PHARMACOGNOSTICAL AND QUALITY CONTROL PARAMETERS OF ORIGANUM MAJORANA LINN. STEM AND ROOT.

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ABSTRACT

Objective: To study and evaluate various pharmacognostical and quality control parameters of both stem and root part of Origanum majorana Linn (Family: Lamiaceae). Methods: Morphology, microscopy, physicochemical analysis, preliminary phytochemical screening, quantitative estimation and other standardisation parameters as per WHO guidelines. Results: Stems are reddish square in shape having descending, multi-branched branches with weak, hairy, round and green with red speckles all over it. The stem specimen are usually 40-150 cm long and 0.5 to 1.5 mm in diameter. The fracture is short, whereas roots are 0.2 mm to 0.6 mm in diameter, sub - cylindrical in shape and longitudinally wrinkled with transverse fissures. Fractures are long, irregular and fibrous. Rootlets and root scars are also present. Powder microscopy shows the presence of parenchyma, phloem fibres and xylem vessels in stem and parenchyma, phloem fibres and cork cells in roots. Phytochemical screening of both the parts reveals presence of terpenoids, flavonoids and tannins in ethanol extracts whereas saponins and carbohydrates are present in aqueous extract and their quantitative estimation is reported. Conclusion: The parameters reported in the present paper, may be helpful in authenticity and adds to the existing knowledge and would be useful for quality control of the plant.

Keywords: Origanum majorana, World Health Organization, Pharmacognostical standardisation.

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INTRODUCTION

*Origanum majorana* Linn. is a tender perennial herb of the mint family (Labiatae), which was formerly classified as *Majorana hortensis* Moench[1]. It is commonly known as sweet marjoram. It is an herbaceous perennial plant, native to Cyprus and the Eastern Mediterranean region. It is cultivated in India, France, United States and Hungry. Marjoram was initially used by Hippocrates as an antiseptic agent. It is a well-liked home remedy for chest infection, cough, sore throat, rheumatic pain, nervous disorders, cardiovascular diseases, epilepsy, insomnia, skin care, flatulence and stomach disorders[2-4]. It is widely used in traditional medicines as well as in the food as a spice or condiment and in cosmetic industries. The plant is reported to posses various biological activities such as anti-anxiety [5], anti-convulsant[6], anti-diabetic[7], anti-microbial[8-11], anti-mutagenic[12], anti-ovicidal[13], anti-oxidant [14,15] and anti-ulcer [16]. The *Origanum* herb, reported the presence of large number of constituents in different parts of the plant, especially terpenoids[17-19], phenols[20] and flavonoids [21] as major constituents, due to its aromatic nature and others like steroids[17], fatty acids and vitamins[22] as minor part. A comprehensive literature review revealed that no such work has been undertaken related to stem and root of the plant, till date hence, the present study aims to study the pharmacognostical characteristics and to develop quality parameters of the stem and root of *Origanum majorana*.

MATERIAL AND METHODS

Plant material collection and authentication

Stem and root of the plant were collected from Guru Jambheshwar University of Science and Technology, Hisar in August, 2012 and identified by Dr. K.C Bhatt, Senior Scientist, National Herbarium of Cultivated Plants, National Bureau of Plant Genetic Resources, New Delhi, vide reference no. NHCP/NBPGR/2012-28/. The plant has been deposited in Pharmacognosy division of department of Pharmaceutical Sciences, GJUS&T, Hisar for further references (voucher specimen no.11pg06).

Pharmacognostical evaluation

Various morphological features viz. shape, size, colour, odour, taste and fracture were studied according to the standard methods [23, 24]. Microscopic studies (both histology and powder study) were done using the method described [23]. Photomicrographs were obtained by observing free-hand sections of drug under compound trinocular microscope (Zeiss Primostar).
Standardization parameters
Various standardization parameters viz. moisture content, ash values, extractives values, crude fiber content, swelling index, foaming index, tannin content, bitterness value, microbial contamination, aflatoxins were determined in both parts (stem and root) of marjoram according to procedure mentioned in Indian Pharmacopoeia (1996) [24] and the World Health Organisation (WHO) Guidelines (2011). Preliminary phytochemical analysis was carried out using standard conventional protocol [25]. The stem and root of the plant was analyzed respectively for the presence of heavy metal by using Atomic Absorption Spectroscopy [23, 26]. The standard calibration curves were then prepared. The instrument was optimized as per requirement and results were obtained in ppm levels.

Preliminary phytochemical screening
The ethanol and aqueous extracts of both stem and root were screened for the presence of alkaloids, glycosides, carbohydrates, sterols, phenolic compounds, tannins, flavonoids, saponins, proteins and free amino acids using standard procedures [27-29].

Quantitative estimation of various phytoconstituents
Stem and root extracts (ethanol and aqueous) of Origanum majorana were used respectively for determination of total phenolic, flavonoid and carbohydrate content.

Total Phenolic Content
The concentration of phenols in stem and root ethanol extracts were determined respectively by using spectrophotometric method (Folin-ciocateu reagent method) [30, 31].

Total Flavonoid Content
The total flavonoid content was determined in plant ethanol extracts (stem and root) using spectrophotometric method (Aluminum chloride method) [31, 32].

Total Carbohydrate Content
The total carbohydrate content in stem and root aqueous extracts respectively were determined [33].

RESULTS
Morphological studies
STEM: woody cylindrical, reddish brown in colour, 40-150 cm long and 0.5 to 1.5 mm in diameter. Stems are reddish square in shape and having descending, multi-branched branches
that spill over to create a mound. Branches are straight having weak, hairy, round and green with red speckles all over it. The fracture is short, characteristic aromatic in odour and astringent, non bitter in taste (Figure 1a).

ROOT: *Origanum majorana* has tap roots. They are 0.2 mm to 0.6 mm in diameter. Roots of the herb are sub-cylindrical in shape and longitudinally wrinkled with transverse fissures. The outer surface of root is dark brown in colour and internal surface is light brown in colour. It has aromatic odour and taste is non bitter. Fractures are long, irregular and fibrous. Several long rootlets and root scars are also present (Figure 1b).

**Histology**

Stem: The stem is circular in transverse section consisting of a thick cuticle. The epidermis is composed of single layer rectangular cells. The cortex contains 5-6 layers of closely packed polygonal parenchyma cells. Phloem fibres and phloem parenchyma are clearly distinguished. The medullary rays are two cell thick. Xylem consists of xylem vessels and xylem parenchyma. A prominent parenchymatous pith is present in the centre (Figure: 2a).

Root: Transverse section is circular in outline. It consists of 2-3 layers of rectangular cork cells. The cortex consists of 6-7 layers of closely packed parenchyma. Xylem elements consist of xylem vessels and xylem parenchyma. The medullary rays are composed of 2 cell thick rectangular cells. Phloem is present outer to the xylem. Pith is absent (Figure: 2b).
Fig 2 Transverse section of stem (A) Cuticle (Cu), epidermis (Ep), cortex (C), phloem fibres (Ph.f), phloem parenchyma (Ph.P), xylem fibres (Xy. P), xylem vessels (Xy.V), medullary rays (M) and pith (P); root (B) Cork cells (Ck), phloem parenchyma (Ph.P), xylem parenchyma (Xy.P), xylem vessels (Xy.V) and medullary rays (Mr).

**Powder study**

On microscopic examination, stem powder of the herb showed presence of parenchyma, phloem fibres, xylem vessels whereas root powder showed presence of parenchyma, phloem fibres and cork cells (Figure: 3,4).

**Fig 3 Microscopy of stem powder:** (a) Parenchyma, (b) Phloem fibres, (c) Xylem vessels

**Fig 4 Microscopy of root powder:** (a) Parenchyma, (b) Phloem fibres, (c) Cork cells
Physicochemical parameters
Total ash, acid insoluble ash, water soluble ash and sulphated ash values of stem and root were found to be 7.6%, 1.7%, 4.9%, 2.8%; 16.3%, 2.5%, 8.5%, 4.9% respectively. Extractive values of ethanol and aqueous extract of *O. majorana* (stem and root) were 2.44%, 5.8% respectively through hot extraction method and 6.4%, 5.9% through cold extraction method in case of root whereas, in case of stem it was found to be 9.8%, 3.4% through hot extraction method and 10%, 6.2% through cold extraction method respectively.

The other physiochemical parameters like loss on drying, bitterness value, swelling index, foaming index, crude fibre content, tannin content and aflatoxin presence were summarized in Table 1.

Table 1 Physiochemical parameters

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Parameter</th>
<th>Stem</th>
<th>Root</th>
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<tbody>
<tr>
<td>1.</td>
<td>Loss on drying</td>
<td>6.2 % (w/w)</td>
<td>4.03 % (w/w)</td>
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<tr>
<td>2.</td>
<td>Foaming index</td>
<td>Less than 100</td>
<td>Less than 100</td>
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<tr>
<td>3.</td>
<td>Swelling index</td>
<td>1ml/g drug</td>
<td>1ml/g drug</td>
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<tr>
<td>4.</td>
<td>Crude fibre content</td>
<td>52.35 % (w/w)</td>
<td>17.8 % (w/w)</td>
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<td>5.</td>
<td>Bitterness value</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>6.</td>
<td>Tannin content</td>
<td>2.25 % (w/w)</td>
<td>2.75 % (w/w)</td>
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<tr>
<td>7.</td>
<td>Aflatoxins (B1, B2, G1, G2)</td>
<td>Nil</td>
<td>Nil</td>
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Heavy metal analysis
The concentration of copper, cadmium, arsenic, lead, iron, zinc and mercury were under permissible limits in stem whereas magnesium concentration was little high. The concentration of copper, cadmium, arsenic, lead and mercury were found to be under permissible limits in roots whereas iron, zinc and magnesium concentration is little high, but safe to be used.

Microbial contamination
Determination of microbial contamination of ethanol and aqueous extracts of stem and root of the plant showed complete absence of *Escherichia coli, Salmonella typhi, Psuedomonas aeruginosa, Staphylococcus aureus, Clostridia and Shigella*.

Preliminary phytochemical screening
The ethanol extract of both stem and root showed the presence of terpenoids, flavonoids and tannins whereas saponins and carbohydrates were present in stem and root water extract.
respectively. Alkaloids, glycosides ad proteins were absent in both of the extracts (root and stem).

Quantitative estimation of various phytoconstituents

The total phenolic content (mg/g) gallic acid equivalent was found to be 25.16 and 24.65 in stem and root ethanol extract whereas flavonoid content, quercetin equivalent (mg/g) was found to be 305.8 and 195.5 in stem and root ethanol extract respectively. The carbohydrate content in stem and root aqueous extracts were found to be 36.38 and 30.94 respectively.

DISCUSSION

Ancient Indian literature incorporates a remarkably broad definition of medicinal plants and considers all plant parts to be potential sources of medicinal substances. However, a key obstacle, which has hindered the acceptance of the alternative medicines in the developed countries, is the lack of documentation and non-compliance of GMP guidelines basically due to poor standardization status. The variability of the constituents in herbs or herbal preparations due to genetic, cultural and environmental factors has made the use of herbal medicines more challenging than it would necessarily have been. The process of standardization can be achieved by stepwise pharmacognostical studies. According to WHO, 2011 the macroscopical and microscopical description of a medicinal plant is the first step towards establishing the identity and the degree of purity of such material. The pharmacognostical parameters and phytochemical studies are one of the major reliable and inexpensive criteria for confirmation of the crude drugs. The standardization of herbal drugs including authentication of genuine drug, harvesting the best quality raw material, assessment of intermediate and finish product and detection of harmful and toxic ingredient. Since, much literature related to Origanum majorana L., especially regarding its stem and root, respectively was unavailable. In conclusion, the present study on O. majorana (stem and root) could be useful to detect the authenticity of the medicinally useful plant and also distinguishing it from its closely related species. It will add to the existing knowledge regarding standardisation and quality control of the plant.

ACKNOWLEDGMENTS

The authors are grateful to express our gratitude to Dr. K.C Bhatt, NBPG, Pusa campus, New Delhi for identification and authentification of the plant for our project.
REFERENCE


