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Preliminary Phytochemical Screening of Root Extracts of *Myxopyrum smilacifolium* Blume

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Abstract

Preliminary phytochemical screening is the key step for finding the chemical constituents which leads to the isolation of lead compounds of medicinal importance. *Myxopyrum smilacifolium* is a large woody climbing shrub belonging to the family Oleaceae. Its root, stem, leaves are of much medicinally active and is employed in many traditional systems of medicines. The main focus of this study is to identify and understand the bioactive chemical constituents of the root extracts by subjecting the root powder to soxhlet extraction in different solvent systems. The yield of each extract was calculated and it was found to be more in methanolic extract. The phytochemical screening showed the presence of alkaloids, phenolics, glycosides, tannins, flavonoids etc which contribute several pharmaceutical applications to humans.

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INTRODUCTION

Since ancient times, people have been exploring the nature particularly plants in search of new drugs. This has resulted in the use of large number of medicinal plants with curative properties to treat various diseases(1). Nearly 80% of the world's population relies on traditional medicines for primary health care, most of which involve the use of plant extracts(2). Knowledge of the chemical constituents of plants is desirable because such information will be of value for the synthesis of complex chemical substances. The study of plants continues principally for the discovery of novel secondary metabolites which possess many pharmacological properties.

In order to promote Indian herbal drugs, there is an urgent need to evaluate the therapeutic potentials of the drugs as per WHO guidelines(3). Plant derived chemicals are a promise for it to be used as the active ingredients of modern medicine or as the lead compounds for new drug discovery.

The preliminary step in any phytochemical screening procedure is the extraction. It involves extracting medicinally active portions of plant tissues using selective solvents through standard procedures. The products so obtained from plants are relatively complex mixtures of metabolites, in liquid or semisolid state or in dry powder form, and are intended for pharmacological applications. During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity(4).

Myxopyrum smilacifolium (Oleaceae) is a woody climbing shrub belonging to the family Oleaceae. The genus Myxopyrum consists of four species distributed in the tropical and subtropical regions of East Asia. Its root, stem, leaves are of much medicinally active candidates. In many traditional systems of medicines this plant extract has been employed. The present study was undertaken for identifying the phytochemicals present in the root extract of M.smilacifolium with suitable solvents such as Benzene, Ethyl acetate, Methanol, Ethanol and water.

MATERIALS AND METHODS

Collection of Plant: Fresh plant roots of *M. smilacifolium* were collected from Botanical garden, Dept. of Botany, University of Kerala, Kariavattom. The roots were washed thoroughly with tap water followed by sterile distilled water. Then roots were dried under shaded condition at room temperature. Roots were crushed to coarse powder and were stored at room temperature in air tight container bottles.

Extract preparation: For both aqueous and solvent extractions, 15 g of root powder of *M. smilacifolium* were extracted serially with 100 ml of solvents (Benzene, Ethyl acetate, Methanol Ethanol and aqueous) in increasing order of their polarity and were then taken into the soxhlet apparatus which was run upto 12 hours. After that the extracts were concentrated in rotary evaporator, dried in oven and stored at 4^0C in airtight bottles and were qualitatively tested for the presence of various phytocompounds.

Extractive Value: The extractive value of root powder in all the five solvent system was calculated using the formula

Extractive Value = (Weight of extract in gm)/(Weight of sample in gm)× 100

Phytochemical Screening

The qualitative phytochemical screening for the five root extracts was done according to Sofowara, (1993) (5), Trease & Evans (1989) (6) and Harborne (1998) (7).

1) **Detection of acids**

To 1ml of plant extract, 0.5 ml sodium bicarbonate was added. Formation of effervescence indicates presence of acids.

2) **Detection of carbohydrates**

Fehling's test: 1ml of extract boiled on water bath with 1ml each of Fehling's solution A and B. The colour change observed. Red precipitate indicates presence of sugars.

Barfoed's test: To 1ml of extract, 1ml of Barfoed's reagent added and heated on boiling water bath for 2 min. The colour change noted and recorded. A red precipitate indicate presence of sugars

Benedict's test: To 0.5 ml of the extract, 0.5 ml of Benedict's reagent added. Mixture heated on boiling water bath for 2 min and results observed. Red precipitate indicates the presence of sugars.

3) Detection of proteins

The extract was filtered through Whatmann No.1 filter paper and the filtrate was subjected to test for proteins

Biuret test: To 2ml of test solution add 2ml of 10% sodium hydroxide. Mix well. Add 2 drops of 0.1% copper sulphate solution. Pink colour in ethanol layer indicated the presence of proteins,

Xanthoproteic test: To 5ml of test solution, add 1ml Concentrated nitric acid. Boil the contents. After cooling add excess 40% sodium hydroxide. On adding acid, yellow colour is noticed. When sodium hydroxide added deep orange colour develops.

4) Detection of Anthraquinones

Few drops of 2% hydrochloric acid added to 0.5 ml of root extract. Appearance of red colour indicates presence of anthraquinones.

5) Detection of Steroids and Phytosteroids

To 0.5 ml of the plant extract add equal volume of chloroform and add few drops of concentrated sulphuric acid. Appearance of brown ring indicates the presence of steroids and bluish ring indicate the presence of phytosteroids.

6) Detection of Cardiac glycosides

2 ml glacial acetic acid and few drops of 5% ferric chloride added to 0.5% of the extract. This was undertaken with 1 ml concentrated sulphuric acid. Formation of brown ring at the interface indicates the presence of cardiac glycosides.

7) Detection of Saponins

2 ml distilled water added to plant extract and shaken for 15 minutes. Formation of 1 cm foam indicates the presence of saponins.

8) Detection of gum and mucilage

Extract dissolved in 5 ml of distilled water and to this added 25 ml of absolute alcohol and with contact stirring. White or cloudy precipitate indicates presence of gums and mucilage.

9) Detection of fat

Saponification test: Few drops of 0.5 N alcoholic potassium hydroxide solution added to small quantity of extract along with drops of phenolphthalein. Mixture heated on water bath for 2 hour. Formation of soap or partial neutralization of alkali indicated presence of oils and fats.

10) Detection of Alkaloids

About 50 mg of solvent free extract stirred with 3 ml of dilute hydrochloric acid and then filtered thoroughly. Then filtrate was tested carefully with various alkaloid reagents as follows.

Mayer's test: To 1 ml of filtrate, few drops of Mayer's reagent added by the side of the test tube. The white or creamy precipitate indicates the test positive.

Dragendorff's test: To 1 ml of filtrate, 2 ml of Dragendorff's reagent added and result observed carefully. A prominent yellow precipitate confirm test as positive.

11) Detection of glycosides

Borntragers test: To a small amount of extract add chloroform and the chloroform layer separated. To this equal quantity of dilute ammonia added. A layer of pink, reddish violet colour indicates the presence of glycosides.

12) Detection of Phenols

Lead acetate test : The extract (5mg) dissolved in distilled water and 3 ml of 10% lead acetate solution added. A bulky white precipitate indicates the presence of phenols.

13) Detection of Flavonoids

An aqueous solution of extract added with ammonium hydroxide solution. The yellow fluorescence indicates the presence of flavonoids.

14) Detection of Phytosterols

Libermann-Buchard test: Extract (5mg) dissolved in 2 ml acetic anhydride and one or two drops of concentrated sulphuric acid was added slowly along the sides of the test tube. Formation of blue green colour indicates the presence of triterpenoids and Phytosterols.

15) Detection of Tannins

To 0.5 ml of extract solution 1 ml of water and 1-2 drops of ferric chloride solution added. Blue colour observed for gallic tannins and green black for catecholic tannins.

RESULTS

Extractive Value and Nature of Extract

The extractive value of root powder in different solvent system showed that the methanolic extract (14.27%) has the maximum yield percentage followed by aqueous (11.73%) and ethanolic (10.93%) extracts. The details are shown in table 1.

Sl.No.	Solvent used for extraction	Physical appearance of the extract	Nature of the extract	Weight of the extract (g) (Mean*)	Yield %
1.	Benzene	Deep brown	Slightly sticky	0.16	0.80
2.	Ethyl acetate	Yellowish	Sticky	0.29	1.93
3.	Methanol	Brown	Highly sticky	2.14	14.27
4.	Ethanol	Reddish	Slightly sticky	1.64	10.93
5.	Aqueous	Reddish brown	Non sticky	1.76	11.73

Table 1: Physical characteristics and yield of five root extracts of *M. smilacifolium*

*Extract weight taken on average of five extractions

Phytochemical Screening

The preliminary phytochemical screening conducted on the root extracts of *M. smilacifolium* revealed the presence of alkaloids, phenols, flavonoids, tannins, glycosides etc as shown in Table 2. Presence of phytoconstituents varied upon different solvent systems.

Phytochemical test	BE	EAE	ME	EE	AE
Acids	+	-	-	-	-
Carbohydrates	+	+	+	+	+
Proteins	+	+	+	+	+
Anthraquinones	-	-	-	-	-
Phytosteroids	-	+	+	-	-
Cardiac glycosides	-	-	+	-	-
Saponins	-	-	-	-	+
Gum & Mucilage	+	-	-	-	-
Fat	-	-	-	-	-
Alkaloids	+	+	+	+	+
Glycosides	-	-	+	+	+
Phenols	-	-	+	+	+
Flavonoids	-	-	+	+	+
Phytosterols	+	-	-	-	-
Tannins	-	+	+	+	+

Table 2: Qualitative phytochemical analysis of *M. smilacifolium* root extracts

BE- Benzene extract, EAE- Ethyl acetate extract, ME- Methanol extract, EE- Ethanol extract, AE- Aqueous extract

Benzene extract showed the presence of acids, carbohydrates, proteins, gums and mucilages, alkaloids and Phytosterols. Ethyl acetate gave positive results for Carbohydrates, proteins, phytosteroids, alkaloids and tannins.

Methanolic extracts gave positive result for the presence of Carbohydrates, proteins, cardiac glycosides, phenols, phytosteroids, phytosterols, alkaloids, glycosides, flavonoids and tannins. However, ethanolic extract showed the presence of carbohydrates, proteins, alkaloids, glycosides, phenols, flavonoids and tannins. The aqueous extract confirms the presence of carbohydrates, proteins, saponins, alkaloids, glycosides, phenols, flavonoids and tannins.

DISCUSSION

The study clearly shows that all the extracts gave positive result for the presence of proteins, carbohydrates, alkaloids. Test for Anthraquinones and fat didn't show positive result for any of the extracts.

Alkaloids are a group of naturally occurring chemical compounds which mostly contain basic nitrogen atoms. It has been reported to have analgesic properties(8). The alkaloids contained in plants could be used in medicine as anaesthetic agents(9). Alkaloids exhibit marked physiological effects when administered to animals and hence their wide use in medicine for development of drugs (10, 11).

Saponins have been traditionally used as detergents, piscicides and molluscicides and also used industrially as foaming agents and have beneficial health effects(12).Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness. It has also been shown that saponins are active antifungal agents (13).Glycosides are known to lower the blood pressure according to many reports (14).Phenolic compounds are of great importance as they have high antioxidant potential which protects human body from oxidative stress, which may lead to diseases including cancer, cardiovascular problems and ageing(15). Natural antioxidant mainly come from plants in the form of phenolic compounds such as flavonoids, phenolic acids etc(16). Flavonoids and tannins are major group of compounds that act as primary antioxidants or free radical scavengers(17).Flavonoids are water soluble phytochemicals which reduce free radicals by quenching, up-regulating or protecting antioxidant defenses and chelating radical intermediate compounds(18). Tannins contribute to the property of astringency i.e. faster healing of wounds and inflamed mucous membrane (19).

For the pharmacological as well as pathological discovery of novel drugs, the essential information's regarding the chemical constituents are generally provided by the qualitative phytochemical screening of plant extracts. In the present study, qualitative tests for all five extract showed significant indication about the presence of metabolites which contribute to the pharmacological activity.

The results obtained in this study thus suggest that the identified phytochemical compounds may be the bioactive constituents responsible for the efficacy of the roots of the plants studied. The presences of some of these compounds have also been confirmed to have antimicrobial, antioxidant as well as anti cancer properties. Hence it could be inferred from the study that the root extracts could be a source for the industrial manufacture of drugs useful in the chemotherapy of some microbial infection. The presence of these phytochemicals could be attributed to the bioactive principles responsible for ethnopharmalological activities of most medicinal plants including the plant under study.

CONCLUSION

Several studies confirmed the presence of these phytochemicals contribute medicinal properties to plants. Therefore, extracts from this plant could be seen as a good source for useful drugs. Preliminary qualitative test is useful in the detection of bioactive principles and subsequently may lead to drug discovery and development. Phytochemical studies revealed that the root extract is rich in many active phytoconstituents which impart physiological response. However, a detailed analysis of the plant material is required inorder to explore the hidden therapeutic potency of this plant. Also, many phytochemical methods should be adopted to isolate, purify, and characterize the active constituents present in this plant which could later become promising for drug development.

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