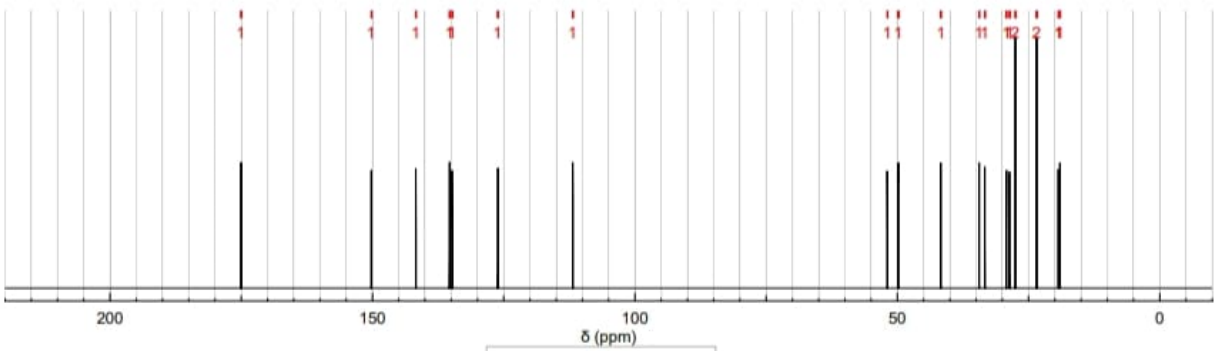

Figure.12.[13]C NMR of D-2

have antioxidant properties. It plays a crucial part in the body's defence mechanism against dangerous reactive oxygen species (ROS), which are created when regular cell aerobic respiration produces such products. Pathological illnesses such ischaemia, asthma, arthritis, inflammation, neurodegeneration, Parkinson's disease, mongolism, the ageing process, and maybe dementia can be brought on by an accumulation of free radicals[14]. The phenolic Compounds with redox characteristics can function as metal chelators, hydrogen donors, reducing agents, or singlet oxygen quenchers. Its phenolic component has antiallergic, anti-inflammatory, antibacterial, antithrombotic, cardioprotective, and vasodilatory properties in addition to antioxidant activity[15]. Characterising the major and secondary phytochemicals of a single component—the leaves of two therapeutic herbal plants, O. tenuiflorum and C. roseus—was the primary goal of the current investigation.

Hydrogen	Chemical Shift(ppm)
25	1.18
26	1.19
27	1.17
28	2.90
29	1.17
30	1.17
31	1.17
32	6.76
36	1.65
37	2.67
38	1.57
39	1.52
40	1.47
41	1.55
42	1.00
43	0.96
44	1.02
45	1.04
46	1.04
47	1.03
48	1.77
49	1.82
50	2.01
51	2.90
52	2.67

Figure.13: Chemical shift of 1H NMR and 13C NMR of D-2

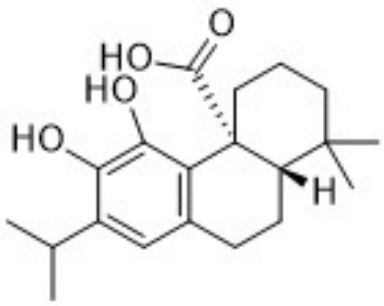

Figure.15: Structure of D-2, (4aR,10aS)-5,6-dihydroxy-7-isopropyl-1,1-dimethyl-1,3,4,9,10,10a-hexahydrophenanthrene-4a(2H)-carboxylic acid

MATERIALS AND METHODS:

Sample collection

Ocimum tenuiflorum Plant are collected from the source of the Sarvoday Nagar Home Garden Raebareli ,UP 229001 India. The *Ocimum tenuiflorum* Plant are collected, washed with Dist—water, dried in shade, Crushed form a Powder and further stored for extraction and bio-analytical study for Research.

Extraction and isolation of *Ocimum Tenuiflorum* Leaf phytoconstituents

After successive extraction in Two different solvents viz. Ethanol (60-70^{0}c), Chloroform ($CHCl_3$) solution, preliminary phytochemical screenings indicate the presence of various constituents like alkaloids, tannins, flavonoids, steroids, glycosides, saponins, phytosterols etc. In TLC Study it was noticed maximum 4 spots obtain in Ethanolic extract of *Ocimum tenuiflorum* Plant Ethanolic extract of *Ocimum tenuiflorum* Plant extracts were subjected to column chromatography. Column chromatography was used to collect five eluted fractions (10-14) and (25-29) using different proportion of Mobile phase N-Hexane (C_6H_{14}): Alcohol methyl (CH_3OH). Two Pure isolates were obtained by column chromatography through a TLC study. Analyzing the fractions of chemicals by column chromatography has always been done using thin-layer chromatograph. Bioactive compounds have been separated using C chromatography technique and thin-layer chromatography (TLC) using various analytical instruments.

2.4 Identification and structural elucidation of organic Compound by Spectrometry

The two pure isolates D-1 and D-2, obtained by column chromatography, undergoes various spectroscopic approaches, mass spectroscopy, 1H NMR, which is 13C NMR, and FTIR for the identification of isolated compounds. In organic chemistry, infrared (I.R.) spectroscopy is helpful because it makes it possible to distinguish between various functional groups. This is because every functional group has certain bonds that consistently appear in the exact locations across the infrared spectrum. the application of Fourier transform in used to identify functional groups (FTIR)spectroscopy. These include vibration bands such as N-H, R-OH, C-H, R-C O. C = C, C = N C = N, and COOH. Atoms and molecules can have their physical and chemical properties ascertained using NMR spectra analysis. Based on the phenomena of nuclear magnetic resonance, it provides extensive details regarding molecules' kinetics, structure, reaction state, and chemical environment. A compound's weight spectrum usually comprises of many signals, the peak at the greatest m/z (molecular ion) value representing the amount of mass of the complete structure.

Identification of D-1 Isolate:

IR of D-1: The FT-IR spectra of Compound D-1 displays many prominent peaks at various ranges: strong O-H stretching intermolecular bonded alcohol present at 3649.69 cm-1; strong O-H stretching vibration(v) at 3271 cm-1 in the presence of alcohol and phenols; strong C=O stretching and saturated five-member ring carboxylic acid confirmed at 1829.11 cm-1; and 1608.56 cm-1, 1098.61 C=C Alkenes are bent C=C trisubstituted stretching alkenes with a beta keto aldehyde as an enol of 1651 cm-1 and a C-C stretching vibration of 1424 cm-1. Strong (C=C) bending alkene conformation, aromaticity, and 730.88 (cm-1) bending alkene structure

1H NMR of D-1: ^{1}H NMR: δ 6.26 (1H, d, J = 2.4 Hz), 6.35-6.48 (2H, 6.40 (s), 6.43 (d, J = 2.4 Hz)), 6.96 (2H, ddd, J = 8.3, 1.1, 0.5 Hz), 7.54 (2H, ddd, J = 8.3, 1.8, 0.5 Hz).

^{13}C NMR of D-1: ^{13}C NMR: δ 96.3 (1C, s), 101.7 (1C, s), 103.4 (1C, s), 105.2 (1C, s), 115.5 (2C, s), 128.5 (2C, s), 130.7 (1C, s), 157.2 (1C, s), 157.4 (1C, s), 161.0 (1C, s), 163.3-163.5 (2C, 163.3 (s), 163.4 (s)), 182.3 (1C, s).

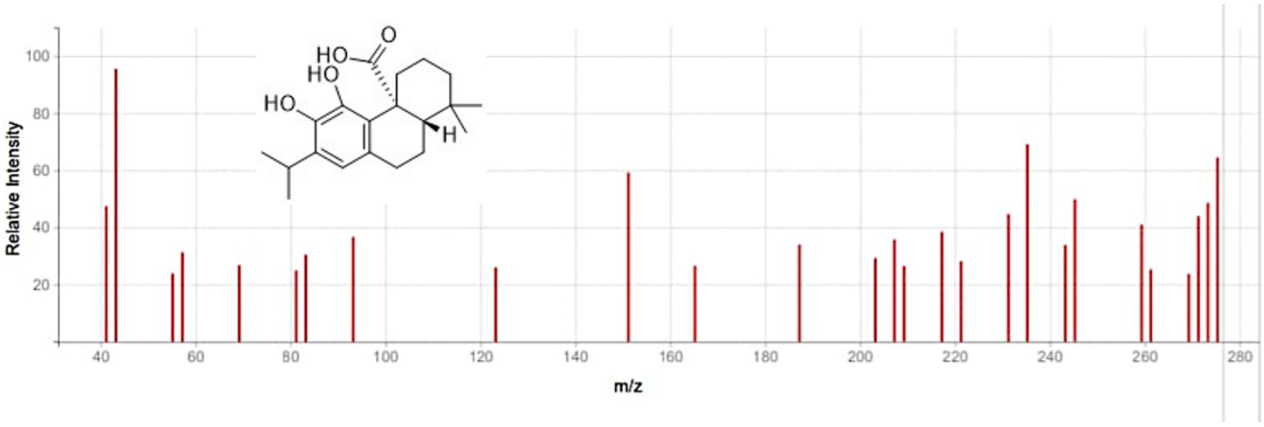

Figure 14: Mass spectrum of D2

Mass spectrum of D-1: Chemical Formula: **$C_{15}H_{10}O_5$** , Exact Mass of compound **270.05**
Molecular Weight: **410.73**, **m/z:** 270.05282 (100.0%), 271.05618 (16.2%), 272.05953 (1.2%), 272.05707 (1.0%) and elemental analysis provide C, 66.67; H, 3.73; O, 29.60.

Identification of D-2 Isolate:

IR of D-2: 1828.92 cm-1 (w intensity C-H bending aromatic compound), 2306.48 cm-1 (s intensity O=C=O stretching), and 3649.77 cm-1 (v intensity free O-H) 1747.90 cm-1 (stretching with an intensity of C=O) and 1655.51 cm-1 (wrestling with an intensity of C=C)m intensity C=C stretching cyclic alkene: 1605.10 cm-1; s intensity carboxylate ions: 1312.38 cm-1'; s intensity carbonyl group: 1241.57 cm-1; s intensity C-O stretching ester: 1160 cm-1 841.61 cm-1 (w intensity isolated aromatic C-H), 931.11 cm-1 (m intensity C = C bending alkene viny lidene).

^{1}H NMR of D-2 ^{1}H NMR: δ 0.92 (6H, s), 1.22-2.01 (15H, 1.28 (d, *J* = 7.0 Hz), 1.37 (ddd, *J* = 13.1, 2.9, 2.7 Hz), 1.42 (ddd, *J* = 13.1, 10.3, 2.9 Hz), 1.55 (dtt, *J* = 13.1, 10.3, 2.9 Hz), 1.66 (dtt, *J* = 13.1, 2.9, 2.7 Hz), 1.70 (dtd, *J* = 13.3, 9.9, 4.0 Hz), 1.73 (ddt, *J* = 13.3, 4.1, 1.9 Hz), 1.81 (ddd, *J* = 12.9, 10.3, 2.9 Hz), 1.81 (dd, *J* = 10.0, 1.9 Hz), 1.93 (ddd, *J* = 12.9, 2.9, 2.7 Hz)), 2.59-2.77 (2H, 2.67 (ddd, *J* = 12.9, 9.9, 4.1 Hz), 2.69 (ddd, *J* = 12.9, 4.0, 1.9 Hz)), 3.00 (1H, sept, *J* = 7.0 Hz), 6.52 (1H, s).

^{13}C NMR of D-2: ^{13}C NMR: ^{13}C NMR: δ 19.0 (1C, s), 19.3 (1C, s), 23.4-23.5 (2C, 23.4 (s), 23.4 (s)), 27.4-27.6 (2C, 27.5 (s), 27.5 (s)), 28.6 (1C, s), 29.2 (1C, s), 33.3 (1C, s), 34.4 (1C, s), 41.7 (1C, s), 49.8 (1C, s), 51.9 (1C, s), 111.8 (1C, s), 126.1 (1C, s), 134.8 (1C, s), 135.3 (1C, s), 141.7 (1C, s), 150.2 (1C, s), 175.0 (1C, s).

Mass spectrum of D-2: Chemical Formula of D-2 is **$C_{20}H_{28}O_4$**, Exact Mass: 332.19, Molecular Weight: 332.44 , m/z: 332.19876 (100.0%), 333.20211 (21.6%), 334.20547 (2.2%)**,** Elemental Analysis of D-2 give C, 72.26; H, 8.49; O, 19.25.

DISCUSSION

From the above, spectroscopic analysis of two isolates collected from ***Ocimum Tenuiflorum*** Leaf by column chromatography gives special structural characterization by interpretation of the spectrum. D-1 compound FTIR spectrum conformed that C-H bending aromatic compound at 1720.92 cm-1, O=C=O stretching at 2306.48 cm-1, free O-H at 3649.77 cm-1, C=O at 1747.90 cm-1, C=C at 1655.51 cm-1, C=C stretching at cyclic alkene: 1605.10 cm-1, carboxylate ions at 1312.38 cm-1', carbonyl group at1241.57 cm-1, C-O stretching at 1160 cm-1, isolated aromatic C-H at 841.61 cm-1, C=C bending alkene vinylidene at 931.11 c 1H NMR of D-1 gives chemical shift of 3-furan at δ 7.42, cyclopentene at δ 3.54, 1 alpha -C*R from methane at δ 1.22, cyclopentene at δ 2.44, cyclohexane at δ 1.20, methyl at δ 3.57, methylene at δ 2.27, 1-ethylene at δ 6.73. 13 C NMR of D-1 gives 1-carboxyl at δ 177, 3-furan at δ 143, cyclohexane δ 86.27, δ 70.15, aliphatic compound at δ 55.48 δ41, 1-ethylene at δ 126, 1-carbonyl at δ202. Mass spectrum of D-1 shown Molecular Weight: **410.73**, m/z: 270.05282 (100.0%), 271.05618 (16.2%), 272.05953 (1.2%), 272.05707 (1.0%) Similarly FTIR of D-2 compound showed that C-H bending aromatic compound at 1828.92 cm-1, O=C=O stretching at 2306.48 cm-1, free O-H at 3649.77 cm-1, C=O at 1747.90 cm-1, C=C stretching cyclic alkene: 1605.10 cm-1, intensity carbonyl group at 1241.57 cm-1, C=C bending alkene vinylidene, 931.11 cm-1.1H NMR of D-2 shown 3-furan at δ 7.42, Cyclopentene at δ 3.55, Cyclohexane at δ 4.37, Methyl at δ 3.72, methylene at δ 2.63, Ar-H at δ 6.74. 13C NMR of D-2 shown 3-furan at δ 146.27, R-CH_3 at δ 24.43, R-C=OH at δ 177, aliphatic compound at δ 88.3, cyclohexane at δ 70.28 .Mass spectroscopy of the D-2 compound showed that Exact Mass: **332.19**, Molecular Weight: **332.44**, m/z: 332.19876 (100.0%), 333.20211 (21.6%), 334.20547 (2.2%)**,**. From the above spectroscopic analysis, we found that the Exact Mass of D-2 is **332.19**, Molecular Weight: is **332.44**, m/z: 332.19876 (100.0%), 333.20211 (21.6%), 334.20547 (2.2%) **,** Elemental Analysis of D-2 give C, 72.26; H, 8.49; O, 19.25 . From the above spectroscopic interpretation, we found that the chemical name of D-1 Is [5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one] common name **Apigenin** and chemical name of D-2 is [(4aR,10aS)-5,6-dihydroxy-7-isopropyl-1,1-dimethyl-1,3,4,9,10,10a-

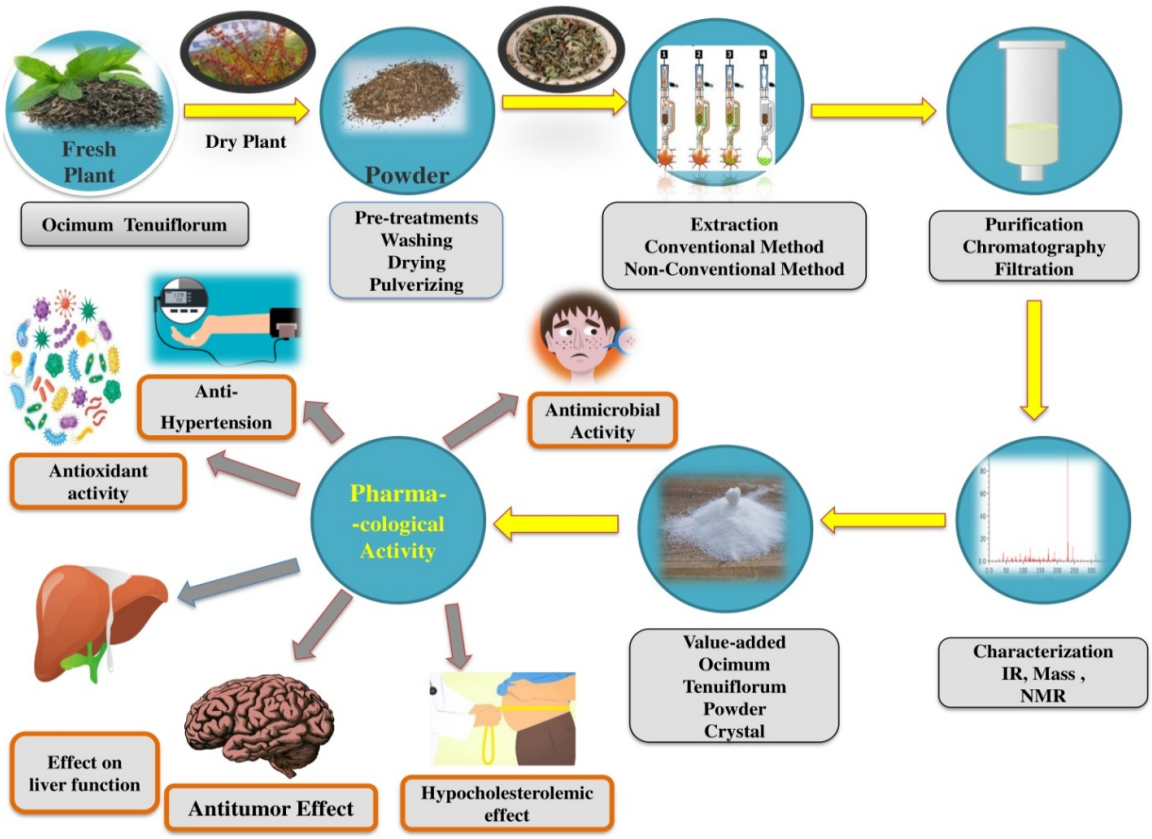

Figure 16: Pharmacological Activity of Ocimum tenuiflorum

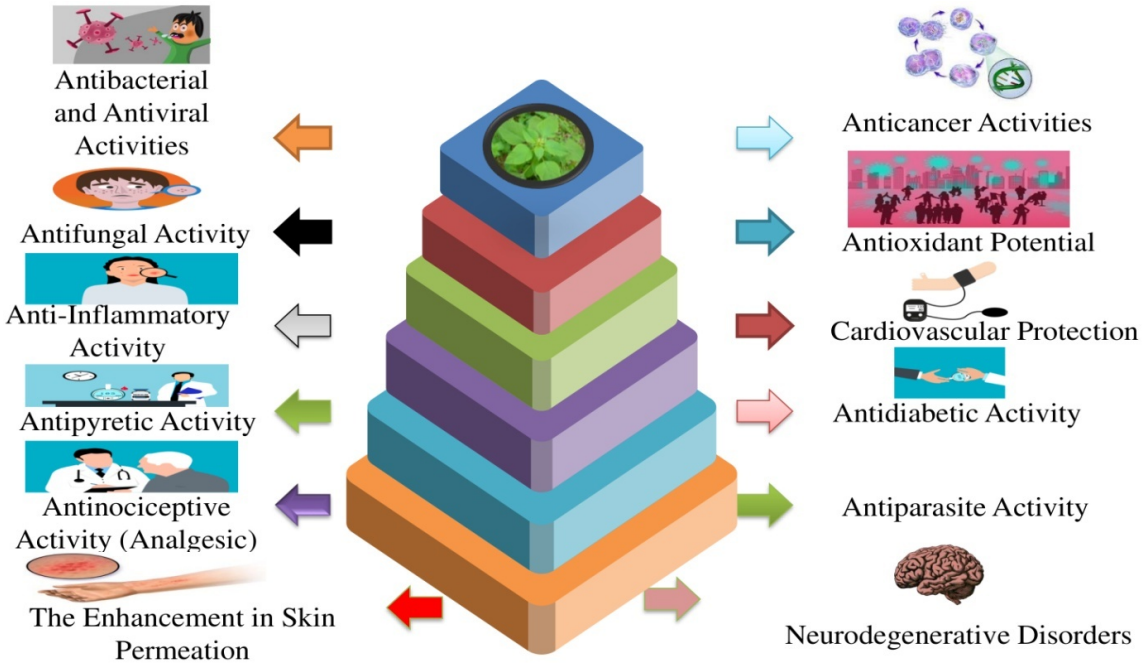

Figure 17: Pharmacological Properties and helath benefits of Ocimum tenuiflorum

hexahydrophenanthrene-4a(2H)-carboxylic acid] shortened form **Carnosic Acid**.

SUMMARY AND CONCLUSION:

Ocimum Tenuiflorum *leaf* is a traditional medicinal plant in India used in Ayurveda for various medicinal use like antibacterial, antifungal, immunomodulatory, Antifungal effects ,Analgesic effects, Anti-allergic Activity , Antimicrobial, Antihypertenssion type medicinal activities

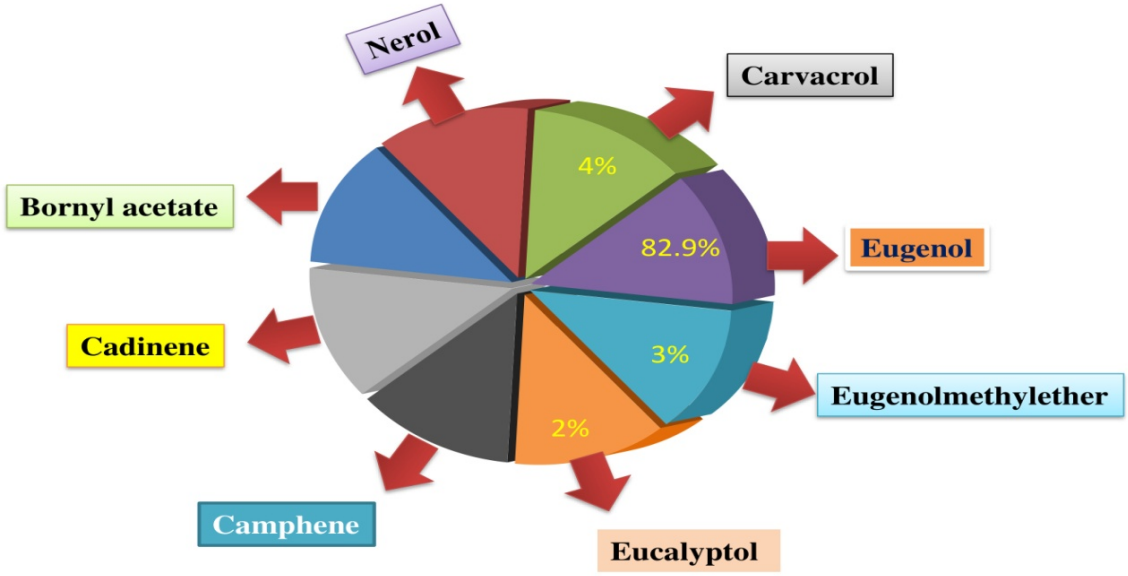

Figure 18: Chemical Molecule present in Ocimum Tenuiflorum

from ancient type. The medicinal activities of ***Ocimum Tenuiflorum*** *plant* are due to the multiple phytoconstituents present in this plant. This low cost bio-analytical method used for this spectroscopic analysis of this medicinal plant, is a very effective technique for isolating and characterizing phytoconstituents present in this medicinal Plant leaf . From the results we have drawn the following conclusions that the D-1 isolate is **Apigenin** and D-2 isolate name is **Carnosic Acid** are two very important phytoconstituents responsible for medicinal activates of ***Ocimum Tenuiflorum***. The methods described in this paper would benefit from further development of bio-analysis of ***Ocimum Tenuiflorum*** *Leaf.*

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