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ISOLATION OF AROMATIC AND MEDICINAL COMPOUNDS FROM *ISODON NILGHERRICUS* (BENTH.) H. HARA - AN ECONOMIC ASPECT

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Keywords:

Aromatic molecules, medicinal compounds, *Isodon nilgherricus*

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ABSTRACT: The secondary metabolites being defensive function in plants, the men also thought of using same to defend the chronic diseases by crud drugs or with some organic aromatic molecules. The phenyl groups and other functional groups are bonded together, forming aromatic organic molecules. These molecules are used in perfumes and medicines. The qualitative and quantitative aromatic and medicinal compounds isolation was carried out from *Isodon nilgherricus* (Benth.) H. Hara. The qualitative analysis was carried out by various solvent extraction tests, and quantitative analysis was carried out by GC-MS technique. The qualitative analysis of essential oil showed the presence of Butenal <2-methyl-> which has 100% area covered with a retention time of 1.905. Quantitative analysis led to the identification of 32 different compounds from total 38 different peaks. The predominant components of the oil detected were Butyl pentanoate, pentanoic acid (13.60%), Norbornene-2-methonol<endo-5->, bicycle (2.2.1) hept-5-ene-2 methanol (1R-endo) with 5.25% concentration. It was followed by Ethyl 3-hydroxyhexanoate, hexanoic acid, 3, hydroxy-ethyl easter (4.45%), Isobutyl angelate (3.10%), Butanoic acid (2.45%). Butyl pentanoate is the major compound that gives the aroma in this plant.

INTRODUCTION: Phytochemicals have gained more attention and popularity than the synthesized chemicals to cure or prevent chronic diseases. Phytochemicals synthesized by the plants are either primary or secondary metabolites. The secondary metabolites being defensive function in plants, the men also thought of using same to defend the chronic diseases by crud drugs or with some organic aromatic molecules. The phenyl groups and other functional groups are bonded together, forming aromatic organic molecules. These molecules are used in perfumes and medicines. Aromatic medicine is the volatile plant extracts given internally.

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So the interest has developed in the use of those phytochemicals in the form of crude drug and aromatic molecules from certain medicinal plants. Even the plant parts are mostly using as a raw material for the synthesis of new targeted molecules against the diseases. Globally, the use of crude herbal drugs, plant parts or their extracts is enhanced. Natural drugs are being used as antimicrobial [1] antioxidant [2] and Cytotoxic Activities [3]. Even most of the workers are finding innovative plant-based drugs with targeted phytochemicals. The phytochemicals, including alkaloids, glycosides, phenolic compounds, steroids, and terpenoid derivatives, are valuable. More than 119 phytochemicals were isolated and identified from higher plants being used worldwide as drugs [4].

Lamiaceae is an important medicinal plant family popular as the mint family due to the aromatic fragrance emitted by the herbs and shrubs of its

members. *Plectranthus* is one of genus and is an aromatic plant. Traditionally this plant is used as a stimulant for, treatment of cough, diuretic, and cytotoxic. The most frequently cited use of *Plectranthus* species is for their medicinal properties, which accounts for over 85% of all uses [5].

In the present investigation, isolation of Aromatic and medicinal compounds from *Isodon nilgherricus* (*Plectranthus nilgherricus*) was carried out.

The qualitative and quantitative phytochemical study was carried out from *Isodon nilgherricus* (*Plectranthus nilgherricus*). The qualitative analysis was carried out by various solvent extraction tests, and quantitative analysis was carried out by GC-MS technique.

MATERIALS AND METHODS:

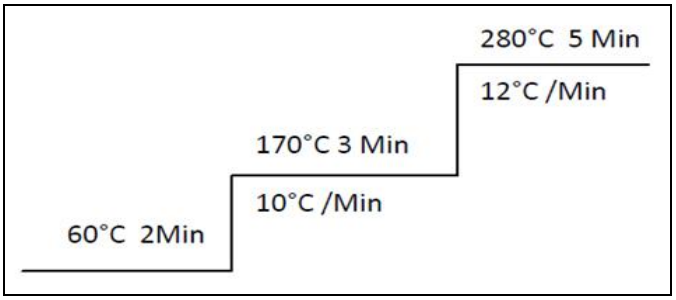
Collection of Plant Material: The plant sampling of *Isodon nilgherricus (Plectranthus nilgherricus)* were collected from hilly regions of Ooty of Tamil Nadu. The live plants were maintained by the pot culture method for further studies. They were identified from Botanical Survey of India, Western Circle, Pune.

The voucher specimens were deposited in the Herbarium, BSI, Pune (BSI/WRC/2016/509), and BSI, Coimbatore (BSI/SRC/5/23/2017/5/1637).

Extraction of the Plant Materials for Essential Oil: The cultured pot plants were harvested at the flowering stage. Plant parts were separated species-wise. Parts were washed thoroughly under running water and blot away. They were air-dried at room temperature, followed by pulverization to powder form using a mortar pestle and grinder. The powder was subjected to extraction of essential oil with the help of the Clevenger apparatus.

Gas Chromatography and Gas Chromato-graphy-Mass Spectrometry Analysis:

Quantitative analysis by Gas Chromatography: GC Model: Chemito GC-8610 Column: Capillary Column - TRB0-5 (25 mtr × 0.32 mm × 0.5 µm); Detector: FID; Injection Volume: 0.2 µl; Temperature: Injector Temperature: 220 °C, Detector Temperature: 270 °C; Carrier Gas: Nitrogen Carrier; Gas Pressure: 1.0 bar; Air Pressure: 1.0 bar, Hydrogen Pressure: 1.0 bar; Run time: 30.00 Min; Sample Injected: 0.2 µl neat.



OVEN PROGRAM

Qualitative Analysis by Gas Chromatography-Mass Spectrometry: The GC-MS analysis was carried out using Hewlett-packed gas chromatography (model 6890 series) equipped with a flame ionization detector and Hewlett Packard 7633 series indicator, MS transfer line temperature of 250 °C. The GC was equipped with a fused silica capillary column HP -5MS (30 x 0.25 mm), film thickness 1.0 µm. The oven temperature was held at 50 °C for 5 min holding time and raised from 50 to 250 °C at a rate of 2 °C/min, employing (99.99%) helium gas as a carrier at a constant flow rate of 22 cm/s. 1.0 micron of extract (1 mg dissolved in 1ml absolute alcohol), at a split ratio of 1:30, was injected. MS analysis was carried out on Agilent. Technology network Mass spectrometer (model 5973 series) coupled to a Hewlett Packard Gas chromatography Model 6890 series) equipped with NIST08 Library software database. Mass spectra were taken at 70 ev/200 °C scanning rate of 1 scan/s.

Identification of Compounds: Interpretation of mass spectrum of the unknown component was conducted by comparing the mass spectra with the spectrum of the known components stored in the data system National Institute Standard and Technique library (NIST-2008, Turbo mass Ver. 5.4.2). The relative percentage of each component was calculated by comparing its average peak area to the total areas.

RESULTS AND DISCUSSION:

Analysis of Essential Oil by Gas Chromato-graphy (GC) and Gas Chromatography-Mass Spectrometry (GC-MS): Aromatic plants have pharmaceutical properties. These plants produce essential oils, which are used as flavoring additives to cosmetics, antibacterial agents and also used in various ailment preparations. The same genera in different geographical locations and in variable

seasons have different phytochemicals and have different biological activities. The essential oil obtained from the *Plectranthus* by hydro-distillation was a yellowish to white liquid with a specific herbal scent and sweet smell. GC-MS analysis of essential oil obtained from three *Plectranthus* species by hydrodistillation utilizing Clevenger type apparatus led to the identification of different compounds. These essential oils have also been reportedly used in traditional and modern medicines [5, 6]. Furthermore, chemical investigations on *Plectranthus* species revealed that the genus is a rich source of diterpenoids [7, 6, 8]. The essential oil of *Plectranthus* is a good source of natural bioactive compounds containing numerous analgesic and antioxidant agents [9]. The volatile plant oils are mostly composed of chiefly terpenoids: mono-, sesque- and di-terpenes, with frequently occurring aromatic compounds arising from the phenyl-propanoid pathway. The essential oils are common to many species as α-pinene, limonene, camphor, *etc*. [10]

Quantitative Analysis of Essential Oil by GC: Quantitative analysis of the hydrodistilled essential oil of *Isodon nilgherricus (Plectranthus nilgherricus* led to the identification of 32 different compounds from total 38 different peaks **Fig. 1**. Identified compounds are with their Retention time, chemical formula, molecular weight, and the peak area **Table 1**. The predominant components of the oil detected were Butyl pentanoate, pentanoic acid or butyl ester (13.60%), Norbornene-2-methonol<endo-5->, bicycle (2.2.1) hept-5-ene-2 methanol (1R-endo) with 5.25% concentration. It was followed by Ethyl 3-hydroxyhexanoate, hexanoic acid, 3-hydroxy-ethyl easter (4.45%), Isobutyl angelate (3.10%), Butanoic acid (2.45%). Butyl pentanoate is the major compound that gives the aroma in this plant.

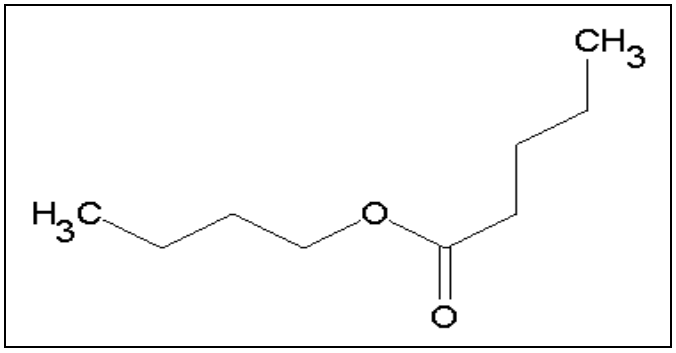


FIG. 1: STRUCTURE OF BUTYL PENTANOATE

Butyl pentanoate (13.60%),

Molecular Formula: $C_9H_{18}O_2$

Molecular Weight: 158.241 g/mol

Butyl pentanoate is a flavouring agent

In addition to these compounds, many more minor compounds as ocimene (Perfuming agent), Terpineol, isocitral, phenal 1,2 allyl, and pinene<β, Menta were also detected by GCMS. These minor but important compounds may play role in various pathways as well as in various ailments. Presence of Ocimene, isocitral, pinene<β are some of the monoterpenoids in the essential oil could be related to anticonvulsant and sedative effects [11]. Terpineol is one of the minor constituents of *Plectranthus nilgherricus* reported as antioxidant and has cardiovascular effects. There is some evidence indicate that α-terpineol functions as a potent antioxidant and radical scavenger [12, 13]. Studies [14] showed the importance and role of α-Terpineol, which is one of the bioactive phytochemical constituents of medicinal plants. They have concluded that α-terpineol has a better effect on morphine-induced dependence and tolerance in mice. It was clarified [15] that the mechanisms by which *α*-terpineol elicit its cardiovascular effects in rats. The major Finding of the study were that the monoterpene *α* -terpineol induces marked hypotension and tachycardia in conscious rats that could be attributed, at least in part, to decreased peripheral vascular resistance mediated by increased nitric oxide release and consequent activation of the nitric oxide GMPc pathway. Beta-pinene (β-pinene) is a monoterpene, a natural compound found in *Plectranthus nilgherricus* Benth. It is one of the two isomers of pinene.

The antimicrobial activities of pinene were evaluated against bacterial and fungal cells and showed that only the positive enantiomers of the α- and β-isomers of pinene were active [16]. Ethanolic extract of *Plectranthus amboinicus* (Lour.) Spreng leaves can be formulated and used as mouthwash which has antibacterial activity against *Staphylococcus aureus* and *Streptococcus mutans* bacteria [17]. Similarly essential oil of *Plectranthus amboinicus* Lour (malvariço), has been used by the people for the treatment of diseases of the oral cavity, alone or as a mouthwash against a strain of

Streptococcus mutans [18]. Very recently, the bacteriostatic activity [19] of *Plectranthus amboinicus* was determined. The bacteriostatic performance of *Plectranthus amboinicus* was effective, which had shown its effect on gram-positive and gram-negative bacteria and yeasts.

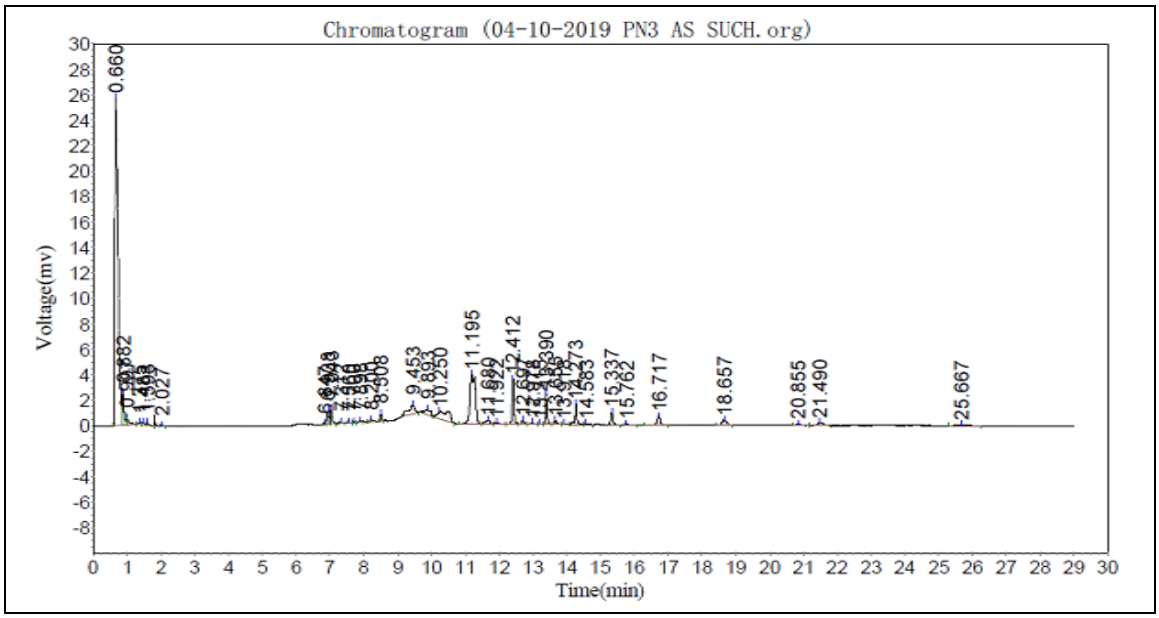


FIG. 2: QUANTIFICATION CHROMATOGRAM OF *ISODON NILGHERRICUS* (BENTH.) H. HARA (BASIONYM: *PLECTRANTHUS NILGHERRICUS* BENTH.) BY GC

TABLE 1: QUANTIFICATION OF CHEMICAL COMPOSITION FROM *ISODON NILGHERRICUS* (BENTH.) H. HARA (BASIONYM: *PLECTRANTHUS NILGHERRICUS* BENTH.)

Retention time	Common Name	Chemical name	Molecular formula	Molecular weight	Concentration (%)
6.93	Isopropyl tiglate	2-butenoic acid, 2-methyl-,1 methylethyl ester (E)-	$C_6H_{14}O_2$	142	1.37
7.04	Pinene < β >,	bicyclo (3.1.1) heptanes, 6,6-dimethyl 1-2-methylene	$C_{10}H_{16}O$	136	1.31
9.45	Isobutyl angelate	2-butenoic acid, 2-methyl-, 2-methylpropyl ester (Z)-	$C_9H_{16}O_2$	156	3.10
9.89	Thiophene <2-butyl->	thiophene, 2-butyl-	$C_8H_{12}S$	140	1.54
10.30	Norbornene-2-methanol <endo-5->	Bicycle(2.2.1)hept-5-ene-2-methanol, (1R-endo)-	$C_8H_{12}O$	124	5.25
11.19	Butyl pentanoate	pentanoic acid, butyl easter	$C_9H_{18}O_2$	158	13.60
12.40	Ethyl 3-hydroxy hexanoate	Hexanoic acid, 3-hydroxy-, ethyl easter	$C_8H_{16}O_3$	160	4.45
12.70	Ocimene <allo->	2,4,6-octatriene, 2,6-dimethyl-, (E,Z)-	$C_{10}H_{16}$	136	0.53
12.97	Terpineol <trans-dihydro-β->	Cyclohexanol, 1-methyl-4-(1-methylethyl)-, trans-	$C_{10}H_{20}O$	156	0.24
13.21	Isocitral <exo->	6-octenal, 7-methyl-3-methylene	$C_{10}H_{16}O$	152	0.11
13.51	Butanoate <3-methyl-2-butenyl 3-methyl->	Butanoic acid, 3-methyl-, 3-methyl-2-butenyl ester	$C_{10}H_{18}O_2$	170	2.46
14.27	Benzene <1,3-dimethoxy->	Benzyne, 1,3-dimethoxy-	$C_8H_{10}O_2$	138	2.24

Qualitative Analysis by GCMS: The qualitative analysis of essential oil showed the presence of Butenal <2-methyl-> which has 100% area covered with retention time of 1.905 **Fig. 4**. The highest peak RT-39.5 in mass to charge (m/z) spectra showed presence of 3-butyn-1-ol 2-methyl with the maximum abundance 6683057.5 **Fig. 5**. It plays an important role as a flavouring agent. An attempt was made to investigate a physicochemical and antifungal activity of leaf extracts of *Plectranthus amboenicus* and *Achyranthes aspera*. Alkaloids and saponins were detected in this plant [20].

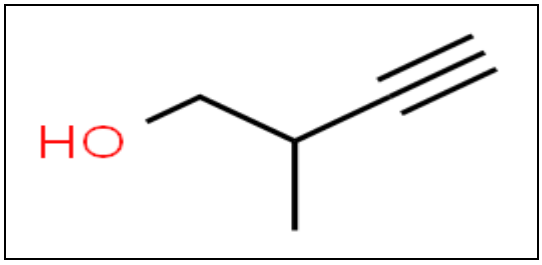
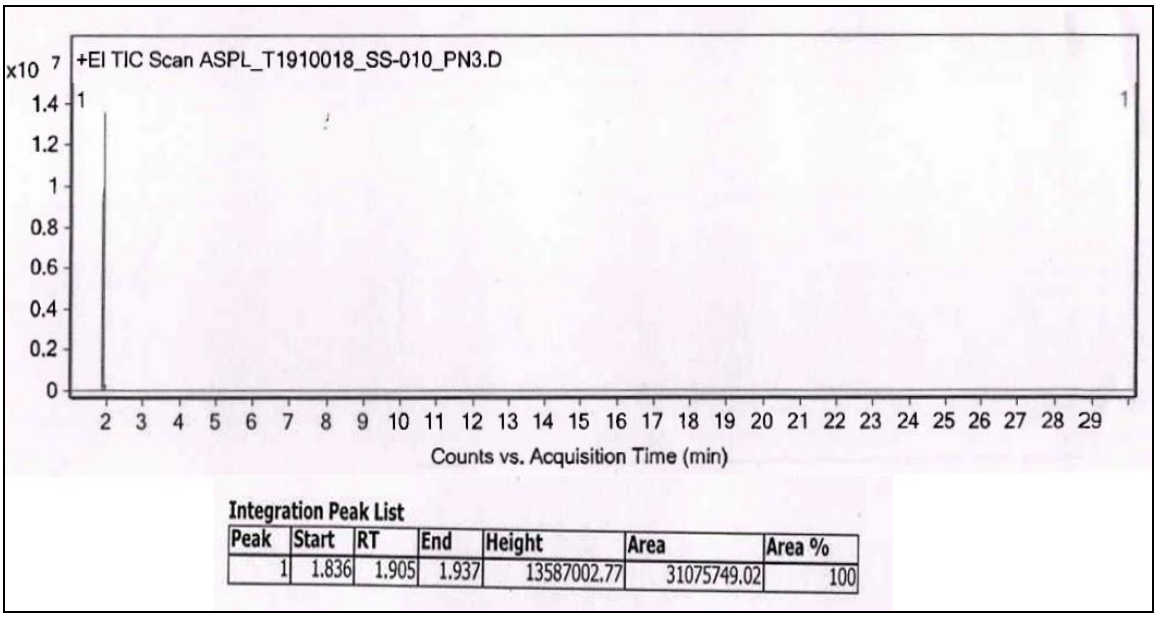


FIG. 3: 2-Methyl-3-butyn-1-ol
Molecular Formula: C_5H_8O
Function: Flavouring agent in plants



Integration Peak List

Peak	Start	RT	End	Height	Area	Area %
1	1.836	1.905	1.937	13587002.77	31075749.02	100

FIG. 4: QUALITATIVE ANALYSIS OF CHEMICAL COMPOSITION FROM *ISODON NILGHERRICUS* (BENTH.) H. HARA (BASIONYM: *PLECTRANTHUS NILGHERRICUS* BENTH.) COUNTS *vs.* MASS TO CHARGE (m/z) BY GC-MS

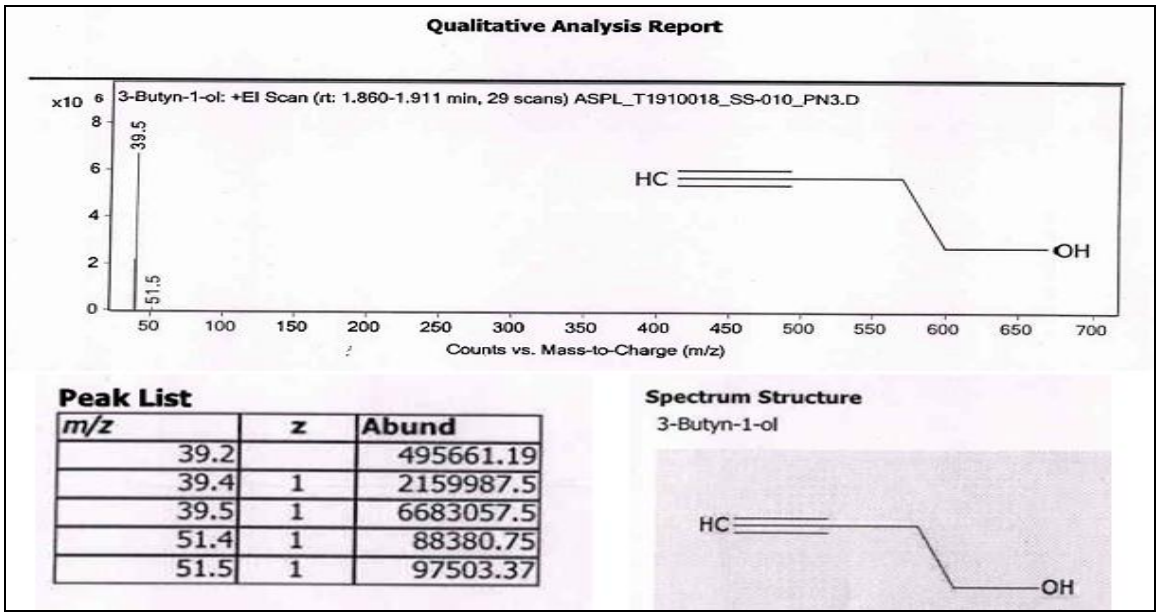


FIG. 5: QUALITATIVE ANALYSIS OF CHEMICAL COMPOSITION FROM *ISODON NILGHERRICUS* (BENTH.) H. HARA (BASIONYM: *PLECTRANTHUS NILGHERRICUS* BENTH.) COUNTS *vs.* MASS TO CHARGE (m/z) BY GC-MS

CONCLUSION: The qualitative analysis was carried out by various solvent extraction tests, and quantitative analysis was carried out by the GC-MS technique. The qualitative analysis of essential oil showed the presence of Butenal <2-methyl-> which has 100% area covered with retention time of

1.905. The quantitative analysis led to the identification of 32 different compounds from total 38 different peaks. The predominant components of the oil detected were Butyl pentanoate, pentanoic acid (13.60%), Norbornene-2-methonol<endo-5->, bicycle (2.2.1) hept-5-ene-2 methanol (1R-endo) with 5.25% concentration.

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CONFLICTS OF INTEREST: We have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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