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QUALITATIVE PHYTOCHEMICAL SCREENING OF LEAF EXTRACTS OF *CISSAMPELOS PAREIRA* L. IN DIFFERENT SOLVENT SYSTEMS.

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Abstract: *Cissampelos pareira* linn. is a medicinally valuable plant belongs to the Family Menispermaceae. It is a perennial climbing herb/ shrub with small greenish-yellow flower. It is commonly referred to as midwife's herb and it has been traditionally used to combat women's ailments such as preventing miscarriages, uterine hemorrhages and also a lacto-stimulator. Leaves are used as an antiseptic against inflammations and can be put on wounds in order to heal sores. The present study is to analyze the phytochemical constituents of leaf extracts of *Cissampelos pareira.* The leaves were extracted in five different solvents such as Aqueous, Methanol, Ethyl acetate, Acetone and hexane. The preliminary phytochemical screenings of *Cissampelos pareira* for its phytochemical constituents were performed using generally accepted laboratory techniques. The plant extracts contains major phytochemicals such as alkaloids, flavanoids, coumarines, steroids, terpenoids, cardiac glycosides etc. The present study proves that the solvent extracts of *Cissampelos pareira* contain medicinally important bioactive compounds and this justifies the plant species as traditional medicine for the treatment of various diseases.

Key Words: *Cissampelos pareira*, phytochemical screening, phytochemicals, bioactive compounds.

INTRODUCTION

Herbal plants used in traditional medicine contain a wide range of bioactive compounds that was used since the dawn of civilization to maintain health and to treat diseases. Amongst them the substances having medicinal value have been extensively used for treating various diseases. Herbs being easily available to human beings have been explored to the maximum for their medicinal properties. Various parts of the plants like roots, leaves, bark, exudates etc. are used as per medicinal properties (1).

Cissampelos pareira (family-*Menispermaceae*) is a sub-erect or climbing herb, known as abuta or laghupatha in ayurvedic medicine (2). The plant is common in orchards, hedges, parks and gardens on moist soils distributed throughout tropical and subtropical regions, ascending up to an altitude of 2000 m, either creeping or twining around other plants; also common on the hilly tracts along watercourses. The stem is woody, flexible and slender and twines for support. The pith and rays of stem cross section resemble a wagon wheel with spokes; annual rings are not visible. The root system consists of flexible, light-brown lateral roots with sinkers and moderately abundant fine roots. The petioles are 3 to 7 cm long. Venation is palmate in widely oval or nearly round 4-10 cm blades (3). The fruits are juicy red, red-orange or yellow hairy drupes 4 to 5 mm in diameter. The leaves are palate or orbicular-reniform with truncate cordate base, glabrous or hairy above up to 3-12cm long (4).

Bisbenzylisoquinoline alkaloids are the main active components of *Cissampelos pareira*. Protoberberine alkaloids have been found in their roots (5). Another important alkaloid is hayatinin methochloride which possesses curariform activity. The cytotoxic alkaloid, cissampareine, has been reported to possess anti-tumor activity (6). The tropolo-isoquinoline alkaloids, pareirubrines A and B, from *C. pareira* have been reported to possess the anti-leukemic action (7). Cissampelo flavone, a chalcone-flavone dimer from the aerial parts has been reported to have activity against *Trypanosoma cruzi* and *T. brucei rhodesiense* and to have low toxicity to the human KB cell line (8)

Another well-known alkaloid berberine which has been documented to have hypotensive, antifungal, and antimicrobial actions. This chemical has been used for the treatment of irregular heartbeat , cancer, *candida*, diarrhea and irritable bowel syndrome (9). Another alkaloid cissampeline is sold as a skeletal muscle relaxant drug in Ecuador. Ethanolic rhizome extracts have shown antihistaminic, hypotensive, antispasmodic and anticonvulsant properties. In a test to confirm the antifertility use of this plant, a methanol extract of the leaves administered to rats caused a significant increase in the duration of the diestrus stage and a reduction in the number of litters in albino mice (10).

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Cissampelos pareira is widely employed in herbal medicine today as a diuretic and as a tonic, as well as to reduce fever and relieve pain. It is often employed for menstrual cramps, difficult menstruation, excessive bleeding and uterine hemorrhages, fibroid tumors, pre- and postnatal pain, colic, constipation, poor digestion, and dyspepsia (11). The roots are used in the treatment of dysuria and renal calculi. The Wayapi Indians use a decoction of the leaf and stem as an oral analgesic. In ayurvedic system of medicine, the leaves are used in the treatment of indolent ulcers and diarrhea. The leaves are eaten as potherb, and are reported to be cooling. Crushed leaves are boiled with rice and given as a tonic for heart complaints and fresh juice is applied for eye-diseases (12). The plant is considered to be antiseptic and on account of this property, it is used in the treatment for urinary tract infections (13). Expressed juice is given for migraine (14). It *has* a long history of use in muscle inflammation, snakebite, rheumatism, diarrhea, dysentery, and menstrual problems (15). The present study aims to assess the phyto-constituents present in different leaf extracts of *Cissampelos pareira.*

MATERIALS AND METHODS

Chemicals

The chemicals used includes Ethyl acetate, Methanol, Acetone, hexane. All the chemicals used were of the analytical grade.

Preparation of plant extracts

The medicinal plant materials were collected freshly and transported to the laboratory, they were then washed thoroughly, air dried in shade until dried completely . The drying process was continued to decrease the moisture content. After drying, the plant materials were macerated with mixer grinder. Then the powder was stored in airtight containers and kept in refrigerator for further use.
For the percolation process, the macerated plant powder are soaked in solvents such as methanol, ethyl acetate, acetone, aqueous, hexane individually. Extraction was done by soaking one part of plant powder to three parts of liquid solvent (1:3) and kept for percolation process for 3-5 days. Then the crude extracts were filtered using Whatman No.1 filter paper, evaporated and concentrated into solid extracts under room temperature.

Qualitative phytochemical screening

Chemical tests were carried out using solvents such as methanol, ethyl acetate, acetone, aqueous, hexane to identify various constituents using standard methods of Sofowora, Trease & Evans, Kokate, Harbone and Raman (16, 17, 18, 19, and 20).

Detection of Acids

1ml of plant extract was treated with Sodium bicarbonate solution. Formation of effervescence indicates the presence of acids.

Detection of Betacyanin

To the 2ml of plant extract, 1ml of 2N NaOH was added and heated for 5 minutes at 100°c. Formation of yellow colour indicates the presence of betacyanin.

Detection of Quinones

To the 1ml of plant extract 1ml of concentrated H_2So_4 was added. Formation of red color indicates the presence of quinones.

Detection of Coumarins

A few drops of ammonia was added on a filter paper. To this, a drop of plant extract was added and fluorescence was observed. This indicates the presence of coumarins.

Detection of Carbohydrates

Molisch's Test: The plant extracts were treated with 2 drops of alcoholic α-napthol solution in a test tube and 2ml of conc. H_2So_4 was added carefully along the sides of the test tube. The formation of dull violet or red ring at the interphase indicates the presence of carbohydrates.

Detection of Alkaloids

Mayer's Test: Plant extracts were treated with Mayer's reagent (1.36 mercuric chloride and 5gms of potassium iodide was dissolved in 100ml distilled H_2O). The formation of yellow cream precipitate indicates the presence of alkaloids .

Wagner's Test: Plant extracts were treated with Wagner's reagent (1.27g iodine, 2gm potassium iodide in 100ml distilled H_2O). The formation of brown or reddish precipitate indicates the presence of alkaloids.

Detection of Proteins and Aminoacids

Ninhydrin Test: To the plant extract, 0.25% Ninhydrin reagent was added and boiled for a few minutes. Formation of blue color indicates the presence of amino acids.

Biuret Test: The plant extracts were treated with 1ml of 10% NaOH solution and heated. To this, a drop of 0.7 % $CuSO_4$ solution was added. The formation of purplish violet color indicates the presence of proteins .

Detection of Reducing Sugars

Benedict's test: The plant extracts were treated with Benedict's reagent and heated on a water bath. Formation of an orange red precipitate indicates the presence of reducing sugars.

Fehling's Test: 1ml of Fehling's solution A and 1ml of Fehling's solution B were mixed and boiled for 1minute. To this, equal volume of aqueous extracts was added. Then the test tubes were heated on boiling water bath for 5-10 minutes. Brick red precipitate indicates the presence of reducing sugar.

Detection of fixed oils and Fats

Stain Test: Small quantities of plant extracts were pressed between 2 filter papers . An oily stain on filter paper indicates the presence of fixed oils and fats.

Saponification Test: To a small quantity of crude extract, few drops of 0.5N alcoholic potassium hydroxide solution was added to which a few drops of phenolphthalein was added separately and heated in a water bath for 1 hour. Formulation of soap indicates the presence of fixed oils and fats.

Detection of Flavonoids

Ferric chloride Test: Test solution when treated with few drops of $FeCl_3$ would result in the formation of blackish red color indicating the presence of flavonoids.

Alkaline Reagent Test: Plant extracts were treated with few drops of NaOH solution. Formulation of intense yellow colour, which becomes color less on addition of dilute acid, indicates the presence of flavonoids.

Lead Acetate Test: Plant extracts were treated with few drops of lead acetate solution. Formation of a yellow precipitate indicates the presence of flavonoids.

Detection of Gums and Mucilages

About 10ml of plant extract was slowly added to 25ml of absolute alcohol under constant stirring. The appearance of precipitation indicates the presence of gums and mucilages.

Detection of Steroids

2ml of acetic anhydride was added to 0.5g ethanol extract of each sample with 2ml of H_2SO_4. The color changes from violet to blue or green indicates the presence of steroids.

Detection of Tannins

To the 1ml of plant extract, few drops of 1% $FeCl_3$ solution were added . The appearance of blue, black, green or blue green precipitate indicates the presence of tannins.

Detection of Resins

To 0.5gm of sample, 5ml of boiling ethanol was added. This was ground and filtered through Whatman No.1 filter paper. Then the filtrate was diluted with 4ml of 1% aqueous HCl. Formation of heavy resinous precipitate indicates the presence of Resins.

Acetone -H_2O Test: Plant extracts were treated with acetone. To this small amount of water was added and shaken. The appearance of turbidity indicates the presence of resins.

Detection of Phlobatannins

About 2ml of aqueous plant extract was added to 2ml of 1% HCl and the mixture was boiled. The deposition of a red precipitate was an evidence for the presence of phlobatannins.

Detection of Terpenoids

Salkowski Test: To the 1ml of plant extract, 2ml of chloroform was added. Then 3ml of conc. H_2SO_4 was added carefully to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

Detection of Phenols

Ferric Chloride Test: To the 1ml of plant extracts, 3ml of distilled H_2O was added . To this few drops of neutral 5% $FeCl_3$ solution were added. A dark green colour indicates the presence of phenolics.

Detection of Saponins

Foam Test: About 2ml of distilled H_2O and 1ml of plant extract were mixed and shaken vigorously. A stable persistent forth indicates the presence of Saponins.

Froth Test: Plant extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15minutes. Formation of 1cm layer of foam, which is stable for 15 minutes indicates the presence of Saponins.

Detection Cardiac Glycosides

Kella- Killani Test: Plant extract was dissolved in glacial acetic acid containing traces of $FeCl_3$. Then the tube was held at an angle of 45°, 1ml of conc. H_2SO_4 was added down the side purple ring at the interface indicates cardiac glycosides.

Detection of Anthroquinones

Borntrager's Test: Small portion of the plant extract was shook well with 10ml benzene and filtered 5ml of 10% ammonia solution was added to the filtrate and stirred. The formation of a pink, red or violet color indicates the presence of free anthroquinones.

Detection of volatile oils

To 1ml of plant extract, 1ml of 90% ethanol was added followed by the addition of few drops of $FeCl_3$. A green color formation indicates the presence of volatile oils in the given sample.

Detection of emodols

The dry extract was added to 25% ammonia solution. A cherry-red solution indicates the presence of emodols.

Detection of Starch

To 1ml of plant extract 10ml of saturated NaCl solution was added and heated. After heating, starch reagent was added. A blue-purplish colour formation indicates the presence of starch.

RESULT AND DISCUSSION

The results confirmed the presence of constituents which are known to exhibit medicinal as well as physiological activities. Acids, betacyanin, quinones, coumarins, carbohydrates, alkaloids, amino acids, fixed oils and fats, flavonoids, steroids, tannins, resins, terpenoids, phenols, cardiac glycosides, volatile oils and starch were present in different extracts of leaf. The result was reported in table 1.

The alkaloids contained in plants are used in medicine as anaesthetic agents (10). The presence of saponins in plants have been reported to be responsible for the tonic and stimulating activities observed in Chinese and Japanese medical herbs (21). The result obtained in this study thus suggest that the identified phytochemical compounds may be the bioactive constituents responsible for the efficacy of the leaves of the plant studied and the presence of some of these compounds have also been confirmed to have antimicrobial activity. Hence it could be inferred that the plant extract could be a source for the industrial manufacture of drugs useful in the chemotherapy of some microbial infection (22).

Flavonoids have been referred to as nature's biological response modifiers, because of their inherent ability to modify the body's reaction to allergies and virus and they showed their anti-allergic, anti-inflammatory, anti-microbial, and anti-cancer activities (23). Glyscosides, flavonoids, Tannins and alkaloids have hypoglycemic activities (24). The terpenoids have also been shown to decrease blood sugar level in animal studies. Steroids and Triterpenoids showed analgesic properties. Saponin is used as detergents and in intracellular histochemical staining. It is also used to allow anti-body access in intracellular proteins. In medicines it is used in hypercholesterolemia, hyperglycemia, antioxidant, anticancer, antifungal, anti-inflammatory etc. (25).

Table 1: Qualitative phytochemical analysis of extracts of *Cissampelos pareira leaves* in different solvent systems.

TESTS		Aq	Hx	EtAc	Ac	M
1.	Acids	-	-	-	+	+
2.	Betacyanin	-	-	+	-	-
3.	Quinones	-	-	-	+	+
4.	Coumarins	-	+	+	+	+
5.	Carbohydrates	-	+	-	-	-
6.	Alkaloids	-	-	-	+	+
7.	Proteins and	-	-	-	-	-
	Amino acids	+	-	-	-	-
8.	Reducing sugars	-	-	-	-	-
9.	Fixed oils &Fats	+	-	-	-	-
10.	Flavanoids	+	-	-	-	-
11.	Gums and mucilages	-	-	-	-	-
12.	Steroids	-	-	+	+	+
13.	Tannins	-	-	+	+	+
14.	Resins		-	-	-	-
15.	Phlobatannins	-	-	-	-	-
16.	Terpenoids	+	-	-	-	-
17.	Phenols	-	-	+	-	-
18.	Saponins	-	-	-	-	-
19.	Cardiac glycosides	-	-	-	+	+
20.	Anthroquinones	-	-	-	-	-
21.	Volatile oils	-	-	+	+	+
22.	Emodols	-	-	-	-	-
23.	Starch	-	+	-	-	-

(+), presence. (-), Absence.

Aq- Aqueous, Hx- Hexane, EtAc- Ethyl Acetate, Ac- Acetone, M- Methanol

CONCLUSION

The use of plants and plant preparations has been in existent since prehistory. *Cissampelos pareira* plant belongs to Menispermaceae family. It is used as an ayurvedic medicine and called as laghupatha. The leaves of *Cissampelos pareira,* extracted in five different solvents are evaluated for phyto-constituents present in them. Based on phytochemical screening the plant extracts contains alkaloids, flavonoids, tannins, starch, terpenoids etc. The present study promotes guidance that solvent extracts of *Cissampelos pareira* contained medicinally improved bioactive compounds. This justifies the use of plant species as traditional medicines for various diseases.

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