Int J Pharm Bio Sci 2015 April; 6(2): (P) 67 - 72

Research Article **Pharmacognosy**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

PRELIMINARY PHYTOCHEMICAL INVESTIGATION OF WHOLE PLANT OF *ALYSICARPUS MONILIFER* (L.) DC

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ABSTRACT

To evaluate the phytochemical activity of different extract of aerial parts of *Alysicarpus monilifer* belonging to the family *Fabacea.* The aerial parts were collected and extract prepared from petroleum ether, ethyl acetate and methanol by hot continuous percolation method in a Soxhlet apparatus for 24 hrs. The preliminary phytochemical investigation shows presence of alkaloids, proteins & free amino acids, glycosides, phytosterols, saponins,carbohydrates & free reducing sugars, tannins & phenolic compounds and flavonoids. Each active compound shows different activities against different types of diseases like cancer, liver disorders, diabetes, atherosclerosis and inflammatory diseases etc. According to their characteristics, they can be involved in the medicinal plant category.

KEYWORDS: ***Alysicarpus monilifer*, Petroleum ether, Ethyl acetate, Methanol**

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INTRODUCTION

Medicinal plants are great importance to the health of individuals and communities in general. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body[1].Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. Phytochemicals are primary and secondarymetabolites. Chlorophyll, proteins and common sugars are included in primary constituents and secondary constituents are terpenoid, alkaloids and phenolic compounds[2].About 1500 plants are systematically used in indigenous system of medicine, like Ayurveda, Unani and Siddha. However, the ethnopharmacologists, botanists, microbiologists and natural-product chemists world over today is constantly still in search of medicinal efficacy of plants and their phytochemicals, since the reported data so far available on plants are comparatively meager before the vast number of plant population[3].Remedial plant's contents are used for the improvement of novel drug compounds that are used in the treatment of various types of diseases like liver and heart problems, cancer, diabetes and atherosclerosis etc[4].*Alysicarpus monilifer* is a low growing much branched annual or perennial herb, 5-15 (-50) cm tall. Leaves simple; ovate, elliptical or lanceolate, cordate at the base, 2.5-7.5 cm long, prominently nerved, glabrous or sparsely pubescent beneath. Racemes spicate, axillary and terminal, 1-15 cm long; flowers lax in dense along racemes. Pods distinctly moniliform, 3-5 jointed, 1-2 cm long, calyx not longer than first joint; glabrous or sparsely pubescent; articles 2.5-3 mm long and 2-3 mm wide, with a smooth to reticulate surface sculpture.*Alysicarpus monilifer* L. (DC.) (Fabacea), commonly known as Samervo (Gujarati) or Juhi ghas (Hindi), is a turf forming legume and native to Africa and Asia. In India it is distributed throughout the plains-Madras, Jammu, Bombay, Punjab, Gujarat-except Kutch and Bulsar, Madhya Pradesh and Uttar Pradesh. It is a prostrate, procumbent or decumbent perennial herb; stem of which is around 12- 60cm long, woody at the base. It is a branched; branches are terete clothed with covering trichomes. The herb is up to 50cm in length and hairy when young [5].

Traditional uses of the plant

This plant is used traditionally as an anti-inflammatory and in stomach ache[6].It is an antidote to snake bite [7]. It is also used in skin diseases and as a diuretic [8, 9]. The leaves are used in fever[10] and jaundice [11].

MATERIALS AND METHODS

(i) Collection and Identification of plant material

The aerial parts of *Alysicarpus monilifer* were collected from authentic dealers from Tirunelveli, Tamilnadu. The identification of the plant materials was confirmed by consulting the Research officer- Botany (Scientiest-C), Central Council for Research in Ayurveda & Siddha, Govt. Of India (Retired), Tirunelveli, Tamilnadu. The whole plant of *Alysicarpus monilifer* were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

(ii) Preparation of extracts

The collected plant material was dried (30±2ºC) for 14 days, ground and sieved to get fine powder from which the extracts were prepared by subjecting to the successive extraction, by using a hot continuous percolation method in Soxhlet apparatus[12] with solvents of increasing polarity such as petroleum Ether (60-80), ethyl acetate and methanol.The powdered whole plant (50gm) was first extracted with petroleum ether (1L) for the de - fating purpose. After complete extraction (18 hrs), the solvent was removed by distillation under reduced pressure.The resulting extract was dried using a water bath to get semisolid residue.Similarly , residues were prepared with ethyl acetate and methanol solvents.

PRELIMINARY SCREENING OF PHYTOCHEMICAL TEST

Phytochemical screening of petroleum ether extract, ethyl acetate extractand methanolic extract from *Alysicarpus monilifer.*The extracts

were subjected to preliminary phytochemical screening for the detection of various plant constituents present. The term qualitative analysis refers to the establishing and providing the identity of a substance. The pharmacological actions of crude drugs were determined by the nature of their constituents the phytoconstituents are responsible for the desired therapeutic properties. To obtain these pharmacological effects, the plant materials itself or extract in a suitable solvent or isolated active constituent may be used.The petroleum ether extract, ethyl acetate extractand methanolic extract of *Alysicarpus monilifer* was subjected to the following chemical tests used for the identification of various active constituents[13].

TESTS FOR ALKALOIDS[14]

Dragendroff's Test

A fraction of the extracts were treated with Dragondroff's reagent and observed for the formation of yellow colored precipitate, indicated the presence of alkaloids.

Wagner's Test

A fraction of the extracts were treated with Wagner's reagent and observed for the formation of a reddish brown precipitate, indicated the presence of alkaloids.

Mayer's Test

A fraction of the extracts were treated with Mayer's reagent and observed for the formation of white precipitate or creamy colored precipitate, indicated the presence of alkaloids.

Hager's Test

A fraction of the extracts were treated with Hager's reagent and observed for the formation of yellow precipitate, indicated the presence of alkaloids.

TEST FOR CARBOHYDRATES

Molisch's Test

To 2 mL of the extract, 1 mL of α- napthol solution was added, and concentrated sulfuric acid is added through the sides of the test tube. Purple or reddish violet color at the junction of the two layers revealed the presence of carbohydrates.

Fehling's Test

To 1mL of the extract, equal quantities of Fehling's solution A and B were added, while heating formation of a brick red precipitate that indicated the presence of carbohydrates.

Benedict's test

To 5mL of Benedict's reagent, 1mL of extract solution was added and boiled for 2 minutes and cooled. Formation of a red precipitate showed the presence of carbohydrates.

TESTS FOR GLYCOSIDES

Legal's Test

The extracts were dissolved in pyridine and sodium nitroprusside solution was added to make it alkaline. The formation of pink red to red color showed the presence of glycosides.

Borntrager's Test

A few mL of dilute HCl was added to 1 mL of the extract solution. It was then boiled, filtered and the filtrate was extracted with chloroform. The chloroform layer was then treated with 1 mL of ammonia. The formation of red color showed the presence of anthraquinone glycosides.

TESTS FOR PHYTOSTEROLS[14]

Libermann Burchard Test

Mixed 3mL of the extracts were added with 3mL of acetic acid anhydride. It was heated and then cooled. Few drops of concentrated sulfuric acid were added. Appearance of blue color shows the presence of phytosterol.

Salkowski's Test

Dissolve the extracts in chloroform and equal volume of concentrate sulfuric acid was added. Formation of bluish red to a cherry red color in chloroform layer and green fluorescence in the acid layer represented the steroid components present in the extract.

TEST FOR FLAVONOIDS[15]

Shinodas test

Small quantities of the extracts were dissolved in alcohol. To that some pieces of magnesium were added followed by concentrated hydrochloric acid was added drop wise and heated. Appearance of magenta color shows the presence of flavonoids.

Aqueous NaOH test

Small quantities of various extracts were dissolved separately in aqueous sodium hydroxide. Appearance of yellow color indicates the presence of flavonoids.

Conc. H_2SO_4 test

To the small portion of each extract, concentrated sulfuric acid was added. Yellow orange colour was obtained shows the presence of flavonoids.

TESTS FOR PROTEINS AND FREE AMINO ACIDS

Biuret Test

To the above prepared extracts equal volume of 5% sodium hydroxide and 1% copper sulfate solution were added. The violet color produced shows the presence of proteins and free amino acids.

Millon's test

The above-prepared extracts were treated with a Millon's reagent. Red color formed shows the presence of proteins and free amino acids.

Ninhydrin test

The extracts were treated with Ninhydrine reagent. Purple color produced shows the presence of proteins and free amino acids.

TEST FOR GUMS AND MUCILAGE

Swelling test

A small quantity of various extracts were added separately to 25ml of absolute alcohol with constant stirring and filtered. The precipitate was dried in air and examined for its swelling properties. No swelling was observed indicates the absence of gums and mucilages.

TEST FOR TANNINS AND PHENOLIC COMPOUNDS[16]

Ferric chloride test

1 mL of the extract were added with ferric chloride and observed for the formation of a dark blue or greenish black color indicated the presence of tannins and phenolic compounds.

TEST FOR SAPONINS

Foam test

About 1 mL of extracts were diluted separately with distilled water to 20mL, and shaken in a graduated cylinder for 15 minutes. A 1% 1 cm layer of foam indicated the presence of saponins.

TEST FOR FIXED OILS

Spot Test

A small quantity of the extracts were pressed between two filter papers. Oil stains on the filter paper indicated the presence of fixed oils.

Saponification Test

1 mL of the extracts were added with a few drops of 0.5N alcoholic potassium hydroxide along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hrs. The formation of soap or partial neutralization indicated the presence of fixed oils.

RESULTS AND DISCUSSION

The above powdered materials were successively extracted with petroleum ether, ethyl acetate and methanol by hot continuous percolation method in a Soxhlet apparatus for 24 hours. The results of the phytochemical screening of petroleum ether extract, ethyl acetate extract, methanolic extract of whole plant of *Alysicarpus monilifer* were present in Table-1. Most of the Secondary metabolites were present in ethyle acetate and methanolic extracts. But the carbohydrates and reducing sugars are present in all the three solvent extracts Different types of secondary metabolites such as carbohydrates, glycosides, phytosterols, saponins, proteins, alkaloids and flavonoids were presented.Flavonoids, as antioxidants, may prevent the progressive impairment of pancreatic beta- cell function due to oxidative stress and may thus reduce the occurrence of type 2 diabetes[17]. Saponins are used in hypercholesterolemia, hyperglycemia, antioxidant, anticancer, anti-inflammatory and weight loss etc. According to the medical field, It is a bioactive antibacterial agent of plants[18, 19].

Table No: 1

***Preliminary Phytochemical investigation of different extracts of Alysicarpus monilifer*(L.)DC**

Name of the test	Pet. Ether extract	Ethyl acetate extract	Methanolic extract
Test for Alkaloids			
Dragendroff's test	-	+	+
Mayer's test	-	+	+
Wagners's test	-	+	+
Hager's test	-	-	+
Tests for Carbohydrates &Free reducing sugars			
Molisch's test	+	+	+
Fehling's test	+	+	+
Benedict's test	+	+	+
Test for Glycosides			
Legal's test	-	+	+
Borntrager's test	-	+	+
Test for Flavonoids			
Aqueous NaOH test	-	+	+
Conc. H_2SO_4 test	-	+	+
Shinoda's test	-	+	+
Test for Tannins and Phenolic compound			
Ferric chloride test	-	-	-
Test for Saponins			
Foam test	-	+	+
Tests for Proteins and Free amino acids			
Millon's reagent test	-	+	+
Ninhydrin reagent test	-	+	+
Biuret test	-	+	+
Test for Phytosterol			
Liberman Buchard test	+	+	+
Salkowski test	-	-	+
Test for Fixed Oil and Fats			
Spot test	-	-	-
Saponification test	-	-	-
Test for Gums and mucilage			
Swelling test	-	-	-

Note: (+) Present (-) Absent

CONCLUSION

The plant screened for phytochemical constituents, seemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health. Exploitation of these pharmacological properties involves further investigation of these active ingredients by implementation techniques of extraction, identification, separation, purification and crystallization.

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