Qualitative phytochemical analysis of ethanolic and aqueous extracts of *Origanum majorana*

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Abstract- Preliminary phytochemical analysis has been performed in aqueous and ethanolic extracts of *Origanum Majorana* (leaves and flower), the various phytochemical constituents like, alkaloids, triterpenoids, anthraquinone, emodin, terpenoids. steroids, flavonoids, glycol-sides, tannin, phenol and saponin are present in all the extracts. All the phytochemicals are abundantly found in leaves extract followed by in flower extract.

Key words: *Origanum majorana*, Phytochemical analysis, Extracts

Introduction

Plant parts such as, leaves, seeds, vegetables, fruit, spices etc with value have been used to cure many diseases since ancient time. Today in this modern world, even though synthetic drugs are readily available and highly effective in curing various diseases, there are people who still prefer using traditional folk medicines because of their less harmful effects. There is a wide diversity of compounds, especially secondary meta-bolites (like, alkaloids, triterpenoids, anthraquinone, emodin, terpenoids. steroids, flavonoids, glycosides, tannin, phenol and saponin) found and isolated from plants and studies have shown that these compounds have anticancer, antibacterial, analgesic, anti-inflammatory, antitumor, antiviral and many other activities to a greater or lesser extent **(Cai et al., 2004; Miliauskas et al., 2004).**

Origanum majorana is a medicinal plant of the Lamiaceae family, known as Maruwa in traditional Indian medicine. This plant is distributed around the Mediterranean regions, Asia, and North Africa, in particular, Morocco, Algeria, Egypt, Spain, and Portugal (**Vasudeva P and Neeru 2015).** *Origanum majorana* showed various biological activities such as allergies, fever, hypertension, respiratory infections, anti-diabetic, pain-ful menstruation, Kidney Yang deficiency, stomach ache, cough, rheumatism, head-ache, insomnia, also in intestinal anti-spasmodic. Moreover, *Origanum majorana* exhibits a wide effect spectrum with antioxidant, antibacterial, antifungal, nephroprotective, anti-proliferative, anti-cancer activities **(Hajlaoui H et al, 2016;**

Kozáowska M et al, 2010, Rao S et al, 2014; Abdel-Massih RM et al, 2018]. These effects are mediated by the presence of bioactive compounds such as thymol, carvacrol, tannins, hydroquinone, sito-sterol, cis-sabinene hydrate, limonene, terpinene, camphene, and flavonoids. The objective of this study was to carry out preliminary phytochemical screening and to determine the contents of *Origanum majorana*.

Plant material and sample preparation

Flowers and leaves of *Origanum majorana* were collected from botanical garden of D.A.V. College, Muzaffarnagar. They were rinsed with tap water followed by distilled water to remove the dirt on the surface. They were then air dried for 2 days and then freeze dried until a constant mass was obtained. Dried samples were ground into fine powder and kept in desiccators until extracted. The extraction was carried out in a soxhlet apparatus for 12 hours using 50% of aqueous and ethanolic solvent at room temperature. The extracts were filtered using Whattman filter paper and filtrates were used as an extract. Extracts were kept at 4 °C until the bioassay analyses.

Phytochemical screening

The aqueous and ethanolic solvent extracts of flowers and leaves were tested for the presence of alkaloids, steroids, tannins, flavonoids, terpenoids, saponins and glycosides etc. The qualitative Results are expressed as (+) for the presence and (−) for the absence of phytochemicals using standard procedures to identify the constituents as described by (**Sofowara, 1993; Trease and Evans, 1989; Harborne, 1973 and 1984).**

Test for Tannins: 1ml of every sample is boiled in 20 ml of distilled water in a test tube and then filtered separately. A couple of drops of 0.1% ferrous chloride are added Resultsing into brownish green or a blue-black colouration.

Test for Saponins: 2 ml of every sample is boiled in 20 ml of distilled water in a water bath and filtered separately. 10ml of the filtrate is mixed with 5 ml of distilled water and jolted smartly for a stable persistent froth. The frothing is mixed with three drops of olive oil and jolted smartly, Resultsing into the formation of emulsion.

Test for Flavonoids: 5 ml of dilute ammonia solution were added to a little of the liquid filtrate of every plant extract followed by addition of targeted H_2S0_4. A yellow colouration in every extract indicated the presence of flavonoids. The yellow colouration disappeared on standing.

Test for Steroids: 2 ml of acetic anhydride were added to 1ml of extract of every sample with 2 ml H_2SO_4. The colour modified from violet to blue or inexperienced in some samples indicating the presence of steroids.

Test for Terpenoids (Salkowski test)*:* 5 ml of every extract was mixed in 2 ml of chloroform, and targeted H_2SO_4 (3 ml) is rigorously added to create a layer. A venetian red colouration is pointecd out positive Results for the presence of terpenoids.

Test for Triterpenoids: One ml of extract is added to 1 ml of chloroform; 1 ml of acetic anhydride is additional following the addition of 2 ml of diluted H_2SO_4.

Formation of blood-red violet colour indicates the presence of triterpenoids.

Test for Alkaloids: Mayer's test: To a couple of (one) ml of every extract, a drop of Mayer's chemical agent was added by the side of the test tube. A creamy or white precipitate confirms presence of alkaloids.

Test for Anthraquinones: 5ml of every extract solution is hydrolyzed with diluted H_2SO4 extracted with benzene. 1 ml of dilute ammonia is added to this solution. Pink coloration steered the positive response for anthraquinones.

Test for Polyphenols: Plant product (4 ml) is added to every extract (1ml) and the ensuing resolution is transferred and warmed in a water bath (15 minutes). 3 drops of freshly made ferrous cyanide resolution were added to the extract resolution. Formation of a blue color indicated the presence of polyphenols.

Test for Glycosides (Keller-Killani test): Five ml of every extract is treated with 2 ml of glacial acetic acid containing one drop of ferrous chloride resolution. This is often under layed with 1 ml of targeted H_2SO_4. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring could seem below the brown ring, whereas within the carboxylic acid layer, a green ring could be seen simply step by step throughout skinny layer.

Test for Emodins: 2ml of NH_40H and 3ml of benzene were added to the extract. Appearance of red colour indicated the presence of emodins in the test solution.

Results and discussion

The phytochemical screening of 50% ethanolic and aqueous extracts of leaves and flowers samples of *Origanum majorana* revealed the presence of some biologically active metabolites such as, alkaloids, triterpenoids, anthraquinone, emodin, terpenoids. steroids, flavonoids, glycosides, tannin, phenol and saponin etc as shown in **Table-1.**

Table-1 Qualitative analysis of phytochemical in different parts of *Origanum majorana* in Aqueous and ethanolic extract

S.No.	Name of bioactive compound	Flower		Leaf	
		Aq.	Et.	Aq.	Et.
1	Alkaloids	++	++	++	+++
2	Triterpenoids	+	++	+++	+++
3	Anthraquinones	++	++	++	+++
4	Emodins	++	+	++	+++
5	Glycosides	++	++	++	+++
6	Tannin	+	+	+++	+++
7	Terpenoids	++	+++	-	++
8	Steroids	-	-	-	-
9	Phenols	++	+++	+	++
10	Saponin	++	++	+++	+++
11	Flavonoids	-	++	+++	+++

aq=
aqueous extract, et=ethanolic extract, + = presence, - = absence

Tannins were detected in the leaf extract but not in the flowers extract of *Origanum majorana*. Terpenoids are absent in aqueous leaf extract of *Origanum majorana*. Steroids are found to be absent in all studied parts of *the Origanum majorana*. All the phytochemicals are abundantly found in leaves extract (aqueous and ethanolic) followed by in flower extract. This phytochemical screening is more prominent in ethanolic (organic) extract as compared to aqueous extract, as bioactive compound are organic in nature and soluble in organic solvent.

The phytochemical compounds detected are known to have medicinal importance. The flavonoids and phenols are major compounds that act as antioxidants or free radical scavengers **(Bhandary et al., 2012).** The importance of alkaloids, saponins and tannins in various antibiotics used in treating common pathogenic strains has recently been reported **(Kubmarawa et al. 2007.)**For example, many alkaloids derived from medicinal plants show biological activities like, anti-inflammatory antimalarial ,antimicrobial and pharmacological effects. Similarly, steroids derived from plants are known to have cardiotonic effect and also possess antibacterial and insecticidal properties They are very often used in medicines due to their well-known biological activities. Tannins, according to research, are known to have antibacterial **(**Augusto et al., 2011**;**Benbott et al., 2012**;** Hisanori et al., 2001**).** Phytochemicals such as cardiac glycosides have been used to treat congestive heart failure **(Vladimir and Ludmila, 2001)**.

Conclusion

From the study, it could be concluded that plants are a great source of phytochemicals that could be utilized in curing various ailments. These phytochemical compounds identified in the leaf and flower extracts may be responsible for the biological activities shown by *Origanum majorana* and the reason for their use as a traditional medicine. This phytochemical screening test may be helpful to provide a therapeutic platform to detect and develop new drugs.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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