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Comparison of Chemical constituents of tissue cultured and field grown plants of *Duchesnea indica*, (Andr.) Focke

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ABSTRACT
To investigate the chemical constituents of the herbage extract of the *in vitro* derived plants of Indian Strawberry, *Duchesnea indica*, (Andr.) Focke, the essential oil isolated was subjected to GC-MS analysis. The fresh tissue cultured plants of *Duchesnea indica* was shade dried at room temperature for 30 days and the dried, flaked and powdered plant material was hydrodistilled in a Clevenger apparatus for 4 hours at 100 °C. The phytochemical screening studies identified 25 chemical constituents in the herbage extract; most of them are having proven beneficial activities. The major components analysed was carvacryl acetate, valencene, nona hexacontanoic acid, aistalone, dehydro aromadendrene, eicosane, 2-hexa decan – 1 ol, aromadendrene etc. The chemical constituents of *in vitro* derived plants were different from the components of field grown plants.

Keywords: *Duchesnea indica*, GC-MS Analysis, Phytochemical Analysis.

1. Introduction
Duchesnea indica, commonly called as mock strawberry (Indian Strawberry) is a trailing herb of Rosaceae. It is a common herb widely distributed in south East Asia [1]. The whole plant is used as an anti-cancer herb in Chinese medicine [2]. The plant is anticoagulant, antiseptic, depurative and febrifuge. It can be used in decoction or the fresh leaves can be crushed and applied externally as a poultice. It is used in the treatment of boils and abscesses, eczema, ringworm, stomatitis, laryngitis, acute tonsillitis, snake and insect bites and traumatic injuries. A decoction of the leaves is used in the treatment of swellings. An infusion of the flowers is used to activate the blood circulation. The fruit is used to cure skin diseases. A decoction of the plant is used as a poultice for abscesses, boils, burns etc. [3].
Essential oils, as it indicates, are 'oils of essence' belong to the most vital constituents of spice crops and other aromatic as well as medicinal plants. These natural products were extensively used in pharmacology as biologically active compounds.
This study revealed the chemical constituents of the plant, *Duchesnea indica*, collected from Ooty, Tamil Nadu.
The literature survey revealed the difference in the oil components of *Duchesnea* in different parts of the world [4]. Aerial parts of the plant were shade dried flaked and powdered, hydrodistilled in a Clevenger type Apparatus for 4 hrs. in 100 °C. The oil obtained is collected. The quantity of the essential oil was measured and isolated oil was dried over anhydrous sodium sulphate and stored in small amber coloured bottle and kept at 4 °C. The percentage of oil was calculated on a dry weight basis to avoid faulty estimation that may arise due to difference in water content of the tissues analyzed each time. The isolated oil was subjected to GC-MS analysis and compared the components of both *in vitro* and field grown plants.

2. Materials and Methods
2.1 Essential oil analysis
The fresh herbage of *in vitro* and *in vivo* plants of *Duchesnea indica* (Andr.) Focke was collected from the experimental garden of the Calicut University Botanical garden.

a) Essential oil extraction
Shade dried, flaked and powdered plant material was hydrodistilled separately in a Clevenger apparatus for 4 hours at 100 °C.

The quantity of the essential oil was measured and isolated oil was dried over anhydrous sodium anhydrous sodium sulphate and stored in a small amber coloured bottle and kept at 4 °C. The percentage of oil was calculated on a dry weight basis to avoid faulty estimation that may arise due to difference in water content of the tissues analyzed each time.

b) Gas Chromatography- Mass spectrometry

The volatiles of the essential oil were analysed by the hyphenated system, GC-MS. This was performed on HP 6890 GC/HP 5973 MSD at 70 eV and 250 °C. The GC column used was: H5-5 (DB5) fused silica capillary-0.32 mm X 30 m with film thickness 0.25 µ. The carrier gas used was helium with a flow rate of 1.4 ml/min.

The column temperature programme: initial temperature of 60 °C for 1 min. followed by an increase of 3 °C/min. to 250 °C. Run time was 62 min. The interpretation of mass spectrum was conducted using the database of National Institute Standard and techniques and the components were analyzed and ascertained with the help of Wiley Library 275 combined with the analyzer. The components were compared with the components of field grown plants of *Duchesnea indica*.

c) Chemotaxonomic evaluation

The data obtained from the qualitative analysis of both *in vivo* and *in vitro* plants were subjected to numerical analysis to understand the chemical affinity of both by arriving at a numerical constant, the co-efficient of similitude (CS), using the following formula [5].

$$CS = \frac{\text{Number of similar components}}{\text{Total number of components}} \times 100$$

Table 1: Chemical composition of the leaf essential oil of Field grown and *in vitro* plants of *Duchesnea indica*.

No.	Retention time	Chemical compounds	Class of Compounds	Percentage	
				Parent Plant	*In Vitro* plant
1	11.71	Terpeneol-4	Monoterpenoid	0.07	0.07
2	15.22	Methyl cinnamate	"	1.8	1.8
3	15.98	1,3-dimethyl-2-cyano-3-piperidine	" "	0.02	-
4	16.95	α-copaene	Sesquiterpenoid	0.1	0.1
5	17.48	β-elemene	"	2.0	2.0
6	18.03	β-caryophyllene	"	1.1	1.1
7	18.62	β –selinene	"	1.3	1.3
8	18.86	α-humulene	"	0.8	0.5
9	19.66	Dehydro aromadendrene	"	4.6	4.6
10	19.88	Valencene	"	7.6	7.7
11	22.62	Aromadendrene	"	2.8	2.8
12	22.95	γ- selenene	"	0.1	0.1
13	24.61	P-Nonyl phenol	Phenolic compound	0.26	-
14	25.94	Carvacryl acetate	"	30.51	31.20
15	26.75	Aristalone	Sesquiterpenoid	5.34	5.34
16	29.07	Trans β- farnesene	"	1.7	1.7
17	29.63	β- pregnal	"	2.4	2.4
18	30.06	Hexa- decanoic acid	Fatty acid derivative	2.0	2.0
19	31.16	Geranyl linalool isomer	Monoterpenoid	1.2	-
20	32.76	2-hexa-decen-1-ol	Fatty acid derivative	4.1	4.1
21	35.49	Nonahexacontanoic acid	"	7.2	7.2
22	39.08	Pentacosane	Straight chain alkane	1.0	1.0
23	39.61	Eicosane	"	4.1	4.1
24	42.02	Heptacosane	"	0.4	-
25	43.42	Octacosane	"	0.1	0.1

3. Results and Discussion

The color of the essential oil of the parent plant was pale yellow (yield 0.1%) and that of the variant was yellow (yield 0.1%). The oil collected in an amber colored bottle was subjected to GC - MS analysis.

The results of GC-MS analysis of *in vivo* and in *vitro* plants are listed in the table.

The analysis of the essential oil samples revealed a range of variation in their constituents. The major components identified from the essential oils of *in vivo* and *in vitro* plants were similar (carvacryl acetate, valencene, nona hexa contanoic acid, aristalone, dehydro aromadendrene, 2-hexa-decan-1-ol, eicosane), even though, there is a marked difference in the percentage of occurrence. A considerable increase in the quantity of carvacryl acetate (30.5% and 31.2%) and valencene (7.6% and 7.7%) and a decrease in percentage in the quantity of α- humulene (0.8% and 0.5%) was observed in the *in vitro* plant. The mass spectra of the

compounds identified in the GC-MS analysis are shown. The total number of components detected by GC-MS in the tissue culture derived plant was found to be 21 but in the parent plant the number of components was found to be 25. However the number of similar components in both the plants was 21. The coefficient of similitude between the parent plant and the callus regenerated plant was found to be 84.

Volatile oils are chemically complex mixtures often containing 100 or more individual components. Most oils have one to several components including sesquiterpenoids, monoterpenoids and phenols, out of which the major components impart characteristic odour and taste [6]. But the minor products also play their part in the final product [7]. The economic properties may be due to the essential oil components present in them, which can be effectively exploited to produce fragrance [8] and flavoring agents [9]. The high value of coefficient of similitude (84), obtained on comparing the essential oils of parent and *in vitro* plants show the more similar nature of essential oil composition. The slight dissimilarity arises due to lack of minor oil components, which may be probably due to due to variation in the biosynthetic pathways of essential oils that are genetically controlled. Since the major components are the same in the *in vivo* and *in vitro* plants, the changes due to culture stresses did not affect their biosynthetic pathway.

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