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PHARMACOGNOSTICAL STANDARDIZATION AND HPTLC FINGERPRINT PROFILE OF *ORIGANUM VULGARE* L. (LAMIACEAE)

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ABSTRACT: India is a great source of naturally occurring plant drugs which are having potential pharmacological activities. *Origanum vulgare* L. (Lamiaceae) is one of the most important medicinal plants in India, consisting of aromatic and medicinal properties which is used to cure many ailments. In spite of its plethora of uses as medicine and food additives since ancient times, it has reaped very little scientific interest so far, particularly in India. In such a propitious context, the intention of this study was to enhance the plant as a medicinal herb by determining pharmacognostic standards and evaluating the phytochemical components. Hence, the present study aimed at the pharmacognostical standardization of *Origanum vulgare* L., including the morphology, anatomy, and powder studies pertaining to organoleptic, microscopic, and physical constant evaluations. This study also describes the phyto-chemical screening and HPTLC analysis of major phytochemicals present in the taxa. The pharmacognostic profile obtained from this work can serve as a tool for developing standards for the identification, quality, and purity of *Origanum vulgare* L. The results of phytochemical analysis exhibited a remarkable combination of chemical components, including alkaloids, essential oils, and steroids. The HPTLC fingerprint obtained can be used as a biomarker for identifying the species from the adulterant. In conclusion, the various pharmacognostical constants and HPTLC profiles reported in this study could be useful in authenticating and developing monographs of this plant.

INTRODUCTION: With increasing demand in the field of herbal medicines, it has become essential and relevant to investigate the systematic knowledge about herbal drugs. In fact, there is an urgent need for the application of this knowledge for the authentication of crude drugs.

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Origanum vulgare L. belonging to the family Lamiaceae, is commonly known as "Oregano or Pizza herb" which is commonly served as food material in Lebanese, Italian and Mexican cuisines.

It is an evergreen, aromatic, pleasant-smelling, perennial herb, 30-90 cm high, widely distributed among the Mediterranean and tropical regions. *Origanum vulgare* grows abundantly on stony slopes and rocky mountain areas at a wide range of altitudes of 1500-3600 m [1]. The leaves and tops cut prior to blooming are used to flavor foods. In India, it is generally found in subtemperate and temperate Himalayas and particularly grown in Shimla Hills,

Nilgiris, and in the Kashmir valley [2]. It is widely recognized as a very versatile plant with voluminous therapeutic properties such as diaphoretic, carminative, antispasmodic, antiseptic, tonic, and being applied in traditional medicine systems in many countries [3]. Oregano has been widely used in agriculture and perfumery for its spicy fragrance [4]. In India, *Origanum vulgare* has been used to treat food poisoning, indigestion, bloating, cough, urinary problem, bronchial problems, and headache [5]. Besides these properties, they are consumed worldwide as spices in the food industry as well [6]. It is mainly used for its aromatic properties with a primary role to enhance the taste and aroma of foods [7, 8].

Oregano is the common name for the aroma and taste that comes primarily from more than 60 species of plants used worldwide as spices. In addition, it has also been demonstrated that *Origanums* species have many properties considered important from a culinary and agricultural point of view. Ovicidal, herbicidal and insecticidal activities are some among them [9, 10]. Furthermore, they retards the growth of microorganisms and therefore can also be used as food preservative and flavouring agents as well as disinfectants in perfumes and soaps [11]. Oregano species are known to possess a special composition of essential oil which is of great interest for pharmaceutical and industrial market [12].

The medicinal value of a drug plant is due to the presence of some metabolites, especially the secondary metabolites present in them. There lies the importance of pharmacognostical studies for maintaining the quality, purity, and standard of the plant drug, ensuring authentication. Pharmacognosy has a vital link between Ayurvedic and Allopathic systems of medicines [13, 14]. It includes parameters that help in identifying adulteration in dry powder form too. Standardization is an important step for the quality assurance program for the production of herbal drugs [15]. Since, antique *Origanum* sp. have been used in folk medicine and ethnomedicine [16, 17] to treat numerous infectious diseases, it is important to ensure that their usages are in appropriate amounts because while they confer beneficial properties in therapeutic doses over a short period of time, they, however, can be toxic if taken in excess [18]. Even though this plant has gained scientific importance recently, there is an urgent need for its pharmacognostic standardization. Hence, the current study mainly focused to obtain the standards of this species using pharmacognostical parameters and phytochemical evaluation using HPTLC fingerprinting of *Origanum vulgare* L., employing appropriate tools and techniques in pharmacognosy and phytochemistry.

MATERIALS AND METHODS: The aerial parts of *Origanum vulgare* L. were collected in April-May from the region of Nilgiris, Tamil Nadu, India, and were identified and authenticated at the herbarium of New Udaya Pharmacy & Ayurvedic Laboratories, Cochin, Kerala, India. (Accession numbers N/PG/075). The leaves were air-dried, pulverized, and used for further analysis.

Pharmacognostic Evaluation: Morphological characters of the plant were examined as per standard protocol, which included qualitative and quantitative morphological parameters [19]. The microscopic study included anatomical studies and microscopic foliar features in which assessed the stomatal type, stomatal index, trichomes, vein islet, and vein termination number. Organoleptic evaluation, ash values, and extractive values also were determined as per standard procedures [20, 21]. The mean and standard deviation of quantitative parameters were compared.

Phytochemical Screening: The methodology adopted for phytochemical evaluation included phytochemical screening using methanol extract and HPTLC profiling for major phytochemical groups. The methanol extract was prepared by extracting 30 g of the crude drug in 250 ml solvent in a soxhlet extractor. The extract was then filtered and concentrated in a rotary vacuum evaporator to screen the phytoconstituents using standard procedures [22]. The extract was tested for the presence of different chemical constituents *viz.*, alkaloids, phenolic compounds, steroids, flavonoids, cardiac glycosides, tannins, triterpenoids and saponins. HPTLC analysis was carried out following the standard methods [23]. HPTLC technique was used for the screening of phytochemical groups. In the present study, a CAMAG HPTLC system equipped with Linomat V applicator, TLC scanner 3, Reprostar 3 for photo documentation, controlled

by winCATS software, were used. All the solvents used for HPTLC analysis were obtained from MERCK. The samples (7 µL) were spotted in the form of bands of 8 mm width with a Camagmicrolitre syringe on a pre-coated silica gel glass plate of 60F-254 (E. Merck, Darmstadt, Germany). The loaded plate was developed in the respective mobile phase up to 90 mm using different mobile phases to detect the various classes of phytochemicals. After developing the plate, it was dried and scanned at UV 366 nm using winCATS software. Chromato-grams were viewed under daylight and UV 366 nm after spraying reagents.

RESULTS AND DISCUSSION: *Origanum vulgare* L. have been used for thousands of years as spices and in ethnomedicine in India. It is commonly used in home-type cure therapies, complementary medicine, and modern medicine because of their perceived anti-mutagenic, antitumor and cytotoxic activities as well as anti-oxidant, anticancerous [24, 25], anti-inflammatory [26], antimicrobial [27], antispasmodic, expectorant, carminative and anti-tussive properties [28]. Furthermore, *Origanum* has been used to treat nausea and rheumatism [29], arthritis, hemorrhoids, sexual diseases, animal bites, and poisoning [30] and to control diabetes and obesity [31]. These species have also been used as carminatives, diaphoretics and tonics, and as a source of antimicrobial compounds [32]. As they possess such biological activities, their potential must be revealed by scientific studies and explained to the public since these herbs are available in almost all public markets. It is necessary because once the plant is dried and made into powder form, it loses its morphological identity and easily prone to adulteration. The present study can be helpful in the authentication of the plant, which will assure the safety and efficacy of natural products.

Pharmacognostic Studies:

Macroscopic Characters: *Origanum vulgare* L. is a perennial, aromatic, erect herb with creeping roots, 30-40 cm high, branched quadrangular stems. It has an entire dark green, opposite, oblong - oval with ovate tip leathery leaves (2-2.5 cm long) with petiole (1-1.6 cm long) and bracts with white or purple flowers in a corymbose inflorescence. Quantitative morphological parameters studied were presented in **Table 1**. According to another study, the most important features of Lamiaceae species were the presence of glandular hairs distributed in vegetative and reproductive organs [33].

TABLE 1: QUANTITATIVE MORPHOLOGICAL CHARACTERS OF *ORIGANUM VULGARE* L.

S. no.	Parameters	Observations Mean± SD
1	Stem Width (cm)	1.2 ± 0.26
2	Internodal length	1.1 ± 0.13
3	Petiole Length (cm)	1 ± 0.3
4	Lamina Length (cm)	1.5 ± 0.19
5	Lamina width (cm)	1.3 ± 0.23
6	Lamina ratio	1.5 ± 0.12
7	Leaf area (cm^2)	1.1 ± 0.15
8	leaf serration number	Nil

Microscopic Characters: Microscopical characters were studied in terms of microscopic foliar features and anatomical sections.

Microscopic Foliar Features: The microscopic foliar features *viz.*, stomatal type, stomatal constants, trichomes, vein-islets, and vein-termination patterns are tabulated in **Table 2**. *Origanum vulgare* L. is characterized by the presence of glandular trichomes covering the aerial organs. Trichomes secrete essential oils with aromatic flavour, which mainly due to the presence of its major compounds such as carvacrol and thymol [34]. The presence of diacytic stomata was observed, which is the characteristic feature of Lamiaceae. Stomatal constants are specific standards for the identification of crude drugs.

TABLE 2: MICROSCOPIC FOLIAR FEATURES OF *ORIGANUM VULGARE* L.

S. no.	Parameters	Observations Mean± SD
1	Stomatal number	9.86 ± 1.1
2	Stomatal index	7.5 ± 1.4
3	Epidermal number	24.38 ± 0.78
4	Stomatal type	Diacytic
5	Vein islet number	5.88 ± 1.2
6	Vein termination number	4.2 ± 0.41

Anatomical Study: Both leaves and stem were subjected to investigation, and the results are the following:

T. S. of the Stem: The transverse section of the stem displayed a quadrangular structure with a single layered epidermis with glandular and non-glandular trichomes. Some of them were short, unicellular, while others are long, unicellular, or bicellular. Three to eight layered, closely packed

collenchymatous tissue was found beneath the epidermis at the ridge area, while loosely packed parenchymatous tissue was found in the plain area. Collateral, conjoint and open vascular bundles were seen continuous in the corner portion with phloem parenchyma and xylem vessels **Fig. 1**. The previous study also stressed the importance of hair structures in *Origanum* species [35].

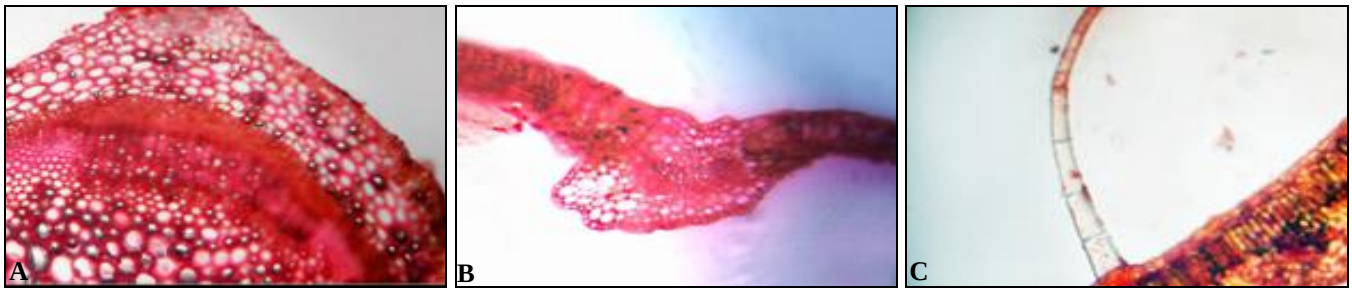

FIG. 1: A- *ORIGANUM* STEM T.S., B- *ORIGANUM* LEAF T.S., C- *ORIGANUM* TRICHOME STRUCTURE

T. S. of the Leaf: The transverse section of *O. vulgare* leaf was dorsiventral in appearance with single-layered epidermis with multicellular epidermal hairs, thin-layered cuticle, and amphistomatic stomata. The mesophyll tissue was divided into a single layered columnar palisade layer and a loosely arranged spongy parenchymatous layer. Three to five layered collenchymatous tissue was found beneath the epidermis. Vascular bundles were conjoint, collateral, and closed with xylem vessels and thin-walled phloem **Fig. 1**. Microscopic analysis and qualitative parameters are generally carried out in order to establish appropriate data that can be used in identifying crude drugs. The microscopic and macroscopic features of *O. vulgare* observed in a previous study were also found to be the same [36].

Organoleptic Evaluation: Colour, odour, taste and texture of the powder were recorded in **Table 3**. The study revealed aromatic odour and taste, which is characteristic to the Lamiaceae family.

TABLE 3: ORGANOLEPTIC EVALUATION OF *ORIGANUM VULGARE* L.

S. No.	Parameters	Observations
1	Odour	Aromatic and pleasant
2	Colour	Dark green
3	Taste	Bitter and astringent
4	Texture	Smooth powder

Evaluation of Physical Constants: To maintain the quality of herbal drugs, it is necessary to reveal the physicochemical parameters of herbs *viz.*, ash values, extractive values, *etc*.

The studied parameters included evaluation of loss on drying on 110 °C, moisture determination, ash analysis, and extractive values. The results are tabulated in **Tables 4** & **5**.

TABLE 4: LOSS ON DRYING AND MOISTURE CONTENT

	Parameters				
O. vulgare **Mean ± SD**	**Loss on drying**	**Moisture Content**	**Total ash (% w/w)**	**Acid insoluble ash (% w/w)**	**Water soluble ash (% w/w)**
	6.94 ± 0.46	6.32 ± 0.46	11 ± 1.8	6.67 ± 0.46	4 ± 0.23

TABLE 5: EXTRACTIVE VALUES

Parameters	*O. vulgare* Mean± SD
Water soluble extractive (% w/w)	1.95 ± 0.85
Alcohol soluble extractive (% w/w)	2.67 ± 0.45
Ether soluble extractive (% w/w)	4.84 ± 0.56
Chloroform soluble extractive (% w/w)	8.34 ± 1.73

Higher extractive values of *Origanum* justify the use of these herbal drugs in the traditional system of medicine. The total ash, acid insoluble ash and water soluble ash of leaf of *O. vulgare* (11%, 6.67%, 4% w/w respectively) were found to be similar to the present investigation [37].

Phytochemical Screening: The detection of phyto-chemicals revealed the presence of secondary metabolites like alkaloids, phenolic compounds, steroids, flavonoids, tannins, triterpenoids, and saponins. The findings converge with another study having the presence of oleanolic, ursolic, caffeic, rosemarinic, lithospermic acids, flavonoids, hydroquinones, tannins, and phenolic glycosides [38].

According to another study on *Origanum vulgare*, there is a wide chemical diversity with reference to essential oils, with a considerable intraspecific qualitative and quantitative variation in constituents was found [39]. The results obtained from the current study are presented in **Table 6**.

TABLE 6: PRELIMINARY SCREENING OF SECONDARY METABOLITES IN THE METHANOLIC EXTRACT OF *ORIGANUM VULGARE* L.

S. No.	Secondary metabolites	Test	Observations
1	Alkaloids	Mayer's test	+
2	Phenolic compounds	Ferrric chloride	+
3	Steroids	Libermann-Burchard	+
4	Flavonoids	Shinoda test	+
5	Cardiac glycosides	Keller Killiani test	-
6	Tannins	$FeCl_3$ test	+
7	Triterpenoids	Libermann-Burchard	+
8	Saponins	Frothing	+

HPTLC: HPTLC profiles with respect to major phytochemicals were carried out using the methanol extracts. Chromatographic fingerprint has been suggested to be a practical and broad approach for identifying the authenticity and evaluating the quality of raw herbal materials and herbal extracts [40]. Different plants would obviously have different chemical fingerprints with respect to both inorganic and organic compounds. In the present study, the HPTLC profiles were developed for the phytochemicals like alkaloids, essential oils and steroids. The methanol extract was subjected to HPTLC analysis by specific solvent system and detected under UV at 366 nm and 254 nm before and after derivatization.

In the present study for chemical fingerprinting using HPTLC, the extract exhibited a good colour pattern with numerous bands, revealing the presence of alkaloids, essential oils and steroids.

Screening of Alkaloids: The HPTLC chromatogram for Alkaloids was best observed at 254 nm & 366 nm before derivatization and 10 bands of Alkaloids were seen to be separated before derivatization at 366 nm. The compounds separated were seen at R_f = 0.01, 0.07, 0.09, 0.12, 0.19, 0.43, 0.59, 0.72, 0.87 and 0.91. Toluene: Methanol: Diethylamine (8:1:1) was found as the best solvent system to observe the above separation and Dragendorff reagent was used for derivatization **Fig. 2** & **3**.

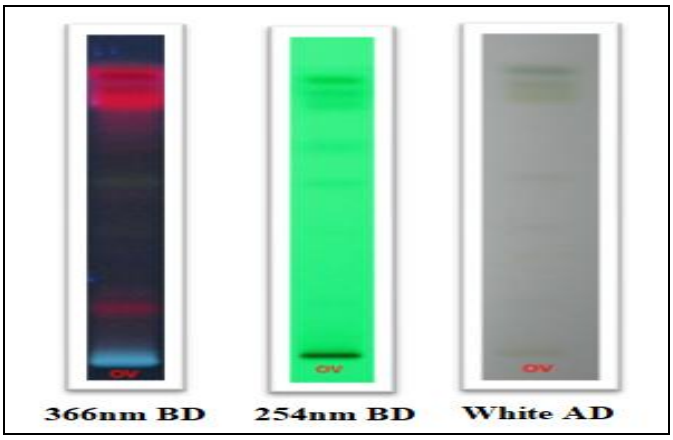

FIG. 2: HPTLC FINGERPRINT OF *ORIGANUM VULGARE* L. OF ALKALOIDS

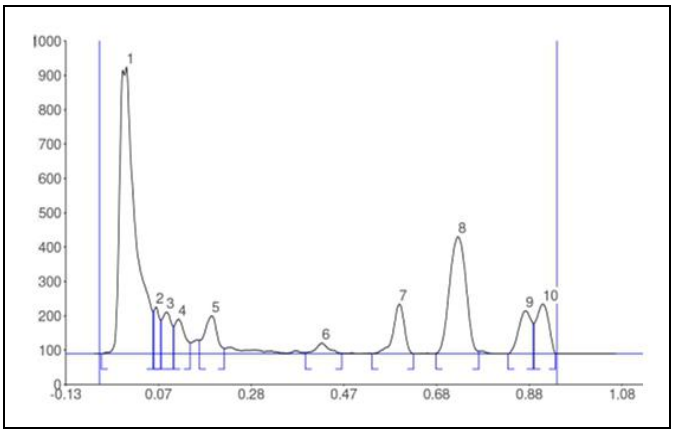

FIG. 3: HPTLC CHROMATOGRAM OF *ORIGANUM VULGARE* L. OF ALKALOIDS

Screening of Essential Oils: The HPTLC chromatogram for Essential oils showed almost 6 bands, and the major compounds separated were seen at R_f = 0.13, 0.24, 0.29, 0.53, 0.70, and 0.82 in the solvent system: Toluene: Ethyl acetate (8.5:1.5). Derivatization was done using Anisaldehyde sulphuric acid **Fig. 4** & **5**. HPTLC profile was developed for ethanol extract of the leaf as a preliminary fingerprinting of the extract, and ten spots were observed in HPTLC profiling of ethanol extract of *Origanum* [41]. *Origanum vulgare* is having all the properties and beneficial activities enumerated earlier due to the presence of different essential oils, which are products of the secondary metabolism of plants. Moreover, it has been demonstrated that the oils present in the whole plant give their properties as anticancerous [42], although essential oils can be obtained from different parts of plants such as flowers, buds, seeds, leaves, branches, barks, fruits, and roots,

they are however commonly obtained from the aerial parts of the plants [43]. Oregano essential oils indicate high antioxidant and moderate antibacterial as well as high antiradical, metal chelating, tyrosinase inhibitory activity, which can be used as a natural source of terpenes, flavonoids and other phytochemicals in medicine, pharmaceutics, cosmetics, and food industry [44-47].

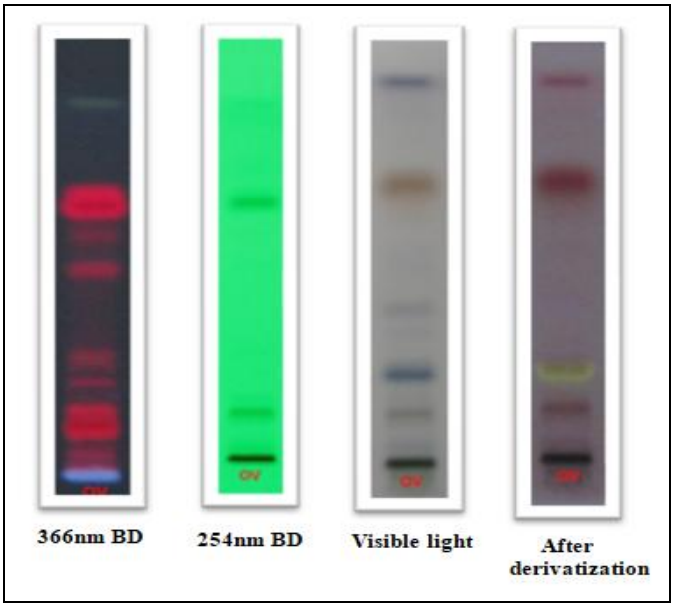

FIG. 4: HPTLC FINGERPRINT OF *ORIGANUM VULGARE* L. OF ESSENTIAL OILS, A: 366 NM BD, B: 245 NM BD, C: VIDILE LIGHT, D: AFTER DERIVATIZATION

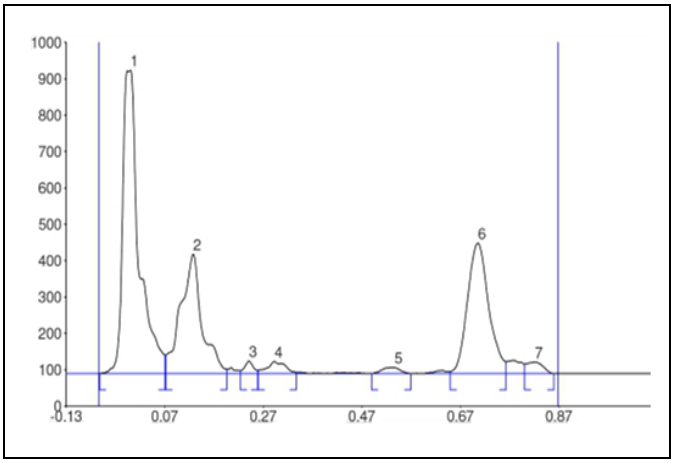

FIG. 5: HPTLC CHROMATOGRAM OF *ORIGANUM VULGARE* L. OF ESSENTIAL OILS

Screening of Steroids: Steroids were best observed at wavelength 254 nm and 366 nm before and after derivatization and 11 bands of Steroids were seen to be separated before derivatization at 366 nm. The major compounds separated were seen at R_f = 0.00, 0.06, 0.22, 0.37, 0.47, 0.54, 0.58, 0.63, 0.68, 0.76 and 0.84 in the solvent system, Toluene: Methanol: Acetone (6:2:2). Anisaldehyde sulphuric acid was used as the derivatizing agent **Fig. 6** & **7**. Steroidal compounds are of great importance and interest in pharmacy due to their relationship with such compounds as sex compounds [48].

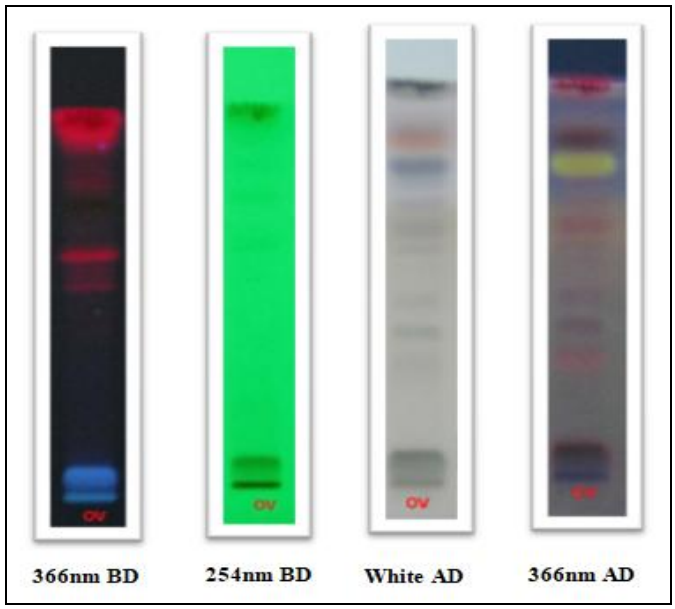

FIG. 6: HPTLC FINGERPRINT OF *ORIGANUM VULGARE* L. OF STEROIDS, A: 366 NM BD, B: 245 NM BD, C: WHITE AD, D: 366 NM AD

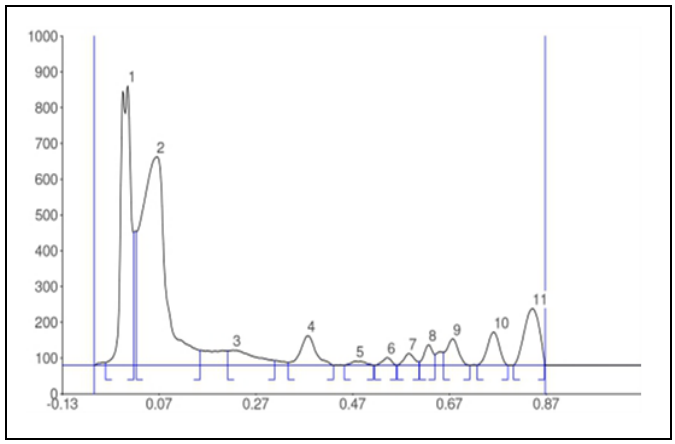

FIG. 7: HPTLC CHROMATOGRAM OF *ORIGANUM VULGARE* L. OF STEROIDS

CONCLUSION: The current surge of interest for herbal products in preference over synthetic products has changed the trade scenario much in favour of natural essential oil and aroma chemicals. During the past few years, there has been a marked increase in many herbal spices, which are used to enhance various kinds of foods. Due to high biological activity, these aromatic plants are widely used these days as herbal spices as well as in the cosmetic and pharmaceutical industry.

Therefore, for different uses, aromatic plants may be grown in different geographic areas. Our study

clearly indicates that the methanolic extract of *Origanum vulgare* from Nilgiris shows a higher concentration of bioactive phytoconstituents like alkaloids, phenolics, flavanoids, terpenes, and steroids that find an amazing wide application in many industries for scenting and flavourings of all types of consumers finished products. They can be used in the perfumery, cosmetics, pharmaceutical, and food as well as flavour industry.

Pharmacognostical and phytochemical studies done so far on *Origanum vulgare* confirms the traditional usage of this plant. Chemical finger-printing has been demonstrated to be a powerful technique for the quality control of herbal medicines, and a chemical fingerprint is a unique pattern that indicates the presence of multiple chemical markers within a sample. So, exploring it will lead to isolation and identification of new compounds which could be used as drugs for curing common and critical diseases.

The macroscopic, microscopic characters, physico chemical values, and HPTLC fingerprinting reported in this paper could be used as the diagnostic tool for the standardization of this medicinally important plant species and also identify the adulterants. The pharmacognostic constants, the diagnostic microscopic features, and the numerical standardization parameters reported in this work could be useful for the compilation of a suitable monograph for the proper identification and authentication of the drug.

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CONFLICTS OF INTEREST: We declare that we have no conflict of interest.

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