Oreganum vulgare Linn. leaf: An Extensive Pharmacognostical and Phytochemical Quality Assessment

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A B S T R A C T

Purpose: Standardization and detailed pharmacognostical studies of *Oreganum vulgare* Linn. leaf for authentication and commercial utilization. **Methods:** *Oreganum vulgare* Linn. leaf was with standardization according to standard procedures described in WHO, 2011 and I.P. 1996. **Results:** The physicochemical parameters total ash, acid insoluble ash, water soluble ash and sulphated ash were found to be 11.5%, 11%, 5, 10.5% w/w respectively. Foaming index was found be <100. The trace elements were found to be copper, lead, cadmium, zinc, cobalt, manganese, nickel and copper in ethanol extract and phytochemical screening of aqueous and ethanol extract showed the presence of carbohydrates, flavonoids, anthocyanins, phenolic compounds etc. **Conclusion**: The standardization parameters viz. physico-chemical parameters, macroscopy, microscopy, taxonomy, anatomy and preliminary phytochemical screening, microbial and aflatoxin count, HPTLC profile is being reported to help in authentication and development of monograph of this plant.

Introduction

The use of medicines from natural resources is as old as evolutionary history of human being. Primitive man started using these herbs by hit and trial method and distinguished whether which combination of herbs is effective in treating any ailment or which is exerting toxicity. Plants are defined as 'biochemical factories' producing bio-chemicals responsible for therapeutic activity or toxicological effect. 80% of the world's population especially in developing countries is dependent on herbal medicines because of less side effects, easy availability and economic.[1,2] Herbal drugs are still at the risk of quality because sources of raw material of herbal drugs are different and an herb is mixture of multi-constituents, and lack of availability of efficient analytical methods. Hence, it is necessary to set official standards to maintain quality, the first step of which must be identification of entire constituents and physicochemical parameters in herbs.[3]

Oreganum vulgare Linn. (Lamiaceae) commonly known as Oregano, is used traditionally as expectorant and spasmolitic agent, as diuretic and antiseptic agent and in dermatological affections.[4,5] In the present paper an attempt was made to standardize and identify phytochemical profile.

Materials and Methods
Plant material

Oreganum vulgare leaves were procured from Aum Agreefresh Pvt. Ltd. and were identified by the same company. The voucher specimen (Pcog1101) was deposited in Department of Pharmaceutical sciences, Guru Jambheshwar University of Science & Technology for future references. The leaves were powdered with the help of a pulvarizer and used for all the studies.

Pharmacognostical Evaluation

Morphological characters like colour, surface texture, odour were examined according to Trease.[6] Organoleptic evaluation, physicochemical parameters viz; ash values, extractive values, heavy metals, microbial counts, phytochemical screening was performed.[6-11]

Transverse section of leaf was performed by free hand sectioning. The transverse sections and coarsely powdered leaves were cleared with chloral hydrate, stained with phloroglucinol and concentrated HCl, mounted in glycerol and studied under microscope for transverse section and powder studies respectively.

Both ethanol and aqueous extracts were analysed for nine elements by atomic absorption microscopy.[10]

Phytochemical screening

For phytochemical screening, aqueous extract was prepared by soaking 20 g of crude drug with 200 ml of distilled water and kept for 24 h. the extract was filtered and concentrated in rotary vacuum evaporator. Ethanol extract was prepared by soaking 20 g of crude drug with 200 ml of ethanol and kept for 24 h. the extract was filtered and concentrated in rotary vacuum evaporator.

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Both the extracts were tested for presence of different chemical constituents viz. alkaloids, glycosides, carbohydrates, sterols, phenolic compounds, tannins, flavonoids, saponins, proteins and free amino acids (Table 1).[12-14]

Table 1. Preliminary phytochemical screening of ethanol and aqueous extracts

Plant Constituents /Reagent used		Leaf extract		Plant Constituents /Reagent used		Leaf extract	
		Ethanol	Aqueous			Ethanol	Aqueous
Alkaloids	Dragendroff's reagent	-	-	Chalcones	Ammonium Hydroxide test	-	-
	Wagner's reagent	-	-	Flavonoids	Alkaline reagent test	+	+
	Hager's reagent	-	-		Shinoda test	-	-
	Mayer's reagent	-	-		Vannilin HCl test	+	+
Carbohydrates	Molish test	+	-		Ammonia test	-	-
	Fehling's reagent	+	+	Flavanonols	Zinc powder test	+	+
	Bennedict 's reagent	+	+	Flavonols	Boric acid test	-	-
	Test for Pentose	-	-	Flavones, Flavanones, Flavolol	Sulphuric acid test	-	-
	Charring test	+	+	Anthocyanins	Sulphuric acid test	+	+
Glycosides	General test	+	+	Leucoanthocyanins	Vanillin test	-	-
Anthraquinone	Borntrager test	+	+	Gelatin	Solubility test	-	-
	Modified Borntrager's test	-	-		Soda lime test	-	-
Cardiac Glycosides	Legal test	-	-		Precipitation test	-	-
	Baljet test	-	-	Lipids	Solubility test	-	-
	Killer killani test	-	-		Grease spot test	-	-
Steroids	Salkovaski test	+	-		Emulsification test	-	-
	Libermann Burchard's test	+	-	Starch	Jelly test	-	-
Coumarins	Ferric chloride test	+	+	Lignin	Phloroglucinol test	-	-
	Fluorescence test	-	-	Proteins	Trichloroacetic acid	-	-
Cynophoric glycosides	Sodium Picrate test	-	-		Xanthoproteic test	-	-
Phenolic compounds and Tannins	Matchstick test	+	+		Ninhydrin test	-	-
	Chlorgenic acid test	+	+		Lead sulphid test	-	-
	Ferric chloride test	+	+		Biuret test	-	-
	Lead acetate test	+	+	Free amino acids	Ninhydrin test	-	-
	Gelatin test	-	-		Phenolic amino acid test	-	-
	Bromine water test	-	-	Resins	Solubility test	-	-
	Vanillin HCl test	+	+		Hydrochloric test	-	-
Phlobatannins	HCl test	-	-		Ferric chloride test	-	-
Saponins	Foam test	-	-		Turbidity test	-	-
	RBC Haemolysis test	-	-				

+ present, - negative

HPTLC profiling

The solvent system used was Toluene: Ethylacetate (5:1.5). After selecting solvent system for each fraction HPTLC studies was done by using 10×10 cm of glass Plates, CAMAG TLC Scanner3 "Scanner3_130716 (1.14.26) attached with UV detector of D2 &W lamp with 281 V, second order optical fibre at 366 nm and controlled by win CATS software.[15]

Results

Pharmacognostic Evaluation

Macroscopic examination

The leaves are dark green in color; oblong ovate in shape; bract hairs are absent; texture is smooth; corolla color is white; calyx is of green color; calyx hairs are sparse; taste: astringent.

Microscopy of leaf powder

Microscopic examination of leaf powder showed the presence of diacytic stomata, glandular and non-glandular trichomes (Figure 1).
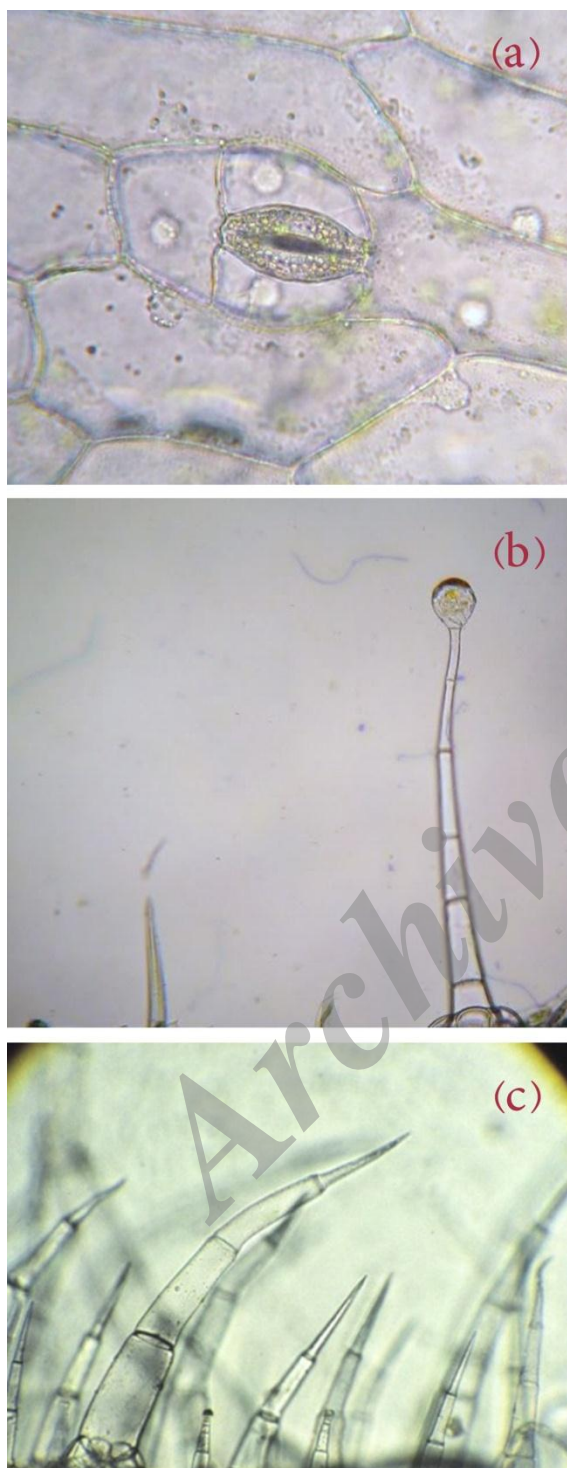

Figure 1. Microscopy of leaf powder (a) diacytic stomata (b) trichomes (c) non-glandular trichomes.

Microscopy (T.S. of leaf)

The transverse section of leaf showed the presence of single layered epidermis with multicellular epidermal hairs, a layer of cuticle and amphistomatic stomata were observed. There was clear cut discrimination between palisade and spongy Parenchymatous cells of Mesophyll. The palisade cells were single layered and columnar. Spongy cells were found to be scattered and loosely arranged. Vascular bundles were conjoint, collateral and closed (Figure 2).
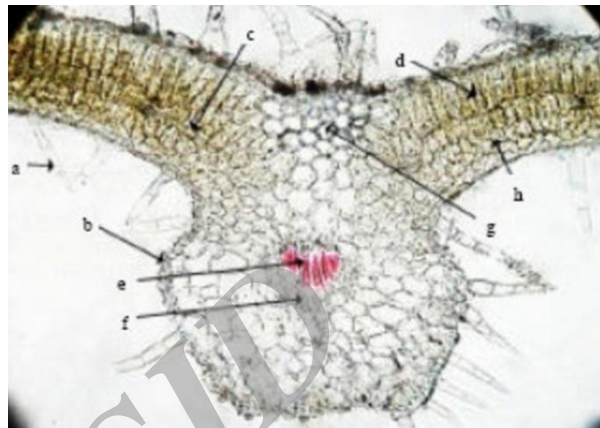
Figure 2. Microscopy T.S. of leaf (a= trichome, b= epidermis, c= mesophyll, d= palisade cells, e= xylem vessel, f= phloem, g= collenchyma).

Determination of physicochemical parameters

The physicochemical parameters total ash, acid insoluble ash, water soluble ash and sulphated ash were found to be 11.5%, 11%, 5, 10.5% w/w respectively. The extractive values of ethanol extract and aqueous extract by hot extraction method were found to be 0.003% and 0.02% w/w respectively and that of by cold extraction method were found to be 0.003%, 0.02% w/w respectively. Swelling index was found to be zero. The drug was not found to be bitter. The foaming index was found to be <100. The % moisture content was found to be 6%. % of volatile oil was found to be 1.67% v/w. crude fiber content was found to be 75% w/w.

Elemental analysis

The Atomic absorption spectroscopy study showed the presence of lead, cadmium, zinc, cobalt, manganese, nickel and copper in ethanol and aqueous extract of leaf but below the permissible limits of WHO and hence are safe to use.

Microbial contamination

The aqueous and ethanol extracts of leaf of *Oreganum vulgare* showed the complete absence of *E.coli, S.typhi, P.aerginosa, S.aureus, Clostridia, Shigella.* Total bacterial count was found to be $<10^3$ and $<10^4$ for ethanol and aqueous extracts respectively.

Preliminary phytochemical screening

The ethanol and aqueous extracts showed the presence of carbohydrates, anthraquinones, coumarins, Phenolic compounds and tannins, flavonoids, flavanonols and anthocyanins. Alkaloids, saponins, cardiac glycosides,

lignin, starch, resins, free amino acids and lipids were absent in both ethanol and aqueous extracts.

HPTLC studies
HPTLC profile was developed for ethanol extract of leaf as a preliminary fingerprinting of the extract. Toluene: ethylacetate (5:1.5) was found to be a suitable solvent system for the separation of constituents of leaf extract. Ten spots were observed in HPTLC profiling of ethanol extract of *Oreganum vulgare* at 254 nm with R_f values of 0.15, 0.16, 0.26, 0.51, 0.58, 0.66, 0.71, 0.75, 0.83 and 0.89 respectively while fifteen spots were observed at 366 nm with R_f values of 0.16, 0.18, 0.25, 0.32, 0.36, 0.38, 0.50, 0.56, 0.58, 0.66, 0.71, 0.75, 0.79, 0.83 and 0.93 respectively indicating the presence of different constituents. HPTLC images and data are shown in Figure 3.
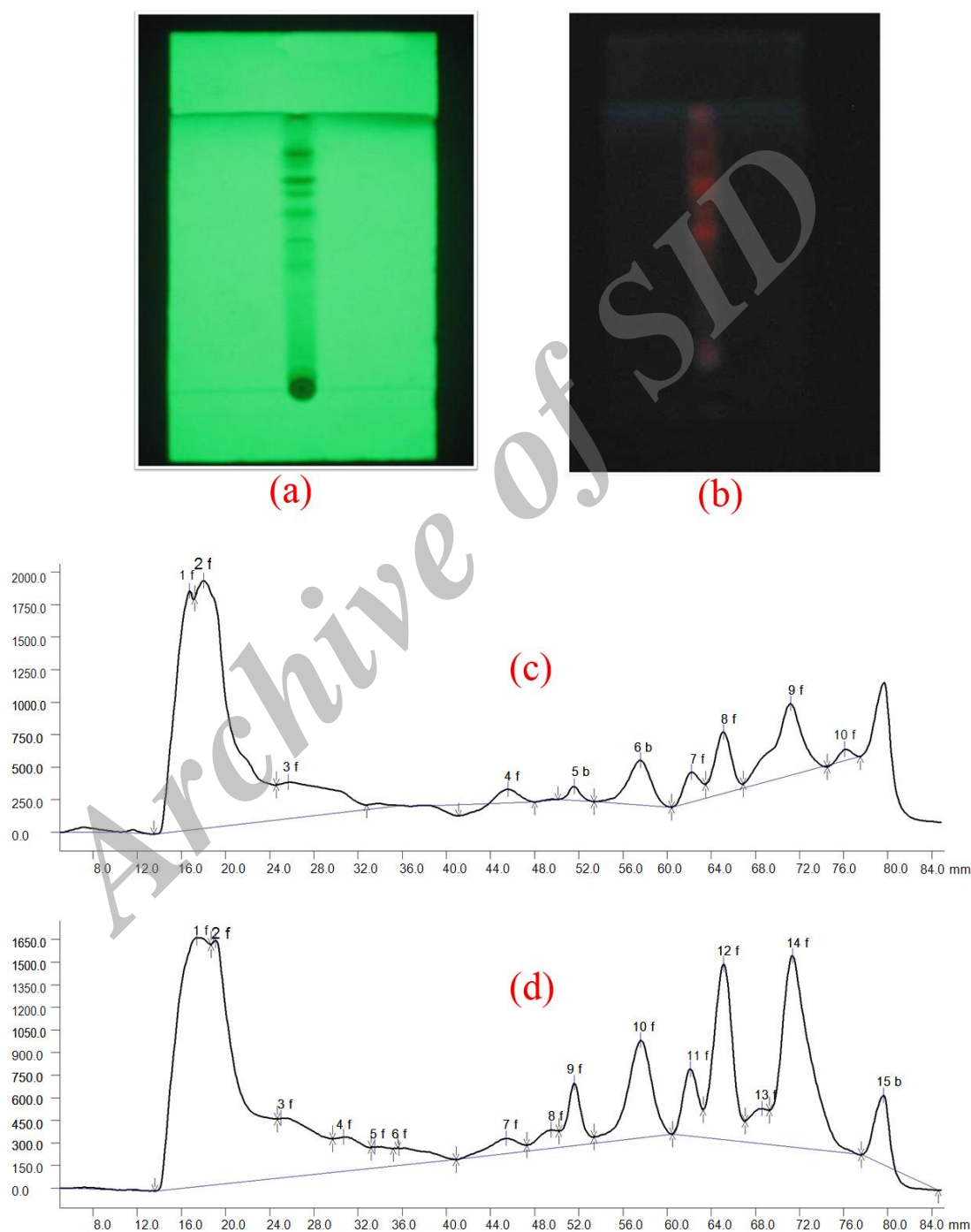
Figure 3. HPTLC profiling of ethanol extract of *Oreganum vulgare* in solvent system Toluene: Ethylacetate (5:1.5) at (a) 254 nm (b) 366 nm (c) R_f value determination at 254 nm (d) R_f value determination at 366 nm.

Discussion

Oreganum vulgare Linn. belonging to family Lamiaceae is commonly known as "Pizza herb" and is commonly served as food material in Lebanese, Italian and Mexican cuisines. It is a perennial herb, distributed among temperate, tropical and subtropical regions. Microscopic analysis and qualitative parameters are carried out in order to establish appropriate data that can be used in identifying crude drug. The phytochemical screening of the test plant was done for their active components present for their medicinal values. Many of the phytochemical tests showed positive results which render the presence of their active compounds. The Total ash, acid insoluble ash, water-soluble ash values and sulfated ash of leaves were observed to be 11.5%, 11%, 5, 10.5% w/w respectively while in a similar study carried out on stem and leaf of *Oreganum vulgare* 10.6%, 5.6%, 0.47% and 9.4% w/w respectively. The microscopic and macroscopic features were found to be same in previous and present study.[16] The present study provides extensive details about microbial count, heavy metal analysis, HPTLC profiling, phytochemical screening, powder studies and physico-chemical features.

Conclusion

Since the plant, *Oreganum vulgare* which is also used for the treatment of various diseases and disorders, it is important to standardize it for use as a drug. The pharmacognostic constants for the leaves of this plant, the diagnostic microscopic features and the numerical standardization parameters reported in this work could be useful for the compilation of a suitable monograph for its proper identification.

Conflict of interest

We declare that we do not have any conflict of interest.

Acknowledgements

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