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Extraction, Isolation, Preliminary Phytochemical Screening and Chromatography of Marrubium Vulgare Linn

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ABSTRACT
Plants possess several secondary metabolites for their defense. Crude extract of aerial parts i.e. leaves of Marrubium vulgare contain flavonoids, saponins, triterpenoids and essentials oils. This plant is aromatic in nature due to the presence of essential oils. These oils are used as bio-pesticidal agent and for fragrance. In the present study, extracts of powdered material of leaves were isolated in methanol and water solvent by using soxhletion extraction methods and percentage yield of the extract were calculated that is found 11.89% in methanol and 10.42% in distilled water w/w with respect to dried powder. Preliminary phytochemical screening of the extract was done for the confirmation of phytoconstituents present in the extract. Thin layer and column chromatography of the extract was also done by using Methanol: Dichloromethane solvent system in different ratio i.e. 95: 05 and 80:20 ratios, respectively and two fraction of green and yellowish color were obtained with RF value 0.142 and 0.857, respectively.

Key-words – Marrubium vulgare, phytochemical analysis, percentage yield.

I INTRODUCTION

Marrubium vulgare L. is commonly known as White horehound. It is a flowering plant in the family Lamiaceae. Horehound serves as raw material for herbal extracts and beverages industries. The plant has also been used as a substitute for hop in beer-breweries. It can be used as ingredients of cough pastilles. The plant is used for traditional medicine, because of its stimulating action on the flow of bile and gastric actions and it is a laxative, a purgative and a cough soother. It is native to Europe, northern Africa, and southwestern and central Asia. It is also widely naturalized in many places, including most of North and South America. It is a grey-leaved herbaceous perennial plant, somewhat resembling mint in appearance and grows to 25-45 centimeters in height. The leaves are 2-5 centimeters long with a densely crinkled surface and are covered in downy hairs. The flowers are white, borne in clusters on the upper part of the main stem. Several modern scientific studies have been conducted on the usefulness of horehound. A study concluded that the essential oil of Marrubium vulgare contains potent anti-microbial and anticancer properties.[1] while another study has found Marrubium vulgare as one of the primary active compounds to possess anti-diabetic, anti-atherogenic and anti-inflammatory properties.[2] Phytochemically, Marrubium vulgare is characterized by the presence of a variety of compounds such as alkaloids, steroids, lactones, tannins and flavonoids. Hence, the present study was proposed to explore phytochemical analysis of the extract of Marrubium vulgare.

II EXPERIMENTAL

(a) Extraction and Isolation

Fresh leaves (400gm) of Marrubium vulgare was collected from surrounding areas of Salamatpur Bridge in Raisen district (M.P.), India and washed thoroughly with tap water. It was identified and authenticated by Dr. P.N. Shrivastava (Taxonomist), S.S.L. Jain P.G. College, Vidisha. A voucher specimen was procured which was deposited in Department of Botany, St. Mary P.G. College, Vidisha (M.P.). Plant materials were dried under shade at room temperature in to the laboratory and after drying, it was found 50% less in amount. The obtained materials were finely grinded using electrical mixer grinder and stored in air tight container for further use. A total of 200 gm of the pulverized plant material was extracted in methanol and distilled water through cold percolation technique for 4 days. Separated extracts were then filtered through Whatman's No. 1 filter paper and the filtrate was then separately condensed to dryness using rotary evaporator. The solvent was then removed under reduced pressure and the percentage yield was obtained in methanol (11.89%) and distilled water (10.42%) w/w with respect to dried powder (Table 1). Dried plant extract was collected in air tight containers and stored at 4°C for further analysis. Moreover, extract of methanol and distilled water were subjected separately to phytochemical study for the identification and presence of different phytoconstituents including flavonoids, phenols, triterpenoids, saponins and lipids.[3, 4]

(b) Phytochemical screening methods

In the present study, the presence of different phytoconstituents viz. alkaloids, carbohydrates, glycosides, flavonoids, tannins, phenolic compounds and terpenoid in the extract of Marrubium vulgare were determined, following standard procedure of Peach and Tracy[5] (Table 2).

(i) Detection of carbohydrates: Extract was dissolved individually in 5 ml distilled water and filtered. Filtrate was treated with 2 drops of alcoholic α-naphthol solution in a test tube and concentrated Sulphuric acid was added but formation of the violet ring at the junction was not seen which indicates the absence of carbohydrates.

(ii) Detection of glycosides: Extracts were hydrolyzed with diluted HCl and then subjected to test for glycosides by treated with sodium nitroprusside in pyridine and sodium hydroxide. Formation of pink to blood red color does not see which indicates the absence of cardiac glycosides.

(iii) Detection of Lipids: Extracts was treated with chloroform and filtered. The filtrate was treated with few drops of acetic anhydride, boiled and cooled. Concentrated Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of lipids.

(iv) Test for saponins: Extract (300 mg) was boiled with 5 ml water for two minutes; the mixture was cooled and mixed vigorously and left for three minutes. The formation of frothing indicates the presence of saponin.

(v) Test for tannins: To an aliquot of the extract (dissolved in water) 2 ml of sodium chloride (2%) was added, filtered and mixed with 5 ml 1% gelatin solution. Precipitation indicates the presence of tannins.

(vi) Test for Triterpenes: Extract (300 mg) was mixed with 5 ml chloroform and warmed for 30 minutes. Few drops of concentrated Sulphuric acid were added and mixed well. The appearance of red color indicates the presence of triterpenes.

(vii) Test for alkaloids: Extract (300 mg) was digested with 2 M HCl and the acidic filtrate was mixed with amyl alcohol at room temperature. Pink colour of the alcoholic layer seen which indicates the presence of alkaloids.

(viii) Test for flavonoids: The presence of flavonoids was determined by using 1% aluminum chloride solution in methanol, concentrated HCl, magnesium and potassium hydroxide solution.

(c) Thin layer and column chromatography

Chromatography means color or to write which is originally described by Tswett.[6] Two types of chromatography were used in the present study viz. TLC and Column. Thin layer chromatography technique was used to determine the number of constituents in the plant extract and to analyze the fractions. In thin-layer chromatography, silica was used as stationary phase. This absorbent was coated on a glass slide creating a thin layer of the silica. Methanol: Dichloromethane (95:05) solvent system was used as the mobile phase. TLC provides a chromatographic measurement known as Rf value. The Rf value was calculated by the formula of Brimley and Barrett[7] as distance travelled by solutes divided by distance travelled by solvents. This value can be calculated for each spot observed on a TLC plate shown in Table (3). According to Stock and Rice[8] column was used as a glass cylindrical container and a sample solution that is the substance to be purified, is poured into the column (Table 4). In the present study, silica gel was used as a stationary phase which was poured in to the column then Methanol: Dichloromethane (80:20) solvent system was used as mobile phase and was run two three times for maintaining its flow by removing air bubbles entered during packaging of silica. Then loaded herbal extracts sample on the top of the column. Then, obtained fractions were separated on the basis of their color characterization and were kept in separate vials for further analysis (Table 4).

III RESULTS AND DISCUSSION

In the present study, the good percentage yield of the extract of Marrubium vulgare was found in methanol (11.89%), followed by distilled water (10.2%), respectively. Almost similar observations have been reported by Elberry et al. (2015),[9] who have isolated successive extract in methanol from the leaves of Marrubium vulgare with a very good percentage yield (12%).

The presence of different phytoconstituents viz. alkaloids, carbohydrates, glycosides, flavonoids, tannins, phenolic compounds and terpenoid in the methanolic extract of Marrubium vulgare were confirmed by following standard procedure of Peach and Tracy[5]. Similarly, a preliminary phytochemical test to identify the chemical

constituents of Marrubium vulgare was carried out according to the methods of Trease and Evans.[10] In the present study, the phytochemical screening of the Marrubium vulgare extract was done for the confirmation of secondary metabolites viz. Flavonoids, saponins, triterpenoids, taanins, alkaloids and lipids and the absence of carbohydrates and glycosides was confirmed. These secondary metabolites are secreted by the plants for their defense which are being used by the peoples for various purposes.

In the present study, thin layer chromatography of the methanolic extract of Marrubium vulgare was done by using Methanol: Dichloromethane (95:05) solvent system and two spots of green and yellowish color with Rf value 0.142 and 0.857, respectively, were obtained. Similarly, the methanolic extract of Marrubium vulgare was analyzed using thin layer chromatography as reported by Wagner and Bladt.[11] TLC on silica gel 60 F 254 plates using chloroform: Methanol (95:5) as a solvent system was used for the identification of terpenoid class compounds after spraying with Komarowsky reagent. The extract was also chromatographed on TLC using ethyl acetate: formic acid: acetic acid: water (100:11:11: 26). TLC was observed under UV 254 and 366 nm, before and after spraying with natural products reagent for the detections of flavonoid class compound. Thus, it was confirmed that terpenoid and flavonoid are most important chemical constituents found in methanolic extract of Marrubium vulgare.

Table 1
Extraction and Isolation of leaves of Marrubium vulgare Linn by cold percolation.

Plant parts used	Wt. of fresh leaves	Wt. of Shade dried leaves	Solvent used	Percentage yield
Marrubium vulgare Leaves	400 gm	200gm	Methanol	11.89%
			Water	10.42%

Table 2
Preliminary Phytochemical screening leaves extract of Marrubium vulgare Linn.

S. No.	Test applied on the extract	Methanol	Distilled water
1	Carbohydrates	–	–
2	Glycosides	–	–
3	Lipids	+	+
4	Saponins	+	+
5	Tannins	+	+
6	Triterpenes	+	+
7	Alkaloids	+	+
8	Flavonoids	+	+

+ (Present), – (Absent)

Table 3
Thin layer chromatography of leaves extract of Marrubium vulgare Linn.

Plant extract	Solvent system used	Fractions Obtained	Distance travelled by solvent	Distance travelled by solute	Rf Value
Marrubium vulgare Leaves Extract	Methanol: Dichloromethane (95:05)	Spot – 1 Spot – 2	7 cm.	1 cm. 6 cm.	0.142 0.857

Table 4
Column chromatography of leaves extract of Marrubium vulgare Linn.

Plant extract	Solvent system used	Fractions Obtained	Color characteristics
Marrubium vulgare Leaves Extract	Methanol: Dichloromethane (80:20)	1 2	Greenish Yellow

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