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ISOLATION, IDENTIFICATION OF NON-POLAR TERPENES AND TOTAL ANTIOXIDANT ACTIVITY FROM CHLOROFORM EXTRACT OF *BOUGAINVILLEA GLABRA* LEAVES

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Keywords:

GC-MS analysis, DPPH method, Spectroscopic techniques, *Bougainvillea glabra* leaves, 1-(1-methyl-decyl)-decahydro-napthalene

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ABSTRACT: This study reports the isolation and antioxidant activity of non-polar terpenes extracted from the leaves of *Bougainvillea glabra* choicy. To characterize the phytochemical constituents of *Bougainvillea glabra* leaves using the GC-MS method. The 95% ethanol extract was filtered, distilled, and concentrated to obtain the solid greenish residue. The residue was further fractioned by n-hexane, chloroform, ethyl acetate, acetone, ethanol, and methanol. The GC-MS analysis of Chloroform extract of *Bougainvillea glabra* leaves revealed the presence of thirteen compounds that could contribute to the medicinal quality of the plant. The first compound identified with less retention time (9.50 min) was 1-(1-methyl-decyl)-decahydro-napthalene. The isolation of organic compounds was done using column and thin layer chromatographic techniques. Compound characterization using various spectroscopic techniques identified the final isolated compound as 1-(1-methyl-decyl)-decahydronapthalene. Antioxidant activity was evaluated by the DPPH method. The method of isolation is simple, cost-effective, and efficient.

INTRODUCTION:

Screening of Phytochemicals: More screening and isolation efforts are necessary to evaluate the pharmacological profile of multi extract preparations. Screening techniques enable us to examine thousands of plant extract and raw materials *via* several test models in a relatively short period of time, followed by the isolation and structure elucidation of all pharmacologically active components, even if they are present in a plant in minor quantities [1].

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These analytical procedures can be supplemented with molecular-biological test models, which have recently been introduced into plant screening techniques. These allow us to determine a hitherto unknown mechanism of action of a given plant extract of raw material at a molecular level, as well as facilitate the discovery of new indications which offer a causality-based therapy. The synergetic effects of components in extract preparations can also be elucidated using molecular-biological methods [2].

Though the use of these techniques for the chemical and pharmacological study of phyto-preparations is the reproducible and internationally recognized clinical results of researchers can for the first time [3]. More than 400 positive, placebo-controlled, randomized, double-blind studies have been completed for around 20 standardized

phytopreparations. These reports are further evidence that the development of phytomedicinal preparations should be of high priority for the near future. Recent Trends in the Evaluation of Antidiabetic screening of traditional medicinal plants has been the source of innumerable therapeutic agent [4]. *Bougainvillea glabra* is a good fit for plants around the house for security to keep people from climb thorns. It makes excellent colors to spread on walls and fences. Bougainvillea flowers are ranging from yellow, pink, red, orange, purple and especially white. The varieties of Bougainvillea's include *Bougainvillea spectabilis* and Bougain-villea harrisi, and these plants are mainly grown for decorative purposes in tropical regions [2]. *Bougainvillea glabra* choicy have been used by a variety of disorders like diarrohea, reduce stomach acidity, cough, and sore throat[5].

MATERIALS AND METHODS:

Collection, Identification and Preparation of Plant Materials: The leaves of the plant *Bougainvillea glabra choicy* collected from Thanjavur district and authenticated by Dr. John Britto, Rapinet Herbarium, St. Joseph's College, Tiruchirappalli. The leaves were cleaned, dried in shadow, and crushed into powder.

Extraction: The powdered sample was extracted with 95% ethanol by using cold method extraction at room temperature for one week. The 95% ethanol extract was filtered, distilled, and concentrated to obtain the solid greenish residue.

The 95% ethanol extract was further partitioned successively with petroleum ether, n-hexane, chloroform, ethyl acetate, ethanol, n-butanol, and methanol. The solvents were recovered under reduced pressure. The chloroform fraction was subjected into GC-MS analysis, isolation, and antioxidant activity.

GC-MS Analysis of Chloroform Fraction: GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising an AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: Column Elite-1 fused silica capillary column (30 × 0.25 mm ID x µM df, composed of 100% dimethylpolysiloxane), operating in electron impact mode at 70eV; helium (99.999%) was used as carrier gas at a constant flow of 1 mL/min, and an injection volume of 0.5 µL was employed (split ratio of 10:1) injector temperature 250 °C; ion-source temperature 280 °C. The oven temperature was programmed from 110 °C (isothermal for 2 min), with an increase of 10 °C / min, to 200 °C, then 5 °C / min to 280 °C, ending with a 9 min isothermal at 280 °C. Mass spectra were taken at 70 eV; a scan-interval of 0.5 seconds, and fragments from 40 to 450 Da. Total GC running time is 36 min.

Isolation: A portion of chloroform fraction was subjected to GC - MS analysis as in 95 % ethanol extract. The remaining portion was subjected separately into silica gel (100 - 200 mesh) column chromatography eluted gradient with chloroform, 9.5: 0.5 and 9:1 chloroform: ethyl acetate mixture, respectively. The 100 % chloroform fraction was crystallized with methanol to give a white colour compound (11 mg).

Antioxidant Activity: The antioxidant activity was evaluated by 95% ethanol, chloroform fraction of plant leaves using the DPPH method [6]. About 0.1 ml of the 95% ethanol, chloroform extracts were taken in test tubes, 6 mL of DPPH solution

was added, and a blank solution was prepared to contain the same amount of respective solvent and DPPH. All the test tubes were kept in the dark for one hour. The colour change from deep violet to light yellow was read at 517 nm.

The difference in the optical density of DPPH solution and DPPH solution + sample was calculated. The decrease in OD with sample addition was used for calculation of the antioxidant activity. The activity was compared with BHT (butylated hydroxyl toluene) standard. Free radical scavenging activity was expressed as the inhibition percentage calculated using the formula.

DPPH scavenging activity (%) = $\{[A_b - A_a] / A_b\} \times 100$ …… (1)

Where, A_b is the absorption of the blank sample and A_a is the absorption of the extract.

RESULTS AND DISCUSSION: GC-MS analysis of chloroform fraction.

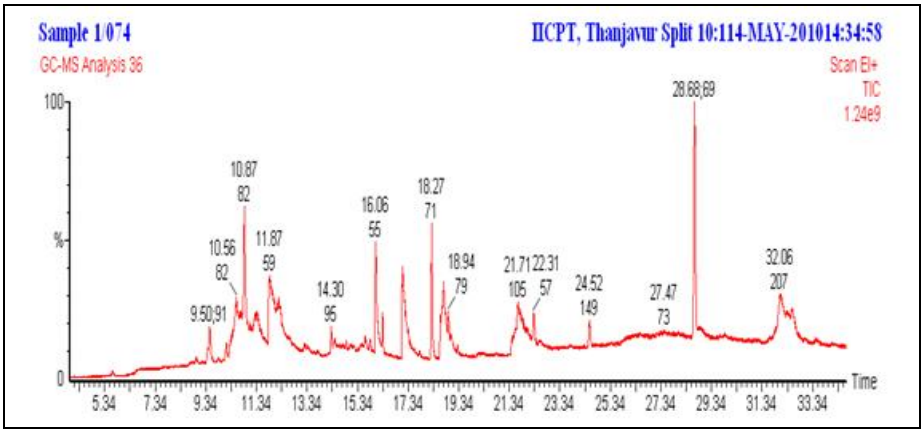

FIG. 1: GC-MS ANALYSIS OF CHLOROFORM EXTRACT OF *BOUGAINVILLEA GLABRA* LEAVES

TABLE 1: GC-MS ANALYSIS CHLOROFORM FRACTION

S. no.	RT	Name of the Compound	Molecular Formula	MW	Peak Area %
1	9.50	1-(1-methyl-decyl)-decahydro-napthalene	$C_{21}H_{40}$	292	12.50
2	10.14	á-Cubebene	$C_{15}H_24$	204	5.77
3	10.56	Acetyl turicine	$C_9H_{15}NO_4$	201	55.61
4	10.87	Cyclohexanemethanol, 4-ethenyl-à,à,4-trimethyl-3-(1-methylethenyl)-, [1R-(1à,3à,4á)]-[Elemol]	C15H26O	222	74.78
5	11.87	2-Hydroxymethyl-5-(1-hydroxy-1-isopropyl)-2-cyclohexen-1-one	$C_{10}H_{16}O_3$	184	70.58
6	16.06	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	41.64
7	16.33	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	6.36
8	17.12	2H,8H-Benzo[1,2-b:5,4-b']dipyran-2-one, 8,8-dimethyl-	$C_{14}H_{12}O_3$	228	75.84
9	18.27	Phytol	$C_{20}H_{40}O$	296	34.97
10	18.74	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	57.03
11	21.71	E-2-Hexenyl benzoate	$C_{13}H_{16}O_2$	204	94.37
12	28.68	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-[All-trans-Squalene]	$C_{30}H_{50}$	410	92.54
13	32.06	1-Monolinoleoylglycerol trimethylsilyl ether	$C_{27}H_{54}O_4Si_2$	498	60.06

GC-MS is one of the best techniques to identify the constituents of volatile matter, long-chain, branched-chain hydrocarbons, alcohols, fatty acids, and esters, *etc*. The GC-MS analysis of Chloroform extract of *Bougainvillea glabra* leaves revealed the presence of thirteen compounds that could contribute to the medicinal quality of the plant. The identification of phytoconstituents was shown in Table and Figure. The first compound identified with less retention time (9.50 min) was 1-(1-methyl-decyl)-decahydro-napthalene, whereas 1-Mono-linoleoylglycerol trimethylsilyl ether was the last compound that took the longest retention time (32.06 min) to identify. The identified compounds possess many biological properties. For instance, n-hexadecanoic acid and hexadecanoic acid, ethyl

ester possesses anti-oxidant, hypocholesterolemic, nematicide, pesticide, lubricant activities and hemolytic5-alpha reductase inhibitors. Phytolditerpene is an antimicrobial, anticancer, anti-inflammatory and diuretic agent. 9, 12-Octadecadienoic acid was found to have potential antioxidant and anticancer activities [6]. All -Trans -squalene shows antitumour activity. It protects against several carcinogens [7]. Mostly mono and sesquiterpenes are active against bacteria, fungi, viruses and protozoa. á-Cubebene and Elemol is a sesquiterpene that exhibits the antimicrobial activity [8]. reported that Euphorbia longan leaves mainly contained n-hexadecanoic acid and 9,12-octadecadienoic acid. These reports are in accordance with the result of this study.

Isolation: Structure identification of isolated compound

Physical State: white amorphous powder

R_f Value: 0.49 (3:2 petroleum ether: hexane solvent system).

Melting Point: 37 ºC.

Screening Test: Compound was subjected to micro thin layer chromatography. The developed TLC plates were placed in iodine chamber; brown color appeared in iodine vapor, indicating the presence of terpenoids.

Vanillin sulphuric acid was sprayed by TLC plates and put in an Oven at 110 ºC. After minutes, a dark brown color appeared. Presence of terpenoids.

Elemental Analysis: Obtained values for $C_{21}H_{40}$; C= 86.22%, H= 13.78 %, Molecular weight 292. ^{1}HNMR- The signals appeared at δppm- 0.93 to 1.5 indicating the presence of CH_3, CH, and CH_2 Protons.

EI-MS: The EI-MS spectroscopy of the present compound exhibited a molecular ion peak at m/z value 292 and fragmented peaks at m/z, m/z, and m/z. In accordance with the above data, the compound may be deduced as 1-(1-methyl-decyl)-decahydro-napthalene. Based on the above results and by comparing them with other similar compounds, the identified structure and spectral data of the isolated terpene hydrocarbon are given in **Fig. 2, 3,** and **4.**

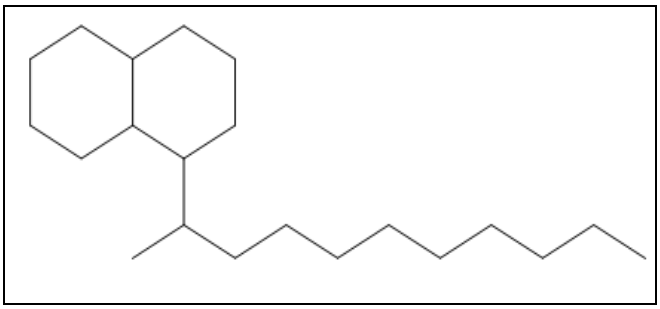

FIG. 2: (1-METHYL-DECYL)-DECAHYDRO-NAPTHALENE

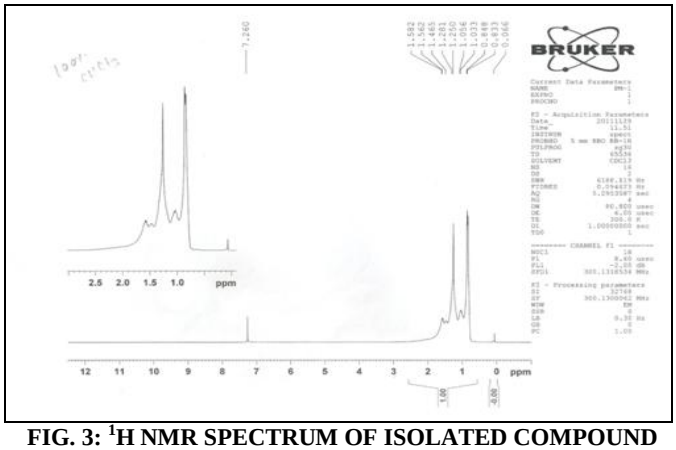

FIG. 3: ^{1}H NMR SPECTRUM OF ISOLATED COMPOUND

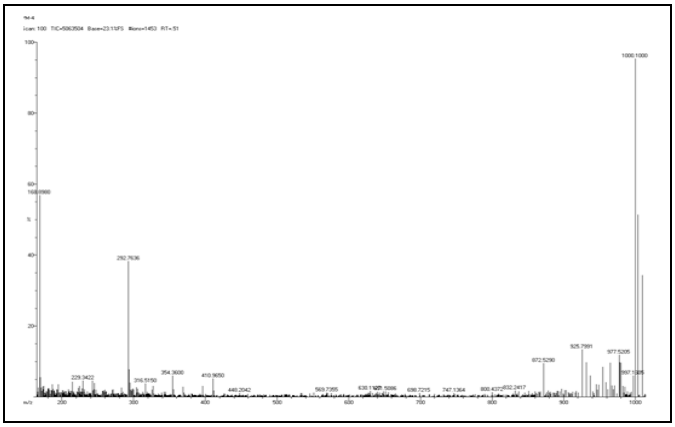

FIG. 4: MASS SPECTRUM OF ISOLATED COMPOUND

Antioxidant Activity: The results of the antioxidant activity are given in **Table 2**. The in vitro antioxidant activity of the two plant extracts reveals significant antioxidant potential compared with standard BHT and ascorbic acid. The antioxidant activity of two plant extracts as measured by the ability to scavenge DPPH free radicals was compared with the standards / ascorbic acid and butylated hydroxyl toluene (BHT). It was observed that ethanol extract of Bougainvillea glabra leaves has higher activity than that of chloroform extracts of plant leaves at a

concentration of 0.1 mg /mL. The antioxidant potential of *Bougainvillea glabra* leaves shows 73.45% and 90.66% of chloroform and 95% ethanol extracts, respectively. The results obtained from *Bougainvillea glabra* leaves show high antioxidant activity as compared to ascorbic acid (100 %) and BHT (94.82 %). The study showed that the plant was potently active. This suggests that the plant extract contains compounds that are capable of donating hydrogen to a free radical in order to remove an odd electron, and it could serve as a free radical inhibitor or scavenger, acting possibly as primary antioxidants. Considering the DPPH radical scavenging activity as indices of the three ornamental plant extracts' antioxidant activity, these findings revealed the potential of *Bougainvillea glabra* as a source for natural antioxidants.

Although no correlation study was carried out, but literature reports showed that the reduction mechanism of DPPH correlated with the presence of hydroxyl groups on the antioxidant molecule [10], which can be inferred that the very good antioxidant activity of this polar extract is probably due to the presence of substances with an available hydroxyl group. This structural requirement could be linked to the presence of flavonols or condensed tannins, which are known to occur in plant species belonging to the Nyctaginaceae family [11] to which *Bougainvillea glabra* belongs.

It scavenges free radicals and is used for treating diseases related to free radical reactions. *Bougainvillea glabra* plant leaves were rich in terpenoids, glycosides, and steroids. These phytochemicals confer antioxidant activity on total plant extracts.

The inhibitory activity may be due to the presence of phytochemicals in the extracts. Noticed phytochemicals also inhibited the free radicals in antioxidant method. The terpenoids [12], glycosides [13], steroids [14] have been found to possess antioxidant properties in various plant studies. Presence of phenols in extracts may explain its potent bioactivities, as tannins are known to possess potent antioxidants. The WHO estimated that 80% of the populations of developing countries still relied on traditional medicine, mostly plant drugs for their primary health care needs. Hence, there is an urgent need to study the screening of antioxidant properties of herbs which will be helpful in the treatment of several diseases [15].

Antioxidants are an inhibitor of the process of oxidation, even at relatively small concentration, and thus have a diverse physiological role in the body. Antioxidants may be synthetic or natural. Synthetic antioxidants such as BHT and BHA have recently been reported to be dangerous for human health. Thus the search for an effective, non-toxic natural compound with antioxidant activity has been intensified in recent years [16]. On the basis of our results, bougainvillea glabra appears to have the potential for the treatment of oxidative stress-related diseases. It should, however, be explored as a functional medicinal plant for isolating the active ingredients along with other models such as lipid peroxidation and *in-vivo* assays will be interesting in discovering few biological antioxidants.

TABLE 2: ANTIOXIDANT POTENTIAL FROM DIFFERENT SOLVENT EXTRACTS OF PLANT LEAVES

Plant Leaves	95 % Ethanol (0.1 mg /mL)	Chloroform (0.1 mg /mL)	Ascorbic Acid (0.1 mg /mL)	BHT (0.1 mg /mL)
Bougainvillea glabra	90.66	73.45	100	94.82

CONCLUSION: Compound characterization using various spectroscopic techniques identified the final isolated compound as - (1-methyl-decyl)-decahydronapthalene, and it showed excellent antioxidant activity. The method of isolation is simple, cost-effective, and efficient.

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CONFLICTS OF INTEREST: Authors are no conflicts of interest.

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