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Phytochemical and Pharmacological Studies of Ethanolic and Aqueous Extract from the Leaf of Folk Medicinal Plant *Ichnocarpus frutescens*



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ABSTRACT

The main aim of this study is to evaluate the phytochemical and Pharmacological Studies of Ethanolic and aqueous extract from the leaf of folk medicinal Plant *Ichnocarpus frutescens*. The plant of *Ichnocarpus frutescens* will be collected from the Kattehakkalu village, Thirthahalli taluk, Shimoga district. Phytochemical test is carried out by the chemical group test. Analgesic activities were studied using the model of Eddy's hot Plate method in mice, In the present study the test samples (Ethanol and aqueous leaf extracts of *Ichnocarpus frutescens* exhibited significant analgesic activity). Analgesic activity at a dose of 200 mg/kg and 400mg/kg b.w. Among these test samples ethanol extract exhibited more analgesic action when compare to control at a dose of 400 mg/kg. The phytochemical investigation has shown the presence of flavonoids, steroids and terpenoids in the presently tested samples. The analgesic activity may be due to the presence of these constituents.



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INTRODUCTION

The importance of medicinal plants in traditional health care practice and in providing clues to new areas of drug research and biodiversity conservation is now well recognized. About 80% of the world's population relies on the use of traditional medicine, which is predominantly based on plant material (WHO 1993). Scientific studies on a number of medicinal plants indicated that promising phytochemical compounds can be developed for many health problems (Gupta 1994). Herbal drugs have gained importance in recent years because of their efficacy and cost effectiveness. These drugs are invariably single plant extracts or fractions or mixtures of fractions/extracts from different plants, which have been carefully standardized for their safety and efficacy. Several plants of kuvempu forest are used for medical purposes. The plants in the kuvempu forest showed the potential as a source of medicinal plant for research and getting the medicinal plant to cure many diseases.

Ichnocarpus frutescens R. Br. (Apocynaceae) is a climbing plant found throughout India. A large, much branched, twining shrub with long, slender, whip-like, finely fulvous to mentose bracelets; leaves simple, opposite, 3.7-7.5cm long, 2-3.8cm broad, ovate oval, rounded at base, acute, glabrous above, slightly hairy and paler beneath¹. Studies on chemical constituents of the plant reveals the presence of phenylpropanoids, phenolic acids, coumarins, flavonoids, sterols and pentacyclic triterpenoids². Whole plant is used as tribal medicine in atrophy, bleeding gums, convulsions, cough, delirium, dysentery, glossitis, haematuria, measles, night blindness, relieves pain due to insect bites, splenomegaly and tuberculosis. Plant is also used in abdominal and glandular tumour^[3-5]. *Ichnocarpus Frutescens* is one such medicinal plant reported to have medicinal properties and was used to cure many disorders. No data available so far in any of highly indexed online and printed journals website database or in books and publications that give exhaustive coverage on natural and medicinal plants. In this contest, an attempt is made to screen the analgesic activity by using hot plate method with the help of aerial parts of the plant *Ichnocarpus Frutescens*.

METHODS

Animals

The experimental protocols were duly approved by the Institutional Animal Ethical Committee and Committee for the Purpose of Control & Supervision of Experiments on

Animals (CPCSEA), (Ref No. NCP/IAEC/CL/08/2015- 16) All the Animals Were Procured From Central Animal House National College of Pharmacy Shivamoga.

Animal	Young Swiss albino mice
Age	4-5 weeks
Average Weight	25-30gm
Purchased	S. S. Medical College, Davanagere.
Condition	They were kept under standard environmental condition for one week for adaptation and was fed mice formulated

PLANT MATERIALS

Plant	<i>Ichnocarpus Frutescens R.Br.</i>
Family	Apocynaceae
Collected	Kattehakkalu Village, Thirthahalli Taluk
District	Shimoga District
State	Karnataka
Identified And Authenticated	Dr. Rudrappa, HOD, S.R.N.M National College Of Applied Science Balraj-Urs, Road, Shivamogga, Karnataka
Herbarium	(NCP/Herbarium No:7)

PREPARATION OF PLANT EXTRACT

The leaves of *Ichnocarpus frutescens* were shade dried and reduced to a coarse powder in a Pulveriser (Sunbeam, Munger, India) using mesh no. 3 and passed through a sieve No. 40 to obtain about 3 kg of powder. Various extracts of the plant material were prepared by soxhlet extraction method. The powdered material of *Ichnocarpus frutescens* was extracted with different solvents (Ethanolic, Aqueous,) in a soxhlet extractor for 48 hrs in 8 batches of 35g each. The extract was concentrated under vacuum using rotary flash evaporator (Buchi, Flawil, Switzerland). The solvent was removed completely over the water bath and finally desiccator dried. The extract so obtained was labelled, weighed and the yield was calculated

in terms of grams percent of the weight of the powdered leaves of the plant. These extracts are then used for the activities.

PHYTOCHEMICAL INVESTIGATIONS OF EXTRACTS^{6,7}

The extracts so obtained from each of the solvents were subjected to the following qualitative tests to detect the major chemical constituents.

1. Test for carbohydrates

- ❖ Molisch's test: To the test solution, few drops of Molisch's reagent and 2 ml of concentrated sulphuric acid were added slowly through the sides of the test tube. A purple ring formed at the junction of the two liquids indicates the presence of carbohydrates.
- ❖ Barfoed's test: To the test solution, Barfoed's reagent was added, boiled on water bath, brick red precipitate was formed.
- ❖ Benedict's test: To the test solution, Benedict's reagent was added and boiled on water bath, reddish brown precipitate was formed.

2. Test for proteins

- ❖ Millon's test: The test solution when treated with Millon's reagent and heated on a water bath, yellow coloration was observed.
- ❖ Xanthoproteic test: Test solution after treating with concentrated nitric acid and on boiling, gave yellow precipitate.
- ❖ Biuret test: When the test solution was treated with 40% sodium hydroxide and dilutes copper sulphate solution, blue colour develops.
- ❖ Ninhydrin test: Test solution when treated with Ninhydrin reagent gives blue colour.

3. Test for tannins

- ❖ Ferric chloride test: Test solution with few drops of ferric chloride solution gives dark red colour.
- ❖ Gelatin test: Test solution when treated with gelatin solution gives white precipitate.

4. Test for saponins

- ❖ Foam test: Saponins when mixed with water and shaken, shows the formation of froth, which was stable at least for 15 minutes.
- ❖ Haemolysis test: 2ml.each of 18% sodium chloride solution is taken in two test tubes. To one test tube 2 ml of distilled water was added and to the other 2 ml. of test sample was added. A Few drops of blood were added to both the test tubes, mixed and observed for haemolysis under microscope.

5. Test for triterpenoids

- ❖ Salkowaski test: A few drops of concentrated sulphuric acid was added to the test solution, shaken and allowed to stand, lower layer turns yellow indicating the presence of triterpenoids.
- ❖ Liebermann-Burchard test: The test solution was treated with few drops of acetic anhydride and mixed. When conc. sulphuric acid was added from the sides of the test tube, deep red colour was formed.

6. Test for flavonoids

- ❖ Ferric chloride test: Test solution with few drops of ferric chloride solution shows intense green colour.
- ❖ Shinoda test: Test solution with few fragments of magnesium ribbon and concentrated hydrochloric acid, shows pink to magenta red colour.
- ❖ Zinc-Hydrochloric acid reduction test: Test solution with zinc dust and few drops of hydrochloric acid shows magenta red colour.
- ❖ Alkaline reagent test: Test solution when treated with sodium hydroxide solution, shows increase in the intensity of yellow colour which becomes colourless on addition of few drops of dilute acid.
- ❖ Lead acetate solution test: Test solution with few drops of lead acetate (10%) solution gives yellow precipitate.

7. Test for steroids

- ❖ Salkowski test: Concentrated sulphuric acid was added to the test solution, shaken and allowed to stand. The lower layer turns red indicating the presence of sterols.
- ❖ Liebermann-Burchard test: A few drops of acetic anhydride were added to the test solution. When concentrated sulphuric acid was added from the sides of the test tube, a brown ring was formed at the junction of the two liquids and the upper layer turned green.
- ❖ Sulphur test: Sulphur when added to the test solution, it sinks to the bottom indicating the presence of sterols.

8. Test for glycosides

- ❖ Baljet test: The test solution when treated with sodium picrate gives yellow to orange colour.
- ❖ Keller-Killiani test: The test solution was treated with few drops of ferric chloride solution and mixed. When concentrated sulphuric acid containing ferric chloride solution was added, it forms two layers, lower layer reddish brown and upper acetic acid layer turns bluish green.
- ❖ Raymond' test: The test solution when treated with dinitrobenzene in hot ethanolic alkali gives violet colour.
- ❖ Bromine water test: Test solution when dissolved in bromine water gives yellow precipitate.
- ❖ Legal's test: Test solution, when treated with pyridine (made alkaline by adding sodium nitroprusside solution), gives pink to red color.

9. Test for alkaloids

- ❖ Mayer's test: When Mayer's reagent (potassium mercuric iodide) was added to the test solution, it gives cream coloured precipitate.
- ❖ Wagner's test: The acidic test solution with Wagner's reagent (iodine in potassium iodide) gives brown coloured precipitate.

- ❖ Dragendorff's test: When Dragendorff's reagent (solution of potassium bismuth iodide) was added to the test solution, it gives an orange brown colored precipitate.
- ❖ Hager's test: When Hager's reagent (saturated picric acid solution) was added to the test solution, it gives yellow coloured precipitate.

Statistical Analysis^{8,9}:

All the values were expressed as mean \pm S.E.M. Statistical analysis was carried out by performing one-way ANOVA followed by pairwise comparisons of Tukey's HSD (honestly significant difference) test. A probability level of $P < 0.05$ was considered moderately significant, $P < 0.01$ is considered as significant and $P < 0.001$ is considered as highly significant.

Table no 1: Qualitative chemical investigations of extracts of *Ichnocarpus frutescens*.

Sl No	Phytoconstituents	Ethanol extract	Aqueous extract
1	Carbohydrates	+	+
2	Proteins	+	-
3	Saponins	+	+
4	Triterpenoids	+	
5	Flavonoids	+	+
6	Steroids	-	-
7	Glycosides	+	+
8	Alkaloids	-	-
9	Tannins	-	-

Acute toxicity study¹⁰

Toxicity test studies conducted as per internationally accepted protocol drawn under OECD guidelines. 425. (OECD guidelines. 425 modified, adopted March 23, 2006) in Swiss albino mice.

Evaluation of analgesic activity

The analgesic activity was determined by using hot plate method according to Eddie and Leimback in mice. Mice were placed on a hot plate maintained at constant temperature $55 \pm 1^{\circ}$ immediately after oral administration of control, standard and different extracts. Latency to exhibit the nociceptive response such as licking paws or jumping was determined

- The hot plate consisting, of an electrically heated surface ($55-56^{\circ}\text{C}$).
- The animals were placed on the hot plate and the time until either licking or jumping across was recorded by a stopwatch.
- The latency was recorded before and after 30, 60, 90 and 120 min.

Group I: Control group treated with 1/10 saline water.

Group II: Received reference standard Diclofenac sodium.

Group III: Received Ethanolic leaf extract of *Ichnocarpus Frutescens* 200mg/kg.

Group IV: Received Ethanolic leaf extract of *Ichnocarpus Frutescens* 400mg/kg.

Group V: Received aqueous leaf extract of *Ichnocarpus Frutescens* 200mg/kg.

Group VI: Received aqueous leaf extract of *Ichnocarpus Frutescens* 400mg/kg.

Table No. 2: Table showing the effect of an ethanolic & aqueous extract of leaf of *Ichnocarpus frutescens* by hot plate model.

SL	Groups	Dose Mg/kg	Mean latency before and after drug administration (s)(min)			
			30min	60min	90min	120min
1	Control	0.5	3.05 ± 0.147	3.067 ± 0.08	3.023 ± 0.07	2.815 ± 0.073
2	Diclofenac Sodium	10ml	6.95 ± 0.252	8.65 ± 0.117	10.0 ± 0.163	11.215 ± 0.386
3	EEIF	200mg/kg	3.76 ± 0.166	3.80 ± 0.165	4.187 ± 0.966 **	4.713 ± 0.291 ***
		400mg/kg	5.10 ± 0.152 *	6.70 ± 0.167* *	7.940 ± 0.210** *	9.282 ± 0.185 ***
4	AEIF	200mg/kg	3.30 ± 0.05	3.56 ± 0.130	3.900 ± 0.132 * *	4.445 ± 0.1904* *
		400mg/kg	3.70 ± 0.143	3.93 ± 0.125	4.355 ± 0.430** **	5.228 ± 0.351 ***

Note: Data was analyzed using one-way ANOVA followed by pairwise comparison. Values are expressed as mean \pm S.E.M. n=6, ***P < 0.001 is considered as highly significant.

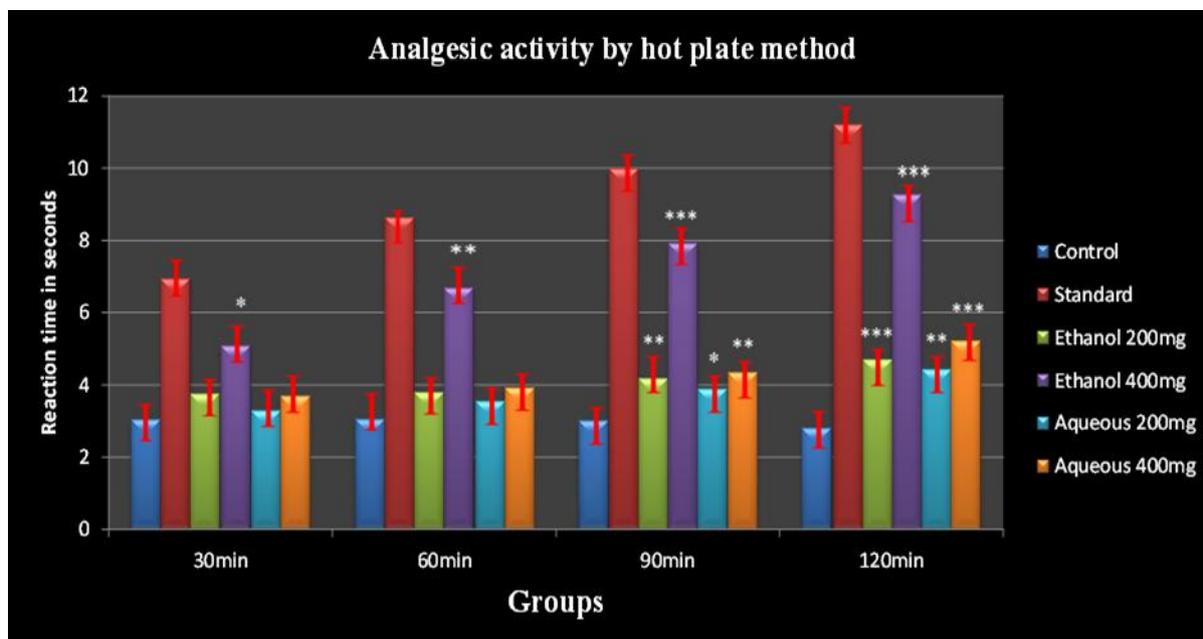


Figure No. 2.1: Histogram showing the effect of an ethanolic & aqueous extract of leaf of *Ichnocarpus frutescens* on analgesic action by hot plate model.

DISCUSSION

In the present study, the test samples of leaf extract of *Ichnocarpus frutescens* belongs to the family Apocynaceae were tested for analgesic activity. In the present study leaf extract of *Ichnocarpus frutescens*, analgesic activity was evaluated by hot plate method and acetic acid induced writhing method. Analgesic can act on peripheral or central nervous system. Peripherally acting analgesic act by blocking the generation of impulses at chemoreceptors site of pain, while centrally acting analgesic not only the threshold for pain but also alter the physiological response to pain and suppress the patient's anxiety and apprehension.

The mechanism of analgesic activity of leaf extract of *Ichnocarpus frutescens* could be probably due to the blockade of the effect or release of an endogenous substance that excite pain nerve endings similar to that of Diclofenac sodium and NSAIDs. Thus, the reduction in the number of writhing indicates that leaf extract of *Ichnocarpus frutescens* might exert analgesic activity by inhibition of prostaglandin synthesis or action of prostaglandin.

In the present study the test samples (Ethanol and aqueous leaf extracts of *Ichnocarpus frutescens* exhibited significant analgesic activity). Analgesic activity at a dose of 200 mg/kg and 400mg/kg b.w. Among these test samples Ethanol exhibited more analgesic action when compare to control at a dose of 400 mg/kg. The phytochemical investigation has shown the presence of flavonoids, steroids and terpenoids in the presently tested samples. The analgesic activity may be due the presence of these constituents

Flavonoids belong to a group of natural substances with variable phenolic structures and are found in fruit, vegetables, grains, bark, roots, stems, flowers, tea, and wine. Flavonoids affect arachidonic acid metabolism in different ways. Some flavonoids specifically block cyclooxygenase or lipoxygenase, whereas others block both enzymes. Flavonoids also inhibit both cytosolic and membranal tyrosine kinase¹¹.

However, further studies are necessary to find the exact mechanism of analgesic effect and to isolate the active compound(s) responsible for this pharmacological activity

CONCLUSION

Analgesic activity of various leaf extracts of *Ichnocarpus frutescens* was carried out by using models namely and hot plate model. In the present study, test samples ethanol and aqueous leaf extracts exhibited analgesic activity among these test samples both ethanol and aqueous extract at the 400 mg/kg exhibited promising analgesic activity at 90 and 120 min when compare to control.

It can be concluded that active constituents responsible for analgesic activity might be present in the leaf extracts. However, further studies are necessary to find the exact mechanism of analgesic effect and to isolate the active compound(s) responsible for this pharmacological activity.

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