

Microscopical characters, phytochemical, and antioxidant screening of hydroalcoholic extract of *Solanum xanthocarpum* and *Mentha arvensis*

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ABSTRACT

Mentha arvensis belonging to the family of Lamiaceae, originally the plant was imported from Japan and is therefore also known as Japanese Mint. The plant is a common garden herb and is extensively cultivated in Northern India for food seasoning as a household remedy and for its industrial use. The *Solanum xanthocarpum* is popularly known as Kateli, Kandakarichunda, Kandankattiri, and Indian *Solanum*. It has sharp and prickly branches that are densely covered with rather a minute star shaped hair. The sparsely hairy egg-shaped leaves, purple colored flowers, and round fruits. The fruit also has smooth seeds. The aim of the present study was microscopic characters of *M. arvensis* and *S. xanthocarpum* leaves, hydroalcoholic extract of the both leaves was used to qualitative screening analyses by color reaction-based identification of active constituents, to determine the total phenolic content, flavonoid content, and antioxidant potential by di-phenyl picrylhydrazyl method.

KEY WORDS: Antioxidant, flavonoid, *Mentha arvensis*, phenolic content, *Solanum xanthocarpum*

INTRODUCTION

Nature of *Mentha arvensis*

This plant is related to the menthe genus, and it is aromatic herbs. Many more species grow wild; some of these cultivated. The chief constituents for which these plants are valued are menthol and peppermint oil. This medicinal plant is an erect, branched herb up to about 55 cm high. The leaves were up to 6 cm long. Flowers small, in small brunches, borne on axile leaves, "medicinal seeds" are also very useful.

Description

M. arvensis is an herbaceous perennial herb that grows to a height of 10-60 cm. This downy herb has running rootstocks and a rigid branching stem. The lanceolate, oblong leaves are simple and sharply toothed, paired in opposites with minute hair. They are 2-6.5 cm in length and 1-2 cm in width. The pale purple flowers (sometimes white or pink) are found in clusters at the stem where each flower measures 2-4 mm in length. The plant is native to the temperate regions of Europe, Western, and Central Asia (eastern Siberia and east of the Himalayas) and North America. It is commonly known as Pudina in Hindi (Blamey and Grey-Wilson, 1989; Huxley, 1992).

Plant Chemicals

Chemical substances that can be extracted from wild mint include menthol, menthone, isomenthone, neomenthol, limonene, methyl acetate, piperitone, beta-caryophyllene, alpha-pinene, beta-pinene, tannins, and flavonoids (Kostka-Rokosz *et al.*, 2015).

Uses of Pudina

Wild mint is often used as a domestic herbal remedy, being valued especially for its antiseptic properties and its beneficial effect on the digestion. Like other members of the genus, it should not be used by pregnant women because large doses can cause an abortion. The whole plant is anesthetic, antispasmodic, antiseptic, aromatic, and has agents that counteract inflammation, that relieve and remove gas from the digestive system, induce sweating, promote or assist the flow of menstrual fluid, promote secretion of milk, relieve fever and thirst, give strength and tone to the stomach, and is a stimulant.

North American Indians made a cold infusion of the plant as a lotion for fever and influenza. A compound infusion was taken, and poultice was applied to the chest for pneumonia.

A decoction of plant parts was taken for stomach pain, colds, swellings, headaches, diarrhea, and fevers. Dried leaves were chewed and swallowed for chest pains and heart ailments. Fresh leaves were put in the nostrils for colds. An infusion of leaves and streams was taken for vomiting, colds, pains, swellings, fevers, headaches, to prevent influenza for stomach troubles, and indigestion. Leaves were used for carious teeth and in the sweat bath for rheumatism. A poultice of crushed leaves was applied to swellings to the gums for toothaches, to areas of pain swellings, for rheumatism and arthritis, and for eye trouble (Khalsa and Tierra, 2008; Kostka-Rokosz *et al.*, 2015).

Nature of *Solanum xanthocarpum*

S. xanthocarpum (Solanaceae) is a perennial herb and is considered as one of the most useful traditional medicines in India. The plant medicinally used to treat for a cough, asthma, and rheumatism. Phytochemical investigation of the *S. xanthocarpum* reported to have number of alkaloids, (Siddiqui and Faizi, 1983) sterols (Manjunath and Shadaksharaswamy, 1942), saponins and flavonoids (Kusano *et al.*, 1973), and their glycosides; and, especially it has high concentration of solasodine, a starting material for the synthesis of cortisone and sex hormones. Pharmacological activities such as antibacterial and antifungal, antinociceptive, antioxidant, hypoglycemic, and larvicidal have been reported in this plant (Tupkari *et al.*, 1972; Debey and Gupta, 1936).

Major Chemical Constitutes

B-Carotene, Disgenin, Carpesterol, Solasodine, Solamargine, B-Solamargine, Solanine, Solasodino-L-Rhamnosy-B-D-Glucoside (Solasurine), Solanocarpine (Solanine-S), Tomatidienol, etc.

MATERIALS AND METHODS

Collection and Authentication of Plant Materials

The plant was of *M. arvensis* L. and *S. xanthocarpum* Burm. F. was collected from Mooligai Pannai, 7 km away from Thanjavur (Tamil Nadu) in the month of August 2009. The plants was identified by local people of that village and authenticated by Dr. M. Jegadeesan, Professor and Head, Department of Environment and Herbal Science, Tamil University, Thanjavur, The Voucher Specimen (TUH273, and TUH 219) is preserved in laboratory for future reference.

Microscopical Examination of Plant Species

Microscopical examination and characterization of plant drugs have always been accorded due to credentials in

the pharmacognostic studies. Anatomical examination of plant drugs may not apparently bear any direct correlation with the pharmacological and phytochemical evaluations. This contention has made many investigators to ask “why anatomical parameters in the study of plant drugs?” one should always remember that botanical identity of the plant drug is an essential prerequisite for undertaking the analysis of medicinal properties of any plant. A researcher may succeed in getting a new compound or may find many pharmacologically active properties in a drug. If the botanical identity of the drug happens to be dubious or erratic, the entire work on the plant becomes invalid. Thus, it is needless to stress that botanical identity of a crude drug is the threshold in the process of pharmacological investigations. The researcher should be equipped with all possible diagnostic parameters of the plant on which the researcher plans to work.

Leaves were collected were fixed in the field immediately after collections. The fixative used was formalin:acetic acid:ethyl alcohol (FAA). The materials were cut into small pieces before fixing. The materials were left in FAA for more than 2 days. Dehydration was carried out employing graded stages of tertiary butyl alcohol and ethyl alcohol mixture as per the schedule given as Sass, 1940.

After dehydration, paraffin infiltration was carried out till supersaturation of tertiary butyl alcohol was achieved. Following supersaturation and materials were transferred to pure paraffin wax for two times, and the materials were cast into paraffin blocks.

Microtoming

Wax embedded materials were sectioned with the help of rotary microtome to the thickness of 10-12 μ m. Sections were stained with toluidine blue (0.25% having a pH of 4.7) as per the schedule given O'Brien *et al.* (1964). Since the stain has metachromatic property, different colors of the cells were obtained depending upon the chemical nature of the cells.

Collection of Plant Extracts

The different ratio of the cold macerated hydroalcoholic standardized dry extract of *S. xanthocarpum* (8:1), (Batch number-7579) *M. arvensis* (3:1), (Batch Number-6086), was collected from AMSAR private Limited Indore, India.

Phytochemical Screening of Hydro Alcoholic Extracts (Trease and Evans, 1996)

Phytochemical analysis of the collected two plants hydroalcoholic extracts was performed by the following

preliminary phytochemical screening methods and the phytoconstituents is reported in Table 1.

Estimation of Total Phenol Content of Hydro Alcoholic Extracts (Singleton and Rossi, 1965)

Total phenols were determined by the method of Singleton and Rossi 25 using the Folin-Ciocalteu reagent. An aliquot (0.25 ml) of hydroalcoholic extract was added to 3.5 ml of distilled water in a screw – capped test tube, followed by 0.5 ml of Folin-ciocalteu solution. After 3 min, 1 ml of 1% sodium carbonate was added, and the contents of the tube were thoroughly mixed before being incubated in a boiling water bath for 1 min. The tube was allowed to cool in the dark. The absorbance of the blue color that developed was read at 685 nm using gallic acid as standard. The results were expressed in mg gallic acid g⁻¹ fresh weight. The report is shown in Table 2.

Estimation of Total Flavonoids (TFs) of Hydro Alcoholic Extracts

The TF content was determined using a reagent containing aluminum chloride and sodium nitrite, giving rise to a pink-colored flavonoid-aluminum complex in the alkaline medium followed by the method of Zhishen *et al.* (1999). A solution corresponding to 30 µl of sodium nitrite (10%), 60 µl of aluminum chloride hexahydrate (20%), 200 µl of sodium hydroxide (1 M), and 400 µl of water was added to 100 µl sample. The absorbance readings at 510 nm were started 5 min after the addition of the sample and performed. Reagent blank is containing water instead of the sample was used. The final absorbance of each sample was compared with a standard curve made from catechin (69-689 µmol/L). The data were expressed as µmol catechin equivalents per gram of dry matter. The report is given in Table 2.

Antioxidant Activities of Hydro Alcoholic Extracts of *M. arvensis*, *S. xanthocarpum*

Instrument Name: Shimadzu: UVVisible Spectrophotometer (UV-1800)

Standard Chemicals used: Butylated hydroxytoluene (BHT)
Di-phenyl picrylhydrazyl (DPPH)

Method: DPPH (Blois, 1958)

The DPPH radical scavenging activity of the hydroalcoholic plant extracts was estimated according to the method of Blois (1958). After mixing 0.1 ml of plants extracts with 0.9 ml of 0.041 mM DPPH in ethanol for 10 min, the absorbance of the sample was measured at 517 nm. Radical scavenging activity was expressed as percent inhibition and was calculated using the following formula. The report is shown in Table 3.

Table 1: Preliminary phytochemical screening of the hydroalcoholic extracts of *Mentha arvensis* and *Solanum xanthocarpum*

Phytoconstituents	<i>Mentha arvensis</i>	<i>Solanum xanthocarpum</i>
Alkaloids	—	+
Aminoacids	—	—
Anthraquinones	—	—
Carbohydrates	—	—
Catechins	—	—
Flavonoids	+	+
Phenolic groups	+	+
Resins/gums	+	-
Saponins	—	—
Steroids	—	—
Tannins	+	+
Triterpenes	+	+

+: Present —: Absent

Table 2: Estimation of total phenolic content and total flavonoids in hydroalcoholic extracts of *Mentha arvensis* and *Solanum xanthocarpum*

Species	Total phenolic content ^a	Total flavonoids ^b
<i>Mentha arvensis</i>	936±71	479±66
<i>Solanum xanthocarpum</i>	463±38	214±49

Values are mean±standard deviation (n=3); ^aµg gallic acid g⁻¹ equivalent weight; ^bµg quercetin g⁻¹ equivalent weight

Table 3: Antioxidant activities of hydro alcoholic extracts of *Mentha arvensis* and *Solanum xanthocarpum*

Sample	Abs at 517 nm	Antioxidant activity (%)
DPPH-control	1.274	-
<i>Mentha arvensis</i> (100 mg/ml)	0.111	91.28
<i>Solanum xanthocarpum</i> (100 mg/ml)	0.093	92.70
BHT (0.1 mg/ml)	1.206	5.33

Scavenging activity (%) = $\{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}) / \text{Abs}_{\text{control}}\} \times 100$.
BHT: Butylated hydroxytoluene

%DPPH radical scavenging activity = $(1 - \text{sample OD} / \text{Control OD}) \times 100$

RESULTS AND DISCUSSION

S. xanthocarpum (Solanaceae)

Morphology

Armed, diffuse subshrub, prickles many, and straight. Leaves: lacerate, chartaceous, prickly along, base, attenuate, apex acute; flowers: stalked, aggregated in extra-axillary; cymes calyx: 5 lobes, persistent, corolla purple color, five lobes, broadly ovate-triangular, acute. Stamens five, attached at the mouth of the short tube, anthers linear-oblong; ovary globose, stigma acute, incurved; fruit berry, globose yellow or white with green blotches, seeds smooth, and circular.

Anatomy of the leaf

The midrib is hemispherical on the adaxial side and widely semicircular on the abaxial side. The midrib measures

950 μm in the vertical plane. The palisade tissue of the lamina extends horizontally up to shoulders of the midrib on the adaxial part [Figure 1a]. Both the abaxial and adaxial epidermal cells are single layered; the cells are smaller, cubical to squarish in shape. The epidermis is single layered followed by two or three layers of compact collenchyma cells. The ground tissue (GT) of the midrib is parenchymatous. The parenchyma cells are thin-walled, compact, and polygonal in shape. The vascular strand is in single, bowl-shaped, wide, and collateral. Xylem elements are arranged in vertical files and are surrounded by a thin band of phloem elements both on the upper and lower sides (Bicollateral).

Lamina

The lamina is 260 μm thick. The adaxial and abaxial epidermis are single layered; the epidermal cells are squarish to rectangular; the cuticle is thin and smooth. The mesophyll is differentiated into palisade and spongy parenchyma cells [Figures 1b and 2b] the palisade tissue consists of cylindrical elongated at right angles to the epidermis and arranged as a row of stakes and occupy one-third of the thickness of the lamina. Spongy mesophyll (SM) tissue is five or six-layered, spherical, or lobed with a conspicuous intercellular space. The system is permeating the tissue [Figure 2c].

Main lateral vascular bundle is similar structure to the midrib [Figure 2a] smallest lateral Vein bundle occur in the median part of the mesophyll, beneath the palisade cells, that is, in the uppermost layer of the spongy parenchyma [Figure 2c].

Calcium oxalate crystals are present in the leaf; they are distributed in the lamina, Sand crystals are present in the SM region [Figure 2b].

M. arvensis Linn (Labiatae)

Morphology

Aromatic herbs by running rootstocks; stems prostrate or ascending; pubescent. The leaves are short-petioled, ovate to broadly lanceolate, weakly toothed, and acute. The flowers are in axillary and dense cymes. Calyx short, campanulate, pubescent, and resin-dotted. Corolla is mostly lilac, lobes five, and statements four.

Anatomy of the leaf

Young leaves have small abaxially projecting midrib and deeply curved arc-shaped lamina [Figure 3a]. Mature leaves have thick, circular midrib with lateral wing like lamina [Figure 4a]. The midrib is 450 μm in vertical plane and 500 μm in horizontal plane. The midrib has a distinct epidermal layer

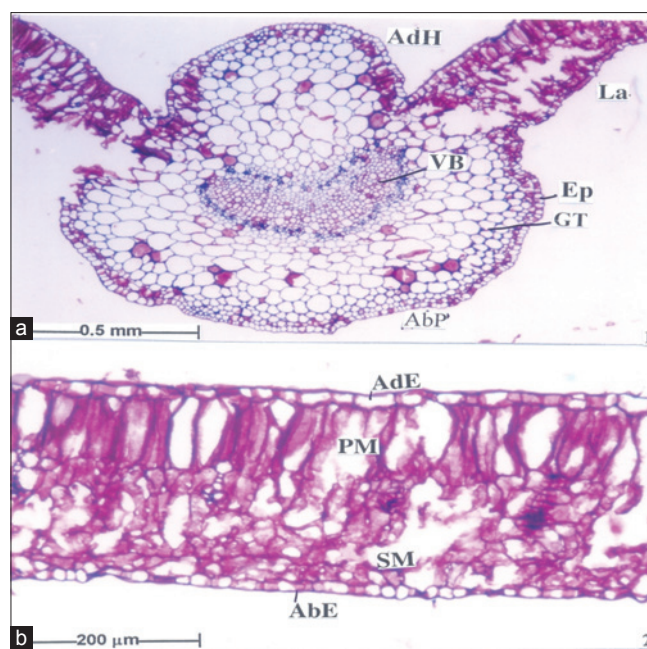


Figure 1: (a) Transverse section (TS) of leaf through lateral vein with lamina (*Solanum xanthocarpum*) midrib. (b) TS of lamina. AbE: Abaxial epidermis, AdE: Adaxial epidermis, AdH: Adaxial hump, Ep: Epidermis, Abp: Abaxial part, GT: Ground tissue, La: Lamina, PM: Palisade mesophyll, SM: Spongy mesophyll, VB: Vascular bundle

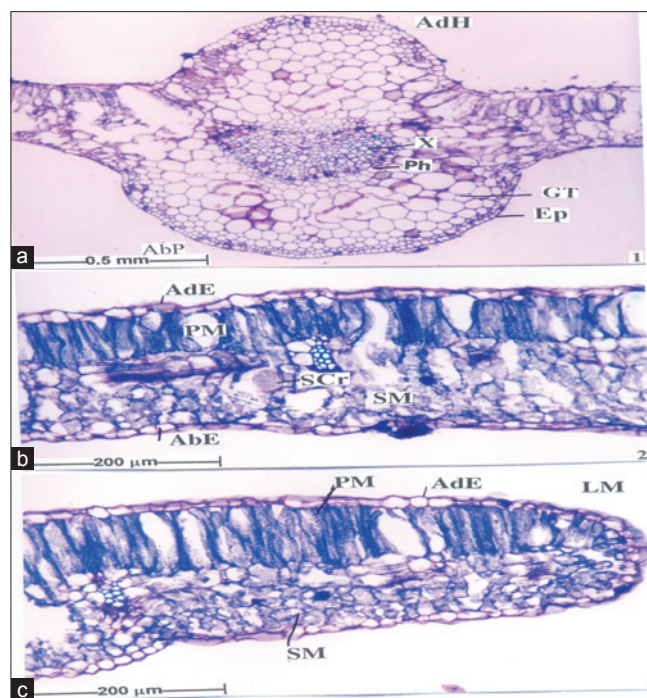


Figure 2: (a) Transverse section (TS) of leaf through lateral vein with lamina (*Solanum xanthocarpum*). (b) TS of lamina showing sand crystal in the mesophyll tissue. (c) TS of LM. AbE: Abaxial epidermis, AbP: Abaxial part, AdE: Adaxial epidermis, AdH: Adaxial hump, Ep: Epidermis, GT: Ground tissue, LM: Leaf margin, ph: Phloem, PM: Palisade mesophyll, Scr: Sand crystal, SM: Spongy mesophyll, X: Xylem

of small thick walled squarish cells. The GT has fairly large; circular thin-walled compact parenchyma cells [Figure 4a].

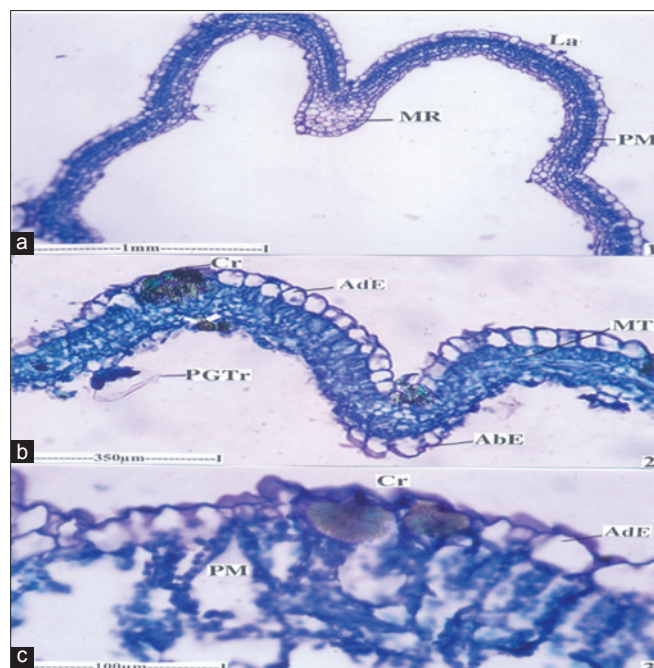


Figure 3: (a) Transverse section (TS) of leaf through midrib with foled lamina. (b) TS of lamina with peltate glandular trichome. (c) Pairs of calcium oxalate crystals on the adaxial epidermis. AbE: Abaxial epidermis, AdE: Adaxial epidermis, Cr: Crystal, La: Lamina, MR: Midrib, MT: Mesophyll tissue, PGTr: Peltate glandular trichome, PM: Palisade mesophyll

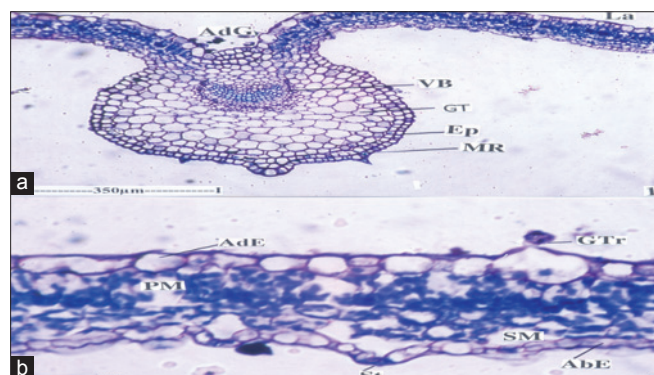


Figure 4: (a) Transverse section (TS) of the mature leaf through midrib with the lamina (*Mentha arvensis*). (b) TS of Lamina. AbE: Abaxial epidermis, AdE: Adaxial epidermis, AdG: Adaxial groove, Ep: Epidermis, GT: Ground tissue, GTr: Glandular trichome, La: Lamina, MR: Midrib, PM: Palisade mesophyll, SM: Spongy mesophyll, St: Stoma, VB: Vascular bundle

The vascular strand is single and semicircular with compact parallel lines of xylem elements and the thin arc of phloem elements.

The lamina is 80 µm thick. It is dorsiventral with an adaxial narrow band of palisade cells and four or five layers of spongy parenchyma [Figure 4b]. Stomata are on the abaxial side.

The young leaves possess abundant glandular trichomes (GTr) on the epidermal cells. The GTr is sessile peltate

glands with a short stalk and wide semicircular head [Figure 3b].

Some of the epidermal cells of the adaxial side are dilated and possess a spherical mass of fine needles (sphaerites) occupying the entire space of the dilated cell. These structures seem to calcium oxalate crystals. The crystals bearing idioblast usually occur in pairs [Figure 3b and c].

Hydro alcoholic extracts of *M. arvensis*, *S. xanthocarpum* were qualitatively screened and are presented in Table 1. Triterpenes, Phenolic groups, and flavonoids were present in both extracts; Alkaloids are particularly present in Hydro alcoholic extract of *S. xanthocarpum*. Tannins mainly present in the *M. arvensis* and *S. xanthocarpum* dry extracts.

CONCLUSION

The microscopical characters of the *M. arvensis* and *S. xanthocarpum* leaves were evaluated by transverse section techniques. The phytochemical analysis was carried out the hydroalcoholic dry extracts of both the plants such as *M. arvensis* and *S. xanthocarpum*. Particularly alkaloids present in the leaf of *S. xanthocarpum* dry extract. Flavonoids, phenolic groups, triterpenes, and tannins present in both extracts. The total Phenolic and flavonoid content of the *M. arvensis* and *S. xanthocarpum* extract values by determined by spectrophotometrically. The antioxidant activity was done by DPPH method and reported.

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