

Spectral Analysis of *Majorana Hortensis* Leaves

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Abstract

All plant species contain some secondary metabolites with marked functions which make them unique. Majority of the disorders and ailments are cured with diverse source of plant species. Most of the plants are bestowed with antioxidant activities. The methanolic extract of the *Majorana hortensis* (*M. hortensis*) leaves proved to be a rich source of antioxidant due to phytochemicals present. Pilot study proved methanolic extract to be most potent and hence it was used for extended assay. Further studies have been carried out in the current paper to identify the different components which contribute to the antioxidant activity. Advanced techniques like Thin Layer Chromatography, High Performance Liquid Chromatography and Infra Red spectrum authenticated the methanolic extract of the *M. hortensis* leaves to be a rich source of antioxidant.

Keywords: *M. hortensis*, HPLC, antioxidant, TLC, spectral analysis.

INTRODUCTION

According to the World Health Organization, about 70–80% of the world populations rely on nonconventional medicines mainly of herbal sources in their healthcare [1]. Two thirds of the world's plant species have medicinal value, in particular, great antioxidant potential that can reduce the oxidative stress in cells and also useful in the treatment of many human diseases [2]. Antioxidant supplements or foods rich in medicinal plants are used to help human body in reducing oxidative damage by free radicals. Currently, research interest has been focused on the role of antioxidant enzymes in the treatment of diseases [3]. The high cost and side effects produced by the chemical drugs paves way for herbal sources. The phytochemicals are the plant chemicals that are naturally present in the plants which play active role in curing diseases without any side effects. Therefore, phytochemicals are called as man friendly medicines [4]. The candidate plant used in the present study is a

Mediterranean herb grown annually and belongs to the lamiaceae or mint family. It is commonly called as sweet marjoram. Some parts of the world call it as sacred plant since the leaves are offered during religious rituals whereas other parts of the world use it for culinary purposes due to the sweet aroma which it carries. Other studies conducted showed that the methanolic extract could improve the antioxidant status of the goat liver slices exposed in vitro to oxidative stress [5]. Previous studies have proved that the *M. hortensis* leaves are rich in alkaloids, phenols, flavonoids, steroids, saponins and tannins [6].

MATERIALS AND METHODS

Plant Material: The plant was grown in pots after collecting saplings from Tamil Nadu Agricultural University, Coimbatore and was identified by Botanical Survey of India, Coimbatore as *Majorana hortensis* Moench. (voucher number BSI/Sc/5/23/08-09/Tech.).

Plant Extract: Methanolic extract of the

leaves was prepared by grinding the fresh washed and pat dried leaves using mortar and pestle. A concentration of 20mg/250 μ l concentration of dimethylsulphoxide were used for the assay.

All the six phytochemical fractions (alkaloids, phenols, flavonoids, saponins, steroids and tannins) were then subjected to TLC analysis. Aliquots (3 μ l) of the extracts were spotted onto the TLC plates and subjected to separation using different solvent mixtures specific for the components, as given below.

TLC of the Phytochemical Fractions

The extracted fractions were subjected to Thin Layer Chromatography on silica gel G60 F254 plates (EMerck). The alkaloid fraction was developed with CH₂Cl₂:ethanol:28% ammonium hydroxide (85:14:1) and sprayed with Dragendroff's reagent. Phenolics were separated with acetic acid: chloroform (45:5) and flavonoids with n-butanol: acetic acid:water (4:1:5) and both were detected with vanillin-H₂SO₄ (10% vanillin in ethanol : concentrated sulphuric acid in 2:1 ratio) spray reagent. The saponin fraction was developed with chloroform: methanol: water (13:7:2) and detected with 10% H₂SO₄. The steroid fraction was developed with chloroform: methanol: water (13:7:2) and detected using iodine vapour. The tannin fraction was developed with chloroform: methanol (19:1) and detected using 5% KOH in ethanol. The R_f values of the spots were calculated as the ratio of the distance travelled by the solute to that by the solvent front.

HPLC Analysis: The residue of the methanolic extract of *M. hortensis* leaves was dissolved in an appropriate volume of HPLC grade methanol and 20 μ l of the

sample was injected into the reverse phase C18 column of the HPLC (Shimadzu, Japan, equipped with a PDA detector). The sample analysis was performed at room temperature, in the wavelength range of 210-440nm at 1000 psi and the mobile phase used was acetonitrile and water in the ratio of 50:50 with a run time of 60 minutes at a flow rate of 1ml/minute.

FT-IR Spectral Analysis: Infrared light from a suitable source passes through a scanning Michelson interferometer and Fourier Transformation gives a plot of intensity versus frequency. When a powdered plant sample is placed in the beam, it absorbs particular frequencies, so that their intensities are reduced in the interferogram and the ensuing Fourier transform is the infrared absorption spectrum of the sample.

RESULTS: The TLC plate, when detected with the alkaloid-specific Dragendroff's spraying reagent, showed six major bands with R_f values 0.83, 0.74, 0.65, 0.53, 0.40 and 0.26 (Plate 1a). The presence of phenolics was analysed using Folin-Ciocalteu reagent as the spraying reagent. The results are shown in Plate 1b, wherein five major spots with R_f values 0.68, 0.66, 0.53, 0.50 and 0.48 were visualized. The investigation of flavonoids separated by TLC, sprayed with 10% vanillin in sulphuric acid, showed four major bands with R_f values 0.81, 0.73, 0.62 and 0.50 as seen in three major bands as indicated in Plate 1c. Saponins were also subjected to TLC analysis and the chromatogram was sprayed with 10% sulphuric acid which showed five distinct bands as seen in Plate 1e. The number of tannin bands was found to be five, with R_f 0.48 and 0.19 respectively, which were developed by spraying 10% sulphuric acid (Plate 1f).

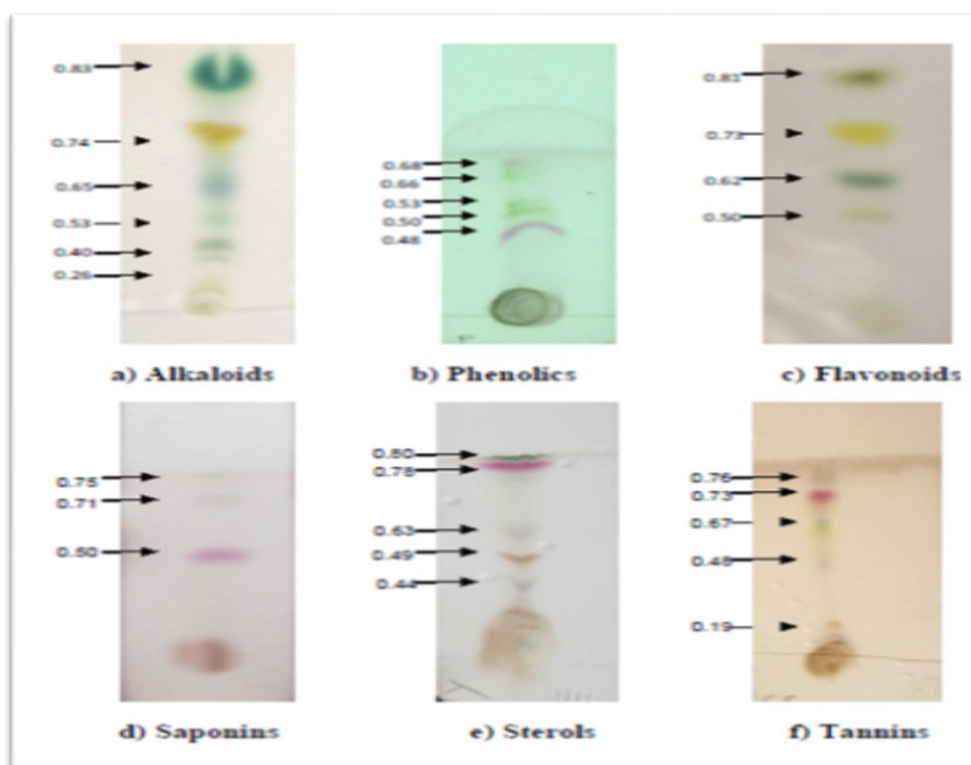


Plate 1: TLC bands of the phytochemical fractions of *M. hortensis* methanolic extract.

HPLC ANALYSIS OF THE METHANOLIC EXTRACT OF *M. hortensis* LEAVES

The HPLC analysis of the methanolic extract of *M. hortensis* leaves was carried out using C18 reverse phase column (Shimadzu equipped with UV detector).

The results obtained are presented in Figure 1. The HPLC spectrum showed 5 peaks (2 major and 3 minor) in the methanolic extract of *M. hortensis* leaves. The retention time of the major and minor peaks along with the peak area of all the 5 peaks are represented in Table 1.



Figure 1: HPLC Analysis of the Methanolic Extract of *M. hortensis* Leaves.

Table 1: Peak table of the methanolic extract of *M. hortensis* leaves subjected to HPLC.

Scanning at 366 nm (UV long range)	
Retention Time	Peak Area
4.288	198311.1
4.958	1102922.8
41.607	1956522.0
43.804	14563.6
44.387	40041.5

IR ANALYSIS OF THE METHANOLIC EXTRACT OF *M. hortensis* LEAVES

The methanolic extract of *M. hortensis* leaves were analysed for the IR spectrum using FT-IR spectrophotometer using KBr pellet method (Figure 2). It exhibited bands at

2910cm⁻¹ along with bands at 1217cm⁻¹ and 1012cm⁻¹ which are characteristic of –OH stretching, C-O-H bending and C-O stretching vibrations. This indicates the presence of –OH group. A band at 1725cm⁻¹ indicates the presence of –CO group. Hence, the extract may contain saponins.

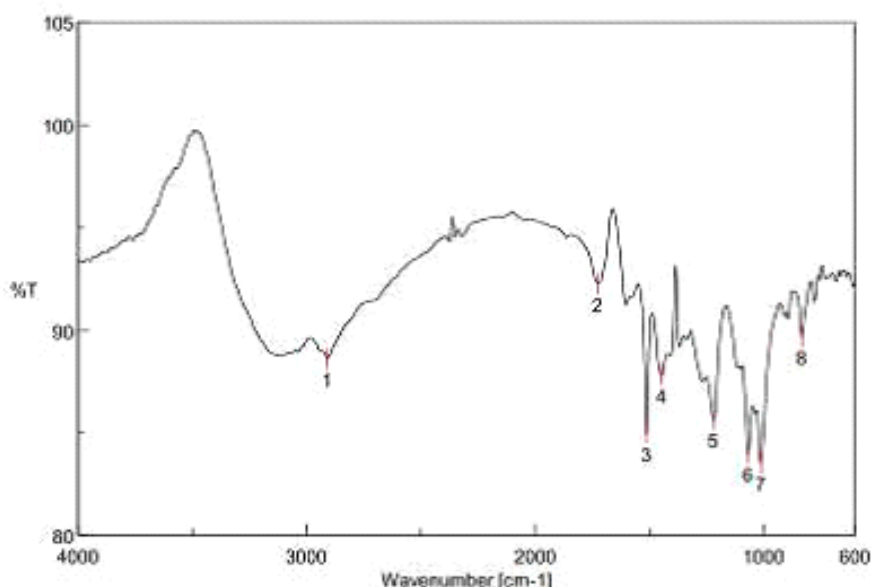


Figure 2: IR spectrum of the methanolic extract of *M. hortensis* leaves.

DISCUSSION

TLC Analysis: Several plant samples have been exposed to TLC as a measure to identify the distinct bands of the various phytochemical fractions present. Similar work was carried out with fractions of *Terminalia chebula*, *Emblica officinalis* and *Piper nigrum* showed presence of a number of polyvalent phytoconstituents [7]. Not only leaves but also the stem bark of *Catunaregam spinosa* possessed various phytoconstituents with different R_f values at various wavelengths which was a result of the TLC [8]. Another study was conducted with the root samples of

Methanol extract of *Pandanus odoratissimus* root which showed the presence of polyphenols [9]. Hence TLC gives a quality evaluation of the different bands that can be obtained from a specified compound which sprayed with the respective dye or reagents.

HPLC Analysis: HPLC though an expensive and higher end analysis for the identification of the phytochemicals present is a reliable technique. Few examples of studies which confirmed the type of secondary metabolites are as follows: *Atropa belladonna* and *Atropa acuminata* contains different types of

alkaloids as determined by HPLC [10]. In another study thirteen samples of *Cordyceps sinensis* and its similar products were determined with the help of HPLC [11]. More than 10 common peaks were derived [12] with HPLC fingerprint with cordyceps from different areas by HPLC fingerprint. The RP-HPLC spectrum of *M. hortensis* carried out in the UV range showed 5 peaks indicating the presence of 5 different active components.

IR spectral Analysis: Fourier Transform Infra Red (FT-IR) spectroscopy is a chemical analytical technique, which measures infrared intensity versus wavelength of light. It detects the vibration characteristics of chemical functional groups in a sample [13]. The IR spectrum of *M. hortensis* leaf extract was indicative of the presence of several carboxyl, hydroxyl and ester groups, which may be attributed to the presence of phenols and saponins. The aqueous extract of *Cacumen platycladi* leaf when subjected to IR spectroscopy was found to contain C=O and C-O groups in abundance which was useful for capping nanoparticles [14]. *Acorus calamus* Linn. extract when exposed to FT-IR analysis showed the presence of several functional groups [15] which further indicates that this technique is used for identification of the functional groups accurately.

CONCLUSION

Methanolic extract of the *M. hortensis* leaves proves to be a strong candidate for antioxidant activities proved by spectral studies like TLC, HPLC and IR. Although it has been proved to contain important phytochemicals like alkaloids, phenols, flavonoids, saponins, steroids and tannins, it can be concluded that the saponin component forms steep curve and could contain a saponin compound which is characteristic of the antioxidant activity compared to the rest of the secondary metabolites. Hence, *M. hortensis* leaves can be used as a rich source of antioxidant

in serval edible preparations or as supplements to enrich the antioxidant diet.

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