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# CHEMICAL COMPOSITION OF ESSENTIAL OIL OF *EQUISETUM DIFFUSUM*: A NOBLE SOURCE OF PHYTOL

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**Keywords:**

*Equisetum diffusum*, Essential oil, Phytochemicals, Phytol

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**ABSTRACT:** Plants as a source of medicine have been inherited and are an important component of the health care system in India. *Equisetum diffusum* D. Don (Equisetaceae) is one of the medicinally important plants, which is a native species to the Himalayan Mountains. It is popularly known as Himalayan horsetail. The present endeavor deals with the exploration of the chemical composition of Himalayan *E. diffusum.* The chemical composition of the essential oil obtained from aerial parts of *E. diffusum* was analyzed, for the first time, by GC–FID and GC–MS. A total of 52 compounds were identified, constituting over 93.54% of oil composition. The oil was strongly characterised by phytol (35.63%), in addition to hexacosane (8.04%), cadin-4-ene-7-ol (*cis*) (5.32%), n-decane (3.31%), heptacosane (2.92%), and phytone (2.95%), bisabolol-epi-α (2.6%), 4-heptanone-2-methyl (2.24%), n-nonane (2.24%) and octacosane (2.05%) as the main components. High phytol content with other major constituents reported here, provide initial evidence of a new and alternative source of substances with medicinal interest. Furthermore, identification and purification of the active compounds responsible for the therapeutic activity may prove the plant of great pharmacological importance.

**INTRODUCTION:** The genus *Equisetum* (horsetail) belongs to the family Equisetaceae and has a sub-cosmopolitan distribution. The fifteen widely known *Equisetum* species form large, long-lived populations worldwide via highly developed rhizomes [1, 2]. According to the recent phylogentic system, this group belongs to phylum Monilo-phytes, where they are considered more towards ferns [3, 4]. Various kinds of health-promoting active compounds are bio-synthesized by Equisetum species.

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Due to the presence of different minerals, collectively with silicic acid, horsetails have been used to treat numerous health issues like wounds, inflammations, fractures, *etc*., especially within the Greek medicinal system [5]. Several species of Equisetum have been used in traditional medicine worldwide. The whole plant of *E. arvense* often used as conventional Polish remedy and within the tea industry [6, 7]. *E. hyemale* is reported to possess antioxidant, anti-inflammatory, and anticancer activities [8, 9].

*E. debile* is used towards jaundice, hepatitis, in addition to urinary tract problems, as well as in kidney stones [5, 10], *E. giganteum* as a diuretic [11], and *E. telmateia* as an antiseptic [12]. Many workers attributed polyphenols in *E. ramosissimum*, *E. arvense* and *E. telmateia* for antioxidant activity [12-

[14], whereas *E. arvense* and *E. palustre* are utilized to treat skin problems because of flavonoid and saponin derivatives [14, 15]. Recently, different extracts from the family are also documented in various hair as well as skin affliction [16, 17]. However, very little information regarding essential oil together with various biological activities of horsetails is available. Radulovic *et al*., [18] reported two dinorditerpenoids (hexahydrofarnesyl acetone and *cis*-geranyl acetone, with 18.34 and 13.74% respectively) as major compounds from *E. arvense* essential oil. One recent study by Zhao *et al*., [19] on the composition of essential oil of *E. hyemale* reported two major components; n-Hexadecanoic acid and (Z,Z,Z)-9,12,15-Octadecatrienoic acid (41.3 and 8.3% respectively). *E. diffusum* D. Don is the sole living representative of Equisetaceae, and native to Himalaya Mountains. It is popularly known as Himalayan horsetail and ethnobotanically reported in the treatment of kidney troubles, the bone fracture [20, 21], bone dislocation [21, 22], diuretic effect [23], fever, urinary troubles, scabies, skin disease [24], gonorrhea, arthritis and as a cooling medicine [25]. With the increasing demand for natural bioactive compounds in pharmaceutical industries, together with known ethnomedicinal uses of *E. diffusum*, present investigation on essential oil composition of this medicinal species becomes relevant.

Due to its folk use and the commerce of derivative products, various species of the genus have been widely characterized [10, 26]. However, to the best of our knowledge, this important ethnomedicinal plant species (*E. diffusum*) has marginally worked out [27]. Thus, the present research was carried out to determine the compositional profile of aerial part using GC-FID and GC-MS techniques. The main volatile components of *E. diffusum* were identified by comparison with the National Institute of Standards and Technology Mass Spectral Library [28], retention indices [29].

## MATERIALS AND METHODS:

**Collection of Plant Material:** The fresh aerial part of *E. diffusum* D. Don was collected from Central Himalaya, India, (asl- 1,725 m) in the month of August. Taxonomic identification of the specimen was made by the plant taxonomists, Late Prof. Y. P. S. Pangtey and Prof. P. C. Pandey, Department of Botany, Kumaun University Nainital. A voucher specimen (KU-BOT-ED01) was also deposited in the Herbarium of the Botany Department, D.S.B. Campus, Kumaun University, Nainital, (Uttarakhand), India for further reference.

**Isolation of the Essential Oil:** The fresh Ariel part of *E. diffusum* was used for the analysis of the essential oil composition. 1 kg of plant material was subjected to hydrodistillation in a Clevenger-type apparatus for 2.5 h for isolation of its essential oil. The obtained essential oil was measured directly in the extraction burette, and content (%) was calculated as volume (mL) of essential oil per 100 g of plant material. The oil was dehydrated over anhydrous $Na_2SO_4$ (SD Fine-Chem limited, Mumbai, India) and kept in a cold and dark place until further analyses.

**Gas Chromatography (GC/FID):** The essential oil was analyzed by using the Shimadzu gas chromatograph (Model Shimadzu GC-2010) fitted with Rtx-5MS fused silica capillary column (30 m × 0.32 mm internal diameter, film thickness 0.25 µm). The oven temperature was programmed from 50 °C to 150 °C at 3 °C/min, then held isothermal at 150 °C for 10 min and finally raised to 250 °C at 10 °C/min using $N_2$ as a carrier. The injection temperature was 250 °C, detector temperature 260 °C, and the injection volume 0.6 µL, (using a 1:4 solution of the oil in n-hexane), and the split ratio 100:1.

**Gas Chromatography-Mass Spectrometry (GC/MS):** The GC-MS analysis of the essential oil was conducted on a Perkin Elmer GC Clarus 680 fitted with ELITE-5MS fused silica capillary column (30 m × 0.25 mm internal diameter, film thickness 0.25 µm) and interfaced with Perkin Elmer Clarus 600 T mass spectrometer. The oven temperature was programmed from 50 °C to 150 °C at 3°C/min, then held isothermal at 150 °C for 10 min and finally raised to 250 °C at 10 °C/min using helium as carrier gas at 1.0 ml/min. The Injector, Ion source and MS transfer line temperatures were 290 °C, 230 °C and 250 °C respectively, the injection volume 1µl (1:4 solution of oil in n-Hexane), and the split ratio 20:1. MS were taken at 70 eV with a mass range of m/z 40-400 amu.

**Identification of Essential Oil Constituents:** Identification was made on the basis of Linear

Retention Index (LRI, determined with reference to a homologous series of *n*-alkanes ($C_8$-$C_{20}$, Fluka) under identical experimental conditions, co-injection with standards (Sigma), MS Library search (NIST), by comparison with MS literature data [29]. Retention time (RT) and retention index (RI) of the marker constituents of known essential oil were also used to confirm the identities of constituents. The relative amount of individual component was calculated based on the GC peak area (FID response) without using the correction factor.

**RESULTS AND DISCUSSION:** The essential oil of hydrodistilled aerial part of *E. diffusum* (yield-0.312 (% v/w) was analyzed using GC/FID and GC/MS. **Fig. 1** demonstrates the constructed chromatogram by GC-MS analysis. A total of 52 constituents, representing 93.54% of the total oil composition, were identified and are summarized in **Table 1**. **Fig. 3** expresses the 2D structures of some predominant compounds detected in the studied essential oil of *E. diffusum*. Phytol (35.63%), hexa-cosane (8.04%), cadin-4-ene-7-ol (cis) (5.32%), n-decane (3.31%), heptacosane (2.92%), phytone (2.95%), 4-heptanone-2methyl (2.24%) and n- nonane (2.24%), were the most abundant components constituting 55.27% of the oil **Fig. 2**.

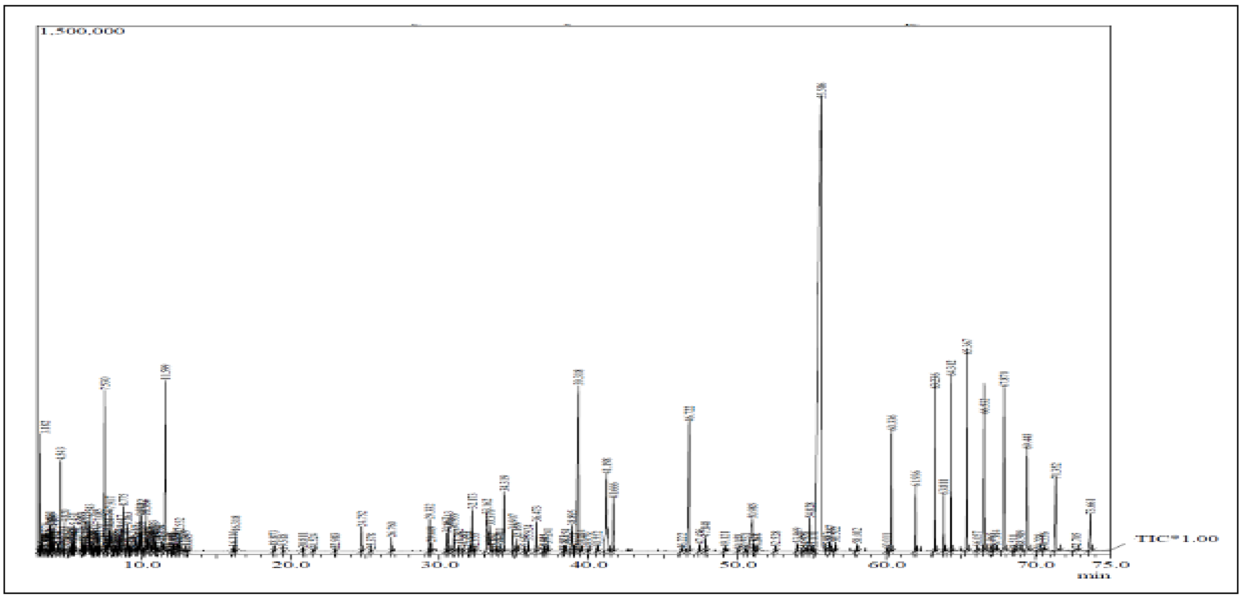


**FIG. 1: GC-MS CHROMATOGRAM OF ESSENTIAL OIL OF *E. DIFFUSUM***

Diterpenoid trans phytol (35.63%) was the major component **Fig. 2**, which is significantly higher than previous reports of other pteridophytes [30-33], as well as other plant groups [18, 34]. In *E. arvense*, Radulovic *et al*., [18] also reported trans-phytol as a major component but in a relatively lesser amount (10.06%).

**TABLE 1: CHEMICAL PROFILE OF ESSENTIAL OIL OF *E. DIFFUSUM***

| S. no. | Components | Chemical formula | RI observed | Percent (%) of contents in oil |
|---|---|---|---|---|
| 1 | 3-methyl heptanes | $C_8H_{18}O$ | 802 | 0.18 |
| 2 | -n Octane | $C_8C_{l8}$ | 823 | 0.83 |
| 3 | Tetra chloro ethane | $C_2C_{l4}$ | 834 | 0.27 |
| 4 | Isopropyl butyrate | $C_9 H_{18}O_2$ | 850 | 0.10 |
| 5 | 3,5,5- trimethyl -Cyclohexane | $C_9H_{18}$ | 857 | 0.27 |
| 6 | 4-heptanone-2methyl | $C_8H_{16}O$ | 876 | 2.24 |
| 7 | Isopentyl acetate | $C_7 H_{14}O_2$ | 890 | 0.42 |
| 8 | (2E)-Hexenol | $C_6H_{12}O$ | 898 | 0.24 |
| 9 | (3Z)-Methylhexene-2-one-5 | $C_7H_{14}O$ | 913 | 0.31 |
| 10 | n- Nonane | $C_9 H_{20}$ | 924 | 2.24 |
| 11 | Cyclohexane,1-ethyl-2-methyl | $C_9H_{18}$ | 930 | 0.26 |
| 12 | 2-ethoxy ethyl acetate | $C_6H_{12}O_3$ | 934 | 0.50 |
| 13 | 4-methyl Heptanone-3 | $C_8H_{16}O$ | 948 | 0.12 |

| | | | | |
|---|---|---|---|---|
| 14 | Octene-1-ol | $C_8H_{16}O$ | 952 | 0.33 |
| 15 | Octane1-ol(2E) | $C_8H_{18}O$ | 957 | 0.66 |
| 16 | Cumene | $C_9H_{12}$ | 964 | 0.30 |
| 17 | Cyclohexyl propanoate | $C_9H_{16}O_2$ | 980 | 0.33 |
| 18 | 4,4-dimethyl-2-butenolide | $C_6H_8O_2$ | 984 | 0.16 |
| 19 | Isopentyl ester | $C_7H_{14}O_2$ | 986 | 0.40 |
| 20 | (2 Z)-Nonenol | $C_9H_{18}O$ | 989 | 0.50 |
| 21 | Dec-1-en-3-ol | $C_{10}H_{20}O$ | 996 | 0.53 |
| 22 | p-trans- methane | $C_{10}H_{20}$ | 1010 | 0.23 |
| 23 | Myrcene | $C_{10}H_{16}$ | 1020 | 0.18 |
| 24 | n- Decane | $C_{10}H_{22}$ | 1025 | 3.31 |
| 25 | 3,3 Dimethyl octane | $C_{10}H_{22}$ | 1047 | 0.29 |
| 26 | *n*- Nonanal | $C_9H_{18}O$ | 1130 | 0.29 |
| 27 | *Z*- Santalol acetate | $C_{17}H_{26}O_2$ | 1316 | 0.42 |
| 28 | Chenopodiol-6-acetate-α | $C_{17}H_{28}O_3$ | 1319 | 0.66 |
| 29 | 2,3,4- trimethyl benzaldehyde | $C_{10}H_{12}O$ | 1346 | 0.32 |
| 30 | *Z*- Patchenol | $C_{11}H_{18}O$ | 1350 | 0.44 |
| 31 | *δ* – Elemene | $C_{15}H_{24}$ | 1358 | 0.41 |
| 32 | *β* –Damascenone | $C_{13}H_{18}O$ | 1387 | 0.78 |
| 33 | γ-Murrolene | $C_{15}H_{24}$ | 1512 | 0.98 |
| 34 | Germacrene-D | $C_{15}H_{24}$ | 1517 | 0.50 |
| 35 | *β*-Bisabolene | $C_{15}H_{24}$ | 1541 | 1.19 |
| 36 | *β*- Sesquiphellandrene | $C_{15}H_{24}$ | 1556 | 0.43 |
| 37 | Geranylbutanoate | $C_{14}H_{24}O_2$ | 1596 | 0.56 |
| 38 | Muurola-4,10 (14) –dien-1- β ol | $C_{15}H_{24}O$ | 1660 | 0.66 |
| 39 | *Cis*- cadin-4-ene-7-ol | $C_{15}H_{26}O$ | 1672 | 5.32 |
| 40 | Bisabolol-epi-α | $C_{15}H_{26}O$ | 1723 | 2.67 |
| 41 | Thujopsenal-cas | $C_{15}H_{24}$ | 1736 | 1.14 |
| 42 | Phytone | $C_{18}H_{36}O$ | 1882 | 2.95 |
| 43 | Hexadecanoic-acid | $C_{16}H_{32}O_2$ | 2012 | 1.61 |
| 44 | Phytol (transphytol) | $C_{20}H_{40}O$ | 2062 | 35.63 |
| 45 | *n*-Decosane | $C_{22}H_{46}$ | 2234 | 1.72 |
| 46 | *n* –Tetracosane | $C_{24}H_{50}$ | 2400 | 1.97 |
| 47 | Pentacosane | $C_{22}H_{52}$ | 2481 | 1.87 |
| 48 | Hexacosane | $C_{26}H_{54}$ | 2571 | 8.04 |
| 49 | Heptacosane | $C_{27}H_{56}$ | 2582 | 2.92 |
| 50 | Octacosane | $C_{28}H_{58}$ | 3192 | 2.05 |
| 51 | Dotriacontane | $C_{32}H_{66}$ | 3200 | 1.80 |
| 52 | 2-methylocacosane | $C_{29}H_{60}$ | 3408 | 1.0 |
| | Total identified % | | | 93.54 |

RI- experimental retention index, calculated on chromatography column

*Cis*- cadin-4-ene-7-ol (5.32%), which also contribute a major proportion of essential oil of *E. diffusum*, was previously suggested responsible for the antimicrobial activity [35, 36]. This oxygenated bicyclic sesquiterpene has also been reported from some angiosperms, *Neolitsea kedahense*-7.7%[36], *Achillea coarctata* [37], but this is the first report from pteridophyte. Kanchanapoom *et al*., studied the chemical composition of the aerial portion of *E. diffusum* growing in Thailand, by using $^{1}$H-NMR and $^{13}$C-NMR and four compounds, sammangaoside A, kaempferol 3-*O*-sophoroside, L-tryptophan and (3*S*, 5*R*, 6*S*, 7*E*, 9*S*)-megastigman-7-ene-5,6-epoxy-3,9-diol 3-*O*-b -D-glucopyranoside were identified [38].

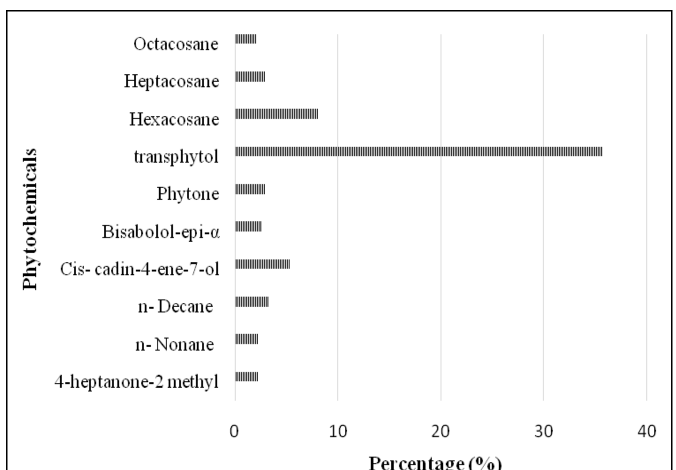


**FIG. 2: COMPARISION BETWEEN MARKER PHYTOCHEMICALS OF *E. DIFFUSUM* ESSENTIAL OIL**

Phytol, which was discussed in the previous section, needs a further mention. Chemically, it is an acyclic diterpene, which acts as the precursor for many biologically important compounds. Recently, it is reported as a precursor of vitamin E [39-41] and K and also used for the manufacture of their synthetic forms [41]. Vitamin K functions in the blood coagulation pathway as well as in bone metabolism [42, 43]. Its deficiency is associated with osteoporotic fracture and low bone mineral density [44, 45].

This effect of vitamin K and the presence of its precursor (phytol) in higher concentration establish a link between its ethnomedicinal use of *E. diffusum* in arthritis and bone fracture; this presumption needs further validation. However, isolation of individual phytochemical constituent and further study of its biological activity will give more fruitful results. Hence, further research is necessary to identify and purify the active compounds responsible for the therapeutic action.

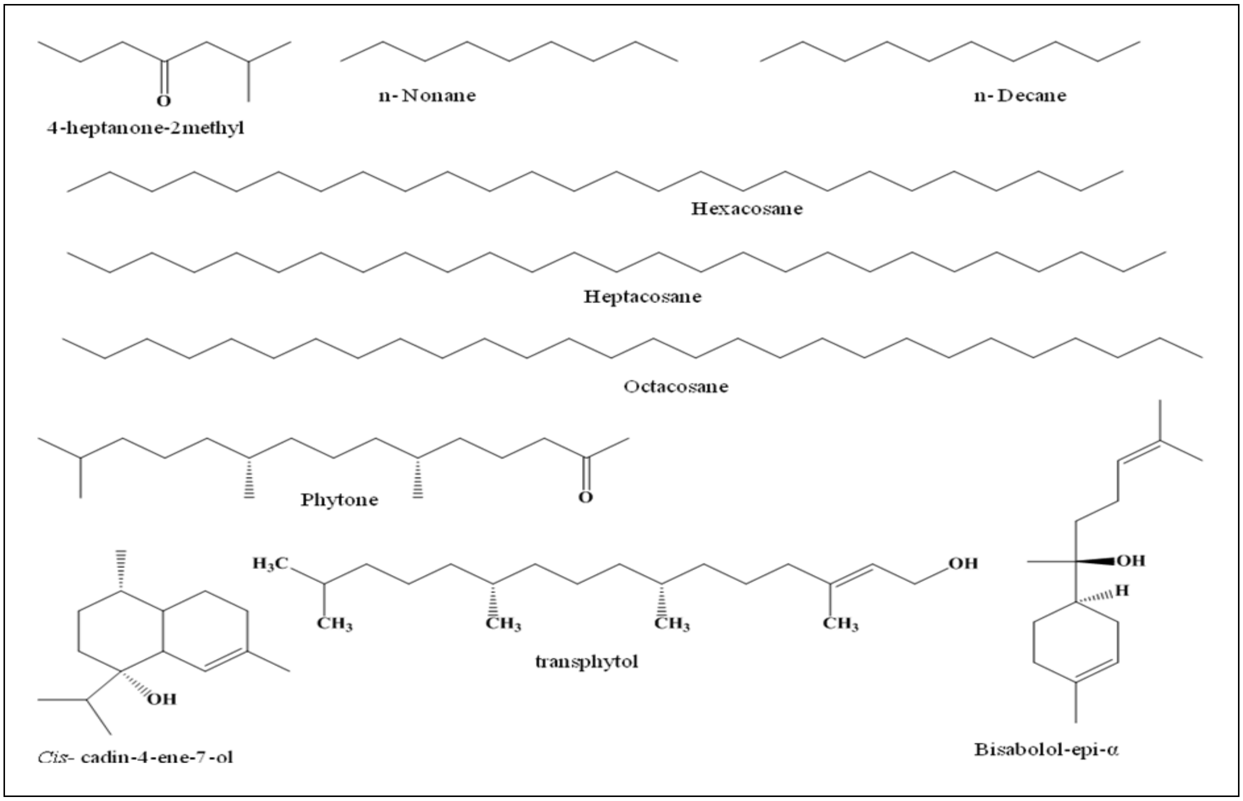


**FIG. 3: STRUCTURE OF THE MAJOR/MARKER CONSTITUENTS OF *E. DIFFUSUM* ESSENTIAL OIL**

**CONCLUSION:** From the medicinal point of view, pteridophytes are primarily ignored as compared to higher plants. Therefore, in this present scenario, an effort is made to scientifically validate *E. diffusum* as a potential alternative natural source of phytol, a precursor of vitamin E and K. Chemical analysis, with highest phytol content, are in correspondence with the ethnomedicinal uses. Many perspectives and expectations emerge from this study in both the scientific and the public health domain.

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