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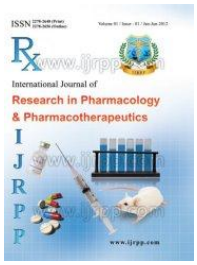

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Studies on physicochemical and phytochemical screening of different extracts of *Ichnocarpus frutescens*

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ABSTRACT

Ichnocarpus frutescens has a broad spectrum in the indigenous system of medicine. It is commonly known as the black creeper belonging to the family of *apocynaceae*. The present study investigates the physicochemical and phytochemical characteristics of three different extracts of leaves of *Ichnocarpus frutescens* by using standard methods. The phytochemical evaluation included colour and consistency, fluorescent behaviour and qualitative analysis. The Physicochemical screening determined extractive value, ash value and moisture content. Results revealed the presence of carbohydrates, glycosides, flavonoids, phytosterols and phenolic compounds. This study of *Ichnocarpus frutescens* will help in quality analysis of crude drug and lay down the standards which can be useful in future experimental studies.

Keywords: *Ichnocarpus frutescens*, phytochemical, physicochemical.

INTRODUCTION

Natural products are the source of synthetic and traditional herbal medicine. Drugs from the plants are easily available, less expensive, safe, and efficient and rarely have side effects. The medicinal importance of a plant is due to the presence of some special substances like alkaloids, glycosides, resins, volatile oils, gums and tannins, etc. The active principles usually remain concentrated in the storage organs of the plants[1]. *Ichnocarpus frutescens*(L) R.Br. commonly known as Black sariva, is an important medicinal plant found throughout India, belonging to family Apocyanaceae. It is considered as a substitute for *Hemidesmus indicus* (Indian sarsaparilla)[2]. It is a branched twining shrub, found almost in all parts of India, ascending to an altitude of 1200 m. Leaves are elliptic oblong or lance-shaped, tip-pointed or tapering base rounded or narrow, 4-10 cm long, 1.5-5cm wide. They are smooth and dark green above, and paler beneath with 2.5-5 mm long stalks.

Pharmacological study revealed hepatoprotective, antioxidant, anti-inflammatory, analgesic, anti-diabetic and antitumor activity[3,4,5,6].

MATERIALS AND METHODS

Collection of plant material :

Leaves of I.frutescens were collected from Kondapalli, Vijaywada after taxonomic verification and were identified and authenticated in the Department of Agricultural sciences, Loyola academy, alwal.

Preparation of crude extracts:

Shade dried powdered plant material (100g) was successively extracted with Soxhlet apparatus using 500 ml each of chloroform, methanol and water for 48 hours. The extract was filtered before drying using whatman filter paper no.2 on a Buchner funnel and the solvent was removed by vacuum rotary evaporator at 35 C. The extract was placed in pre-weighed flasks before drying.

Physicochemical evaluations

Moisture content

An accurately weighed quantity of the shade dried powder of *Ichnocarpus frutescens* (3g) was taken in a tared glass bottle and the initial weight was taken. The crude drug was heated at 105°C in an oven and weighed. This procedure was repeated till a constant weight was obtained. The moisture content of the sample was calculated in the percentage with reference to shade dried plant powder using formula[7].

$$\% \text{ Moisture content} = \frac{\text{Loss in weight of the sample}}{\text{Weight of the sample}} \times 100$$

Ash values[8]

Determination of total ash

An accurately weighed quantity of the shade dried powder of *Ichnocarpus frutescens* (2 g) was incinerated in a crucible at a temperature of 450ÚC in a muffle furnace till carbon free ash was obtained. It was then cooled and weighed. The percentage of total ash was calculated with reference to the shade dried plant powder using following equation.

$$\% \text{ Total ash value} = \frac{\text{Weight of total ash}}{\text{Weight of the crude drug taken}} \times 100$$

Determination of acid insoluble ash

Ash obtained was boiled for 5min with 25 mL of 2 M HCl and filtered using an ash less filter paper. Insoluble matter retain on filter paper was washed with hot water and filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid insoluble ash was calculated with reference to the shade dried plant powder using following equation.

$$\% \text{ acid insoluble ash value} = \frac{\text{Weight of acid insoluble ash}}{\text{Weight of the crude drug taken}} \times 100$$

Determination of water soluble ash

Ash above obtained, was boiled for 5 min with 25 mL of distilled water, cooled and the insoluble matter was collected on an ash less filter paper. Paper was washed with hot water and ignited for 15min at a temperature not exceeding 450ÚC in a muffle furnace. Difference in weight of ash and weight of water insoluble matter gave the weight of water soluble ash. The percentage of water soluble ash was calculated with reference to the shade dried plant powder using formula.

$$\% \text{ Water soluble ash value} = \frac{\text{Weight of total ash} - \text{Weight of water insoluble ash}}{\text{Weight of the crude drug taken}} \times 100$$

Extractive values[9]

Extractive values of shade-dried powder of *Ichnocarpus frutescens* were determined using following methods.

a) Determination of alcohol soluble extractive

An accurately weighed quantity of the shade dried powder of *Ichnocarpus frutescens* (5 g) was macerated with 100 mL of alcohol (Ethanol) in a closed flask for 24 h, shaking frequently during the first 6 h. It was then allowed to stand for 18 h and filtered rapidly to prevent any loss during evaporation. Evaporate 25 ml of the filtrate to dryness in a porcelain dish and dried at 105ÚC and weighed. The percentage of alcohol (Ethanol) soluble extractive was calculated with reference to the shade dried plant powder.

b) Determination of water soluble extractive

Weighed quantity of the shade dried powder of *Ichnocarpus frutescens* (5 g) was macerated with 100 mL of water in a closed flask, shaking frequently during the first 6 hrs and allowed to stand for 18 hrs. Thereafter it was filtered taking precaution against loss of water. Evaporate 25 mL of filtrate to dryness in a tared flat bottom shallow dish dried at 105°C and weighed. The percentage of water soluble extractive was calculated with reference to the shade dried plant powder.

Phytochemical Evaluation

The freshly prepared petroleum ether, methanolic and aqueous extracts of *Ichnocarpus frutescens* were qualitatively analyzed for the presence of major phytochemical constituents using the standard procedures.

1. Detection of carbohydrates[10]

100 mg of extracts were dissolved in 10 mL of water and filtered. The filtrate was used to test the presence of proteins and amino acids.

(a) Molisch's test

To the 1mL of filtrate, 2 drops of Molisch's reagent was added in a test tube and 2 mL of concentrated sulphuric acid were added carefully down the side of the test tube. Formation of violet color at the interface indicates the presence of carbohydrates.

(b) Barfoed's test

1 mL of Barfoed's reagent is heated with 5 drops of filtrate in a test tube on boiling water bath. Formation of a brick-red precipitate within five minutes indicates the presence of monosaccharides. Disaccharides generally don't give any reaction even for ten minutes

2. Detection of proteins and amino acid [11]

100 mg of extracts were dissolved in 10 mL of water and filtered. The filtrate was used to test the presence of proteins and amino acids.

(a) Millon's test

Two mL of filtrate was treated with 2 mL of Millon's reagent in a test tube and heated in a water bath for 5 minutes, cooled and few drops of NaNO2 solution was added. Formation of white precipitate, which turns to red upon heating, indicates the presence of proteins and amino acids.

b) Ninhydrin test

To the 2 mL of filtrate, 2-3 drops of Ninhydrin reagent was added in a test tube and boiled for 2 minutes. Formation of distinct blue colour indicates the presence of amino acids.

(c) Biuret test 2 mL of filtrate was treated with 2 mL of 10% sodium hydroxide solution in a test tube and heated for 10 minutes, a drop of 7% of copper sulphate was added in the above solution. Appearance of violet colour confirms the presence of proteins.

3. Detection of glycosides [12]

0.5 g of extract was hydrolyzed with 20 mL of dilute hydrochloric acid (0.1N) and filtered. The filtrate was used to test the presence of glycosides.

(a) Legal test

One mL of filtrate, 3 mL of sodium nitropruside in pyridine and methanolic alkali (KOH) was added in a test tube. A blue colour in the alkaline layer indicates the presence of glycosides.

(b) Keller-killiani test

One mL of filtrate was shaken with 1 mL of glacial acetic acid containing traces of ferric chloride. One mL of concentrated sulphuric acid was added carefully by the side of test tubes. A blue colour in acetic acid layer and red colour at the junction of the two liquids indicates the presence of glycosides.

4. Detection of alkaloids [13]

0.5 g. of extract was dissolved in 10 mL of dilute hydrochloric acid (0.1N) and filtered. The filtrate was used to test the presence of alkaloids.

(a) Dragendorff's test

To the 2 mL of filtrate add 2-3 drops of Dragendorff's reagent. Formation of reddish brown colored precipitate indicates the presence of alkaloids.

(b) Mayer's test

To the 2 mL of filtrate add 2-3 drops of Mayer's reagent, formation of cream colored precipitate indicates the presence of alkaloids.

(c) Wagner's test

To the 1 mL of the extract, add 2 mL of Wagner's reagent, formation of reddish brown precipitate indicates the presence of alkaloids.

5. Detection of flavonoids [14]

(a) Shinoda test

To the 100 mg of extract, few fragments of magnesium metal was added in a test tube, followed by drop wise addition (3 to 4 drops) of concentrated hydrochloric acid. Formation of magenta colour or light pinkcolour indicates the presence of flavonoids.

(b) Alkaline reagent test

To the 100 mg of extract, few drops of sodium hydroxide solution was added in a test tube. Formation of intense yellow colour that becomes colourless on addition of few drops of dilute hydrochloric acid indicates the presence of flavonoids.

(c) Fluroscence test

To the 100 mg of extract add 0.3 mL boric acid solution (3 %w/v) and 1 mL oxalic acid solution (10 %w/ v) and evaporated to dryness. The residue was dissolved in 10 mL ether; ethereal layer shows greenish fluorescence under UV light indicates presence of flavanoids.

6. Detection of phenolic compounds and tannins [15]

100 mg of extract was boiled with 1 mL of distilled water and filtered. The filtrate was used for the following test.

(a) Ferric chloride test

To the 2 mL of filtrate in a test tube add 2 mL of 1% ferric chloride solution. Formation of bluish to black colour indicates the presence of phenolic nucleus.

(b) Lead acetate test

To the 2 mL of filtrate, few drops of lead acetate solution were added in a test tube. Formation of yellowish precipitate indicates the presence of tannins.

7. Detection of fats and oils [16]

Oily Spot Test

One drop of extract was placed on filter paper and solvent was allowed to evaporate. An oily stain on filter paper indicates the presence of fixed oil.

8. Detection of Saponins [17]

Foam Test

Dilute 1 mL of extract with 20 mL distilled water and shaken in a graduated cylinder for 15 minutes. 1 cm layer of foam indicates the presence of saponins.

9. Detection of phytosterols [18]

0.5 g of extract was treated with 10 mL of chloroform and filtered. The filtrate was used to test the presence of phytosterols and triterpenoids.

(a) Libermann's test

To the 2 mL of filtrate in hot alcohol, few drops of acetic anhydride was added. Formation of brown precipitate indicates the presence of sterols.

(b) Salkowski test

To the extract solution, few drops of concentrate sulfuric acid was added, shaken and then allowed to stand, lower layer turns red indicating the presence of sterols.

Statistical analysis

All data were expressed as mean +-SD and results were analysed using ANOVA, followed by Dunnet's test where $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The preliminary phytochemical screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. Further, these tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds [19].

The present study carried out on *Ichnocarpus frutescens* revealed the presence of medicinal active constituents. Phytochemical analysis was performed on chloroform, methanol and water extracts of I.frutescens separately, and the results are presented in Table 4.

According to Tiwari *et al*., the factors affecting the choice of solvent are; quantity of phytochemicals to be extracted, rate of extraction, diversity of different compounds extracted, diversity of inhibitory compounds extracted, ease of subsequent handling of

the extracts, toxicity of the solvent in the bioassay process, potential health hazard of the extractant[20].

The medicinal value of plants lies in some chemical substances that have a definite physiological action on the human body. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. For example, The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites[21]. They possess biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities[22]. Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds [23, 24]. Natural antioxidant mainly come from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc [25]. The plant extracts were also revealed to contain saponins which are known to produce inhibitory effect on inflammation [27]. Saponins has the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness [26, 28]. Steroids have been reported to have antibacterial properties [29] and they are very important compounds especially due to their relationship with compounds such as sex hormones[30]. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity[31]. Several workers have reported the analgesic[32,33], antispasmodic and antibacterial [34,35] properties of alkaloids. Glycosides are known to lower the blood pressure according to many reports[36]. *Ichnocarpus frutescens* was subjected to physicochemical analysis that to remove all traces of organic matter, aiding pure analytical determination. The physicochemical characteristics are shown in table no 1.The fluorescent character, colour and consistency are given in table no 2 and 3. Qualitative analysis of leaf extracts with different chemical reagents are shown in table no.4.

Table 1: Physicochemical evaluation of leaves of *Ichnocarpus frutescens*

S.No.	Quality Parameters	Results
1.	**Moisture content**	9.15(±0.89)
2.	**Ash value**	
(a)	Total ash value	7.48(±0.67)
(b)	Acid insoluble ash	2.02(±0.34)
(c)	Water soluble ash	4.16(±0.41)
3.	**Extractive value**	
(a)	Methanol soluble extract	15.83(±0.61)
(b)	Water soluble extract	14.28(±1.25)

Table 2: Fluorescence characters of *Ichnocarpus frutescens*

S.No.	Treatment	Visible light	UV light	
			250nm	360nm
1.	Powder	Pale green	Light green	Green
2.	Powder +1N NaOH in water	Pale brown	Parrot green	Brown
3.	Powder +1N NaOH in alcohol	Yellowish green	Fluorescent green	Dark green
4.	Powder + H_2SO_4	Reddish brown	Pale green	Dark green
5.	Powder + HCl	Light reddish brown	Pale brown	Dark brown
6.	Powder + 50% HNO_3	Dark brown	Fluorescent green	Dark green

Table 3 : Colour and Consistency of the extracts.

S.No.	Solvent Extract	*Ichnocarpus frutescens*
1.	Chloroform	Light brown sticky
2.	Methanol	Dark brown sticky
3.	Water	Dark brown sticky

Table 4: Phytochemical analysis of different extracts of leaves of *Ichnocarpus frutescens*

S.No.	Tests	Chloroform Extract	Methanolic Extract	Water Extract
1.	Carbohydrates	Positive	Positive	Positive
2.	Proteins and Amino acids	Negative	Positive	Negative
3.	Glycosides	Positive	Positive	Positive
4.	Alkaloids	Negative	Positive	Positive
5.	Flavanoids	Positive	Positive	Positive
6.	Phenolic Compounds	Positive	Positive	Positive
7.	Tannins	Negative	Negative	Negative
8.	Saponins	Negative	Positive	Positive
9.	Phytosterols	Positive	Positive	Positive
10.	Fats and Oils	Positive	Positive	Positive

CONCLUSION

Ichnocarpus frutescens tend to have the potential to act as a source of useful drug due to the presence of varied secondary metabolites that can be widely used to combat and cure various ailments. The information obtained on the physicochemical tests, fluorescent character, colour and consistency and phytochemical analysis is also helpful in determining quality and purity of crude drug. These parameters are helpful in detection of adulteration in commercial samples

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