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GC-MS Screening of Bioactive Compounds from Seeds of Croton *Bonplandianum baill* (Euphorbiaceae)

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ABSTRACT

The present investigation was carried out to analyze the active constituents present in aerial parts (dry seeds) of Croton bonplandianum Baill (Euphorbiaceae) by GC-MS analysis. The presence of phytochemical compounds was screened by qualitative method. Totally 4 bioactive Phytochemical compounds were identified in the n-hexane extract of Croton species, the components were identified by comparing their relation indices and mass spectra. Fragmentation patterns with those stored on the MS-Computer library and also form the published literatures. The prevailing compounds were Squalene, (9Z, 12Z)-octadeca-9, 12-dienoic acid, methyl 12-oxo-octadec-9-enoate- and phytol. The research revealed the potential of hexane extract of Croton bonplandianum Baill seeds as a good source of phytochemicals that justify the use of this plant for its various ailments by traditional practitioners.

Keywords: Euphorbiaceae, GC-MS analysis, mass spectra Fragmentation patterns and uses.

INTRODUCTION

Plant-derived substances have recently become of great interest owing to their versatile applications. *Croton* is a genus of Euphorbiaceae comprising around 1,300 species, widespread in tropical regions of the old and new worlds. Several species have a long role in the traditional use of medicinal plants in Africa, Asia and South America. Croton bonplandianum Baill. Common name Ban tulsi (Euphorbiaceae), commonly known as “Ban Tulsi”, is a perennial herb found in waste lands and road side areas in India. People use it as both fuel and detergent. First the stems and branches are used as fuel and afterwards the ash is collected and kept in a bottle for 5-6 days. Then the ash is put in warm water and used as detergents for cleaning cotton garments. Flowering and fruiting time of this plant is from September to December [1]. Traditionally, this plant is used to treat liver and skin disease including ring worm infection and also to cure the swelling of body [2]. Bark and roots of C. bonplandianum are alterative and chologogue [3, 4]. Leaves of this plant are medicinally used for the treatment of cuts and wounds, venereal sores and cholera [5]. The seeds are used for the treatment of jaundice, acute constipation, abdominal dropsy and internal abscesses [1]. The genus Croton is rich in secondary metabolites including alkaloids, terpenoids and also possesses toxic components, phorbol esters [6, 7]. Phytochemically, the plant *C.* bonplandianum has been reported to contain rutin ($C_{18}H_{36}O_{19}$) as main constituent, crotsearinine, crotasparine and its methyl derivatives aphorbol [4, 8 and 9].
Literature survey reveals that C. bonplandianum is having wide range of phytochemical compounds. Plant and leaves contain alkaloids sparsiflorine, crotoflorine, crotsparine, crotsparinine, proaporphine, isoquinoline dienone, N-methylcrotsparine and N-methylcrotsparinine. Leaves and stem contains ß-sitosterol and taraxerol, vomifoliol, ursolic acid and tetrahydroglazievine. Leaves also contain rutin. Seeds contain phorbol diesters, phorbol trimesters, cocarcinogen; alkaloid, 3-OMe-4, 6-di-OH-morphinandien-7-one and norsinoacutine. The roots in addition to ß-sitosterol contain phenolic quinonoid alkaloid norsinoacutine and 3-methoxy-4, 6-dihydroxy morphinan-dien-7-one. An unusual finding of this species is the hyper accumulation of copper in it. The seed of Croton bonplandianum

contains diterpines, phorbol ester, including 12-orthotrideconeoly-phorbol-13-acetat (TPA) and myristoyl phorbol acetate (MPA). TPA is a carcinogen, affecting prostaglandin metabolism [10].

Metal oxides play a very important role in many areas of chemistry, physics and materials science. The metal elements are able to form a large diversity of oxide compounds. In technological applications, oxides are used in the fabrication of microelectronic circuits, sensors, piezoelectric devices and fuel cells, coatings for the passivation of surfaces against corrosion and as catalysts [11-19]. We have presented our results on different oxide materials in our earlier studies [20-72]. The present research is an attempt to investigation of more phytochemical compounds in dried seeds of Croton bonplandianum with neutral solvent n-hexane (NH).

MATERIALS AND METHODS

Chemicals: All Chemicals used in the entire study were AR grade obtained from SD fine chemicals, India, Pvt Ltd.,

Plant Material: Dry seeds of Croton bonplandianum Baill. Collection as shown in Figure 1 (in the month of May 2015) from surrounding areas of rural areas of Vijayawada, Krishna District. The plant material was authenticated at the Botanical Survey of India, Howrah and West Bengal, India.

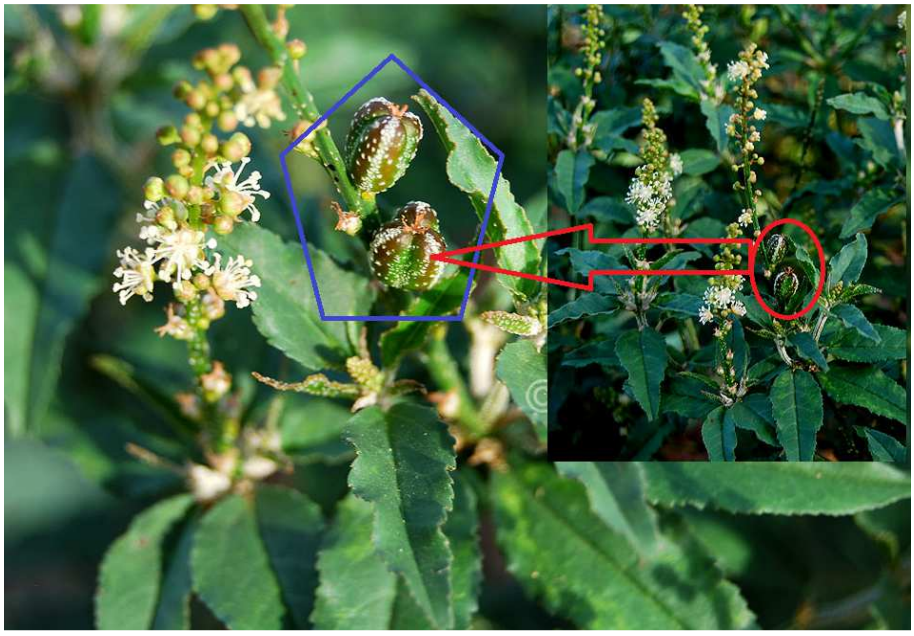

Figure 1: Croton bonplandianum- Fruits, Blooms and Leaves

Preparation of plant extract: The dry material of C. bonplandianum passed through sieve (70 µ). The coarse powdered drug (200grams) was extracted in Soxhlet apparatus for 48 h with n-hexane (60 - 75°C, 2L). N-Hexane extract obtained was concentrated under reduced pressure in rotatory evaporator below 60°C temperature to get semisolid sticky residue (15 gm)

Column chromatography: N-Hexane extract of the plant material (5 g) was subjected to column chromatography using silica gel (80-120 #) as adsorbent and eluted with the mixture of n-hexane: ethyl acetate in gradient manner. N-hexane: ethyl acetate (90: 10) fraction yielded dark brown color liquid.

GC-MS Analysis: GC-MS analysis of the extract was performed using a Perkin-Elmer GC Clarus 500 system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with a Elite-I, fused silica capillary column (30 mm X 0.25 mm 1D X 1 µ Mdf, composed of 100 % Dimethyl poly siloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999 %) was used as the carrier gas at constant flow rate 1ml/min and an injection volume of 2 µl was employed (split ratio of 10:1); Injector temperature 250 °C; Ion-source temperature 280 °C. Mass spectra were taken at 70 eV; a scan interval of 0.5seconds and fragments from 45 to 450 Da. Total GC running time was 36 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a Turbo mass. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of

the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained (Table 1, Table 2 & Figure 2).

Phytochemical screening

Qualitative profiling: n-Hexane extract of Croton was used for qualitative assessment for the major classes of phytochemicals namely tannin, phlobatannin, terpenoids, glycoside, phenolic, flavonoid, steroid, anthraquinone, saponin, alkaloid, carbohydrate and protein etc. The tests were performed according to various standard methods [73]. The tests were based on the visual observation of color change or formation of a precipitate after the addition of specific reagents as shown in Table 3

RESULTS AND DISCUSSION

Herbal medicine represents one of the most important fields of traditional medicine all over the world. The components present in the n-hexane extract of seeds of C. bonplandianum were identified by GC-MS. The GC-MS chromatogram of the 4 peaks of the compounds detected are as shown in Figure 1. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) in the ethanol extracts of aerial parts of C. bonplandianum are presented in Table 1 and Table 2 listed the major phytocompounds and its biological activities obtained through the GC-MS study of the aerial parts of C. bonplandianum. GC-MS profile of the compounds identified is given in the Figures 3, 4, 5 and 6 respectively.

Table 1: Components detected in n-hexane extract of C. bonplandianum

S. No.	RT	Name of the compound	Molecular formula	MW	Peak Area %
1	25.33	Squalene ((6E,10E,14E,18Z)-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14, 18,22-hexaene)	$C_{30}H_{50}$	410	46.21
2	28.78	(9Z,12Z)-octadeca-9,12-dienoic acid	$C_{18}H_{32}O_2$	280	12.21
3	34.35	methyl 12-oxo-octadec-9-enoate -	$C_{19}H_{34}O_3$	310	28.61
4	37.78	Phytol	$C_{30}H_{40}O$	296	9.31

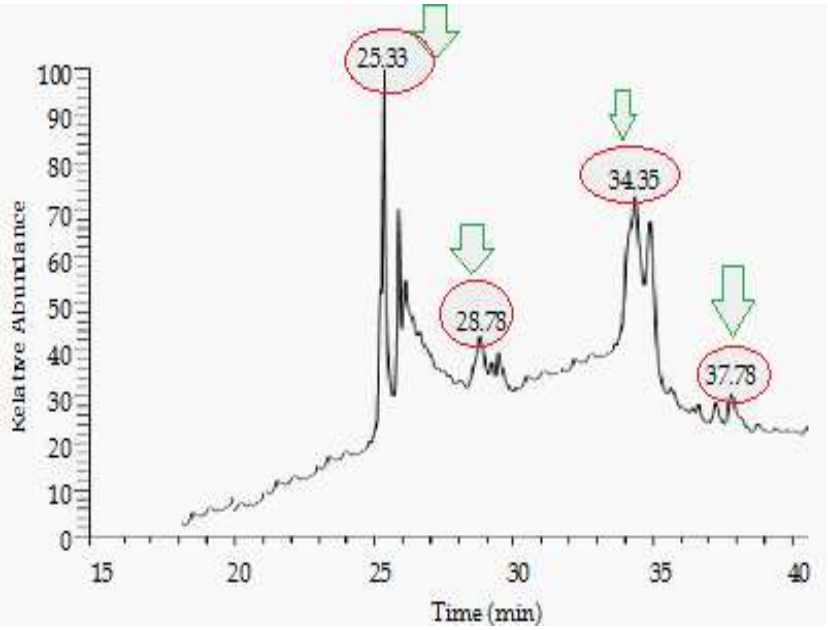

Figure 2: GC-MS Chromatogram of n-hexane extract of C. bonplandianum

Table 2: Listed the major phytocompounds and its biological activities obtained through the GC-MS study of the aerial parts of C. bonplandianum

S. No.	RT	Name of the compound	Molecular formula	MW	Peak Area %	Compd. Nature	Activity≠
1	25.33	Squalene ((6E,10E,14E,18Z)-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene)	$C_{30}H_{50}$	410	46.21	Triterpene	Antibacterial, antioxidant, antitumor, cancer preventive, immune stimulant, chemo preventive, lipoxygenase-inhibitor, pesticide
2	28.78	(9Z,12Z)-octadeca-9,12-dienoic acid	$C_{18}H_{32}O_2$	280	12.21	Linoleate	Hypocholesterolemic, nematicide, antiarthritic, hepato protective, antiandrogenic, hypocholesterolemic 5-alpha reductase inhibitor, antihistaminic, anticoronary, insectifuge, antieczemic, antiacne
3	34.35	methyl 12-oxo-octadec-9-enoate -	$C_{19}H_{34}O_3$	310	28.61	Oxo (Keto) Fatty Acid esters	involved in the Krebs citric acid cycle and in glycolysis
4	37.78	Phytol	$C_{30}H_{40}O_2$	296	9.31	diterpene	Antimicrobial, Anticancer, Diuretic, Anti-inflammatory

≠*Source: Dr. Duke's: Phytochemical and Ethnobotanical databases*

Table 3: Display the presence/ absence of different phytochemicals in the seeds of C. Bonplandianum

Alkaloids	Dragendroff's test/Mayer's test	+
Terpenoids	2ml $CHCl_3$+2ml Con.H_2SO_4	+
Flavonoids	Shinoda test/Lead acetate tests	+
Tannins	Gelatin test/Ferric chloride test	+
Steroids	Salkowski tests/Lieberman Burchardt tests	-
Saponins	Foam test/Froth test	-
Phenols	$FeCl_3$ test/Liebermann's test	-
Cardenolides	Keller Kiliani's test	+
Coumarines	Alcoholic sodium hydroxide	-
Anthraquinone	Borntragers test	–

Chromatogram GC-MS analysis of the n-hexane extract of C. bonplandianum showed the presence of four major peaks and the components corresponding to the peaks were determined as follows: the first set up peak was determined to be Squalene ((6E,10E,14E,18Z)-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene). Squalene, an isoprenoid compound structurally similar to beta-carotene, The primary therapeutic use of squalene currently is as an adjunctive therapy in a variety of cancers. Although epidemiological, experimental and animal evidence suggests anti-cancer properties, to date no human trials have been conducted to verify the role this nutrient might have in cancer therapy regimens [74]. The second peak was indicated to be (9Z, 12Z)-octadeca-9, 12-dienoic acid. It is Linoleic Acid derivative. Linoleic Acid is a polyunsaturated essential fatty acid found mostly in plant oils. It is used in the biosynthesis of prostaglandins and cell membranes [75]. The third peak was indicated to be methyl 12-oxo-octadec-9-enoate - (Figure 2), is fatty acid lipid. The third peak was indicated to be Phytol. The results show that reactive oxygen species promoting substances such as phytol constitute a promising novel class of pharmaceuticals for the treatment of rheumatoid arthritis and possibly other chronic inflammatory diseases Phytol is a key acyclic diterpene alcohol that is a precursor for vitamins E and K. 9, 12, octadecadienoic acid (Z, Z) - has the property of anti-inflammatory and antiarthritic as reported by earlier worker [76, 77]. It could be concluded that, C. bonplandianum seeds contains various bioactive compounds. So it is recommended as plant of pharmaceutical importance. However, further studies are needed to undertake its bioactivity and toxicity profile. The results of the qualitative analysis have been presented in Table 1. Few of the screened phytochemical parameters were present in the seeds of C. bonplandianus, this is due to the dependence of its quantity.

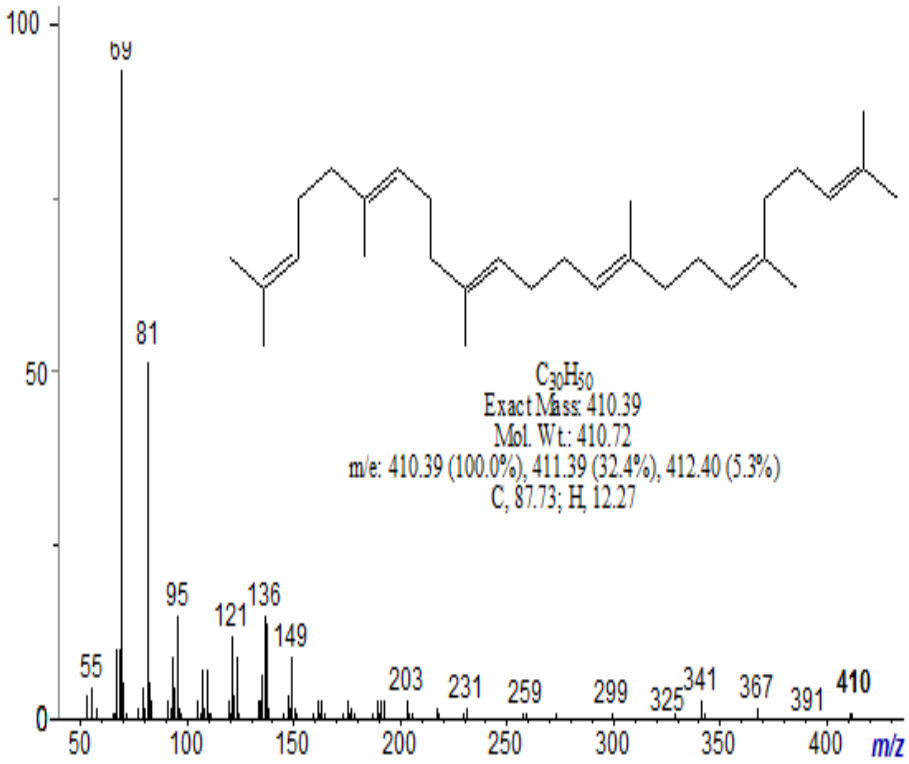

Figure 3: Mass spectrum and structure of Squalene

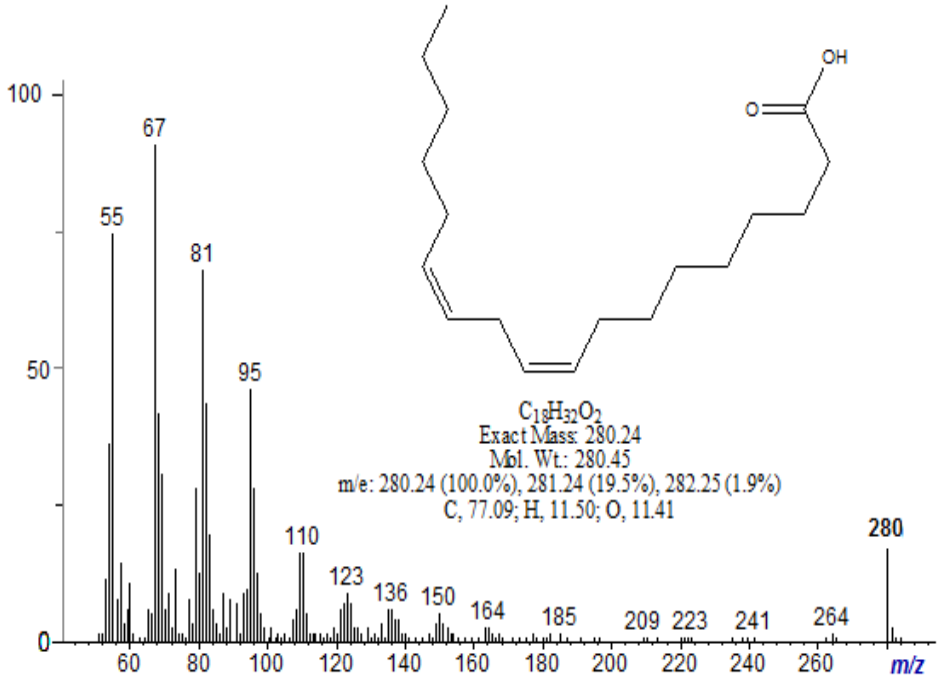

Figure 4: Mass spectrum and structure of (9Z, 12Z)-octadeca-9, 12-dienoic acid

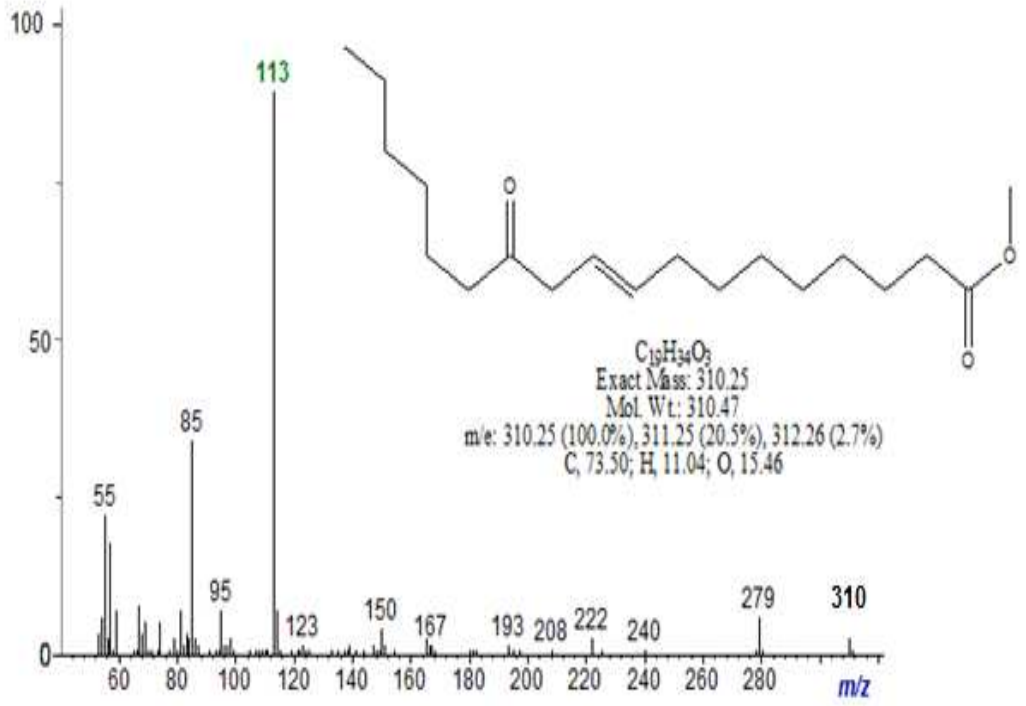

Figure 5: Mass spectrum and structure of methyl 12-oxo-octadec-9-enoate

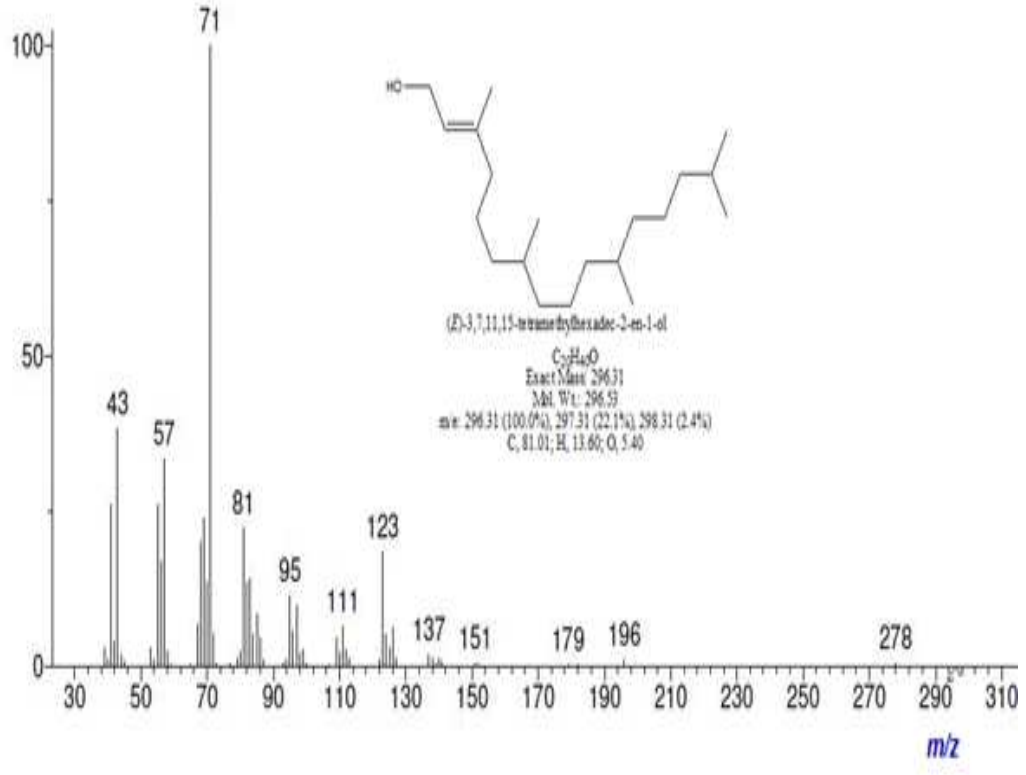

Figure 6: Mass spectrum and structure of Phytol

CONCLUSION

GC-MS analysis was carried out to analyze the active constituents present in aerial parts (dry seeds) of Croton bonplandianum Baill (Euphorbiaceae). GC-MS analysis is the first step towards understanding the nature of active

principles in the medicinal plants. It could be concluded that, C. bonplandianum contains various bioactive compounds. The prevailing compounds were Squalene, (9Z, 12Z)-octadeca-9, 12-dienoic acid, methyl 12-oxo-octadec-9-enoate-and phytol. The research revealed the potential of hexane extract of Croton bonplandianum Baill seeds as a good source of phytochemicals that justify the use of this plant for its various ailments by traditional practitioners. It is recommended as plant of pharmaceutical importance.

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