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DIURETIC ACTIVITY OF THE HYDRO-ALCOHOLIC EXTRACT OF *FICUS RELIGIOSA* LINN IN WISTAR ALBINO RATS

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ABSTRACT

The present study was under taken to Hydro alcoholic extract of *Ficus Religiosa* Linn used for screening of the Diuretic activity using wistar albino rats. The leaves of *Ficus Religiosa* Linn was selected, collected, authenticated, shade dried, granulated and extracted with mixture of distilled water and ethanol (1:1), as per the procedure. The hydro alcoholic extract was subjected to preliminary phytochemical analysis and it reveals the presence of Carbohydrates, Terpenoids, Proteins, Saponins, Steroids, Flavonoids, Tannins and Phenolic, Amino acids, Essential oils. The hydro alcoholic extract of leaves of *Ficus Religiosa* Linn was subjected to diuretic activity at the dose of 200 and 400mg/kg body weight. In Both doses, *Ficus religiosa* exhibits the diuretic activity in a dose dependent manner. The diuretic activity produced by the hydro alcoholic extract 400mg/kg body weight is compared with the standard dose of furosemide (100mg/kg).

KEYWORDS: Diuretic activity, Hydro alcoholic extract, *Ficus religiosa*, Phytochemical analysis, wistar albino rats.

INTRODUCTION

The Plants have been used in conventional medicine for several thousand years. Awareness of medicinal plants has been accumulated in the course of many centuries based on different medicinal systems such as Ayurveda, Unani and Siddha. It was reported that usually 2500 plant species and 100 species of plants serve as a regular sources of medicines. During the few centuries, there has been an increased attention in the study of medicinal plants and their conventional utilization in different parts of the world. The native information through ethnobotanical studies is significant in favour of the conservation as well as consumption of biological resources.

Today, according to the World Health Organization (WHO), as many as 80% of the world's people depends on natural drug for their main healthcare desires. Herbal medicines form the basis of health care throughout the world. The earliest days of mankind are still widely used, and have considerable importance in international trade. Recognition of their clinical, pharmaceutical and economical value is still growing, all though it varies widely between countries.

Medicinal plants are important for pharmacological research and drug development, not only for plant constituents which are used directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds. Regulation of exploitation and exportation is therefore essential, together with international cooperation and coordination for their conservation so as to ensure their availability for the future.

Nature always stands as a golden mark to exemplify the outstanding phenomenon of symbiosis. The biotic and abiotic elements of nature are all inter-dependent. The plants are indispensible to mankind. The three important necessities of life- food, clothing, shelter- and a host of other useful products are supplied to mankind by the plant kingdom. Nature has provided a complete store house of remedies to cure all ailments of mankind. The knowledge of drugs has accumulated over thousands of years as a result of man's inquisitive nature so that, today we possess many effective means of ensuring health care.

Now-a-days many herbal drugs are used to treat various diseases and disorders. Literature review has revealed that the leaves of the plant *Ficus Religiosa* Linn. is prescribed in the treatment of Respiratory Diseases, Sexual Disorders, Central Nervous System disorders, Cardiovascular Diseases, Gastritis, Skin Infection, Anti-Diabetic, Anti-Inflammatory, Anti-Ulcer, Anti-Diarrheal, Anti-Migraine, Hematuria, Anal Fistula, Anti-Anxiety,

Vaginal and urogenital Diseases, Anti-tumour, Anti-bacterial.As per the literature review, so far no scientific study has been carried out on this plant *Ficus Religiosa* Linn (*Family: Moraceae)* to explore the diuretic activity. So, the present study has been under taken to explore the diuretic activity of the hydro-alcoholic extract of the leaf of *Ficus Religiosa* Linn.

MATERIALS AND METHODS

Collection And Authentication Of Plants

As per the literature review, the plant *Ficus Religiosa* Linn (family: *Moraceae*) was selected for our study. The fresh leaves were collected, identified and authenticated by the "ABS Botanical conservation, Research & Training Centre", Kaaripatti, Salem, Tamil Nadu. The fresh leaves was washed, shade dried and then milled to a coarse powder by mechanical grinder.

Preparation Of Extraction Of Plants

The hydro alcoholic extract was prepared by mixing of ethanol and distilled water (1:1). Solvents were removed under reduced pressure. The extracts were concentrated by evaporation. After concentrating the preparation, the extract was stored under cool temperature in refrigerator and used for further experimental studies.

Preliminary Phytochemical Screening

Hydro alcoholic extract of *Ficus Religiosa* Linn were subjected to qualitative test for the identification of various active constituents.

1. Test For Carbohydrates

A small quantity of the extract was dissolved separately in 4ml of distilled water and filtered. The filtrate was subjected to the following tests to detect the presence of carbohydrate and glycosides.

- **Molisch's test**

The filtrate was treated with 2-3 drops of 1% alcoholic α-naphthol solution and 2ml of concentrated sulphuric acid was added along the sides of the test tube. Appearance of brown ring at the junction of two liquids shows the presence of carbohydrates.

- **Fehling's test**

The filtrate was treated with 1 ml of Fehling's solution A and B and heated on the water bath. A reddish precipitate was obtained shows the presence of carbohydrates.

- **Benedict's test**

To 0.5ml of filtrate, 1ml of Benedict's reagent is added. The mixture is heated on boiling water bath for two minutes. A characteristic coloured precipitate indicates the presence of sugar.

2. Test for Fixed Oils And Fats

- **Spot Test**

Small quantity of the extract was pressed between two filter papers, Appearance of oil stain on the paper indicates the presence of fixed oil.

- **Saponification Test**

Add few drops of 0.5N alcoholic potassium hydroxide to a small quantity of various extracts along with a drop of phenolphthalein separately and heat on a water bath for 1-2hrs. The formation of soap or partial neutralization of alkali indicates the presence of fixed oil and fats.

3. Test For Proteins

- **Biuret Test**

To the extract solution (2ml) biuret reagent (2ml) was added, Violet colour indicates the presence of protein.

- **Xanthoprotein Test**

To 5ml of extract solution, 1ml of nitric acid was boiled, yellow precipitate was formed. After cooling it, add 40% sodium hydroxide solution orange colour was formed.

- **Millon's test**

2 ml of the test solution was mixed with millons reagent, white precipitate was formed.

4. Test for Steroids

- **Salkowski's Test**

To 1ml of chloroform solution, few drops of concentrated sulphuric acid was added. Brown colour indicates the presence of phytosterols.

- **Libermann – burchard's test**

The extract was added with few drops of acetic anhydride, boiled and cooled. Then concentrated sulphuric acid was added from the side of the test tube, brown ring was formed at the junction of two layers and upper layer turned green which indicates presence of steroids.

5. Test for Glycosides

General Test

- **Test A**

200 mg of the drug was extracted with 5ml of dilute H_2SO_4 by warming on a water bath. It was filtered and then the acid extract was neutralized with 5% solution of sodium hydroxide. 0.1ml of fehlings solution A and B added until it became alkaline and heated on a water bath for 2 minutes, the quantity of red precipitate formed was noted and compared with that of formed in test B.

- **Test B**

200mg of the drug was extracted with 5ml of water instead of H_2SO_4. After boiled equal amount of water was added. 0.1 ml of Fehling's solution A and B was added until it becomes alkaline and heated on a water bath for 2 minutes. The quantity of red precipitate formed was noted. The quantity of precipitate formed in test B with that formed in test A was compared. And it indicates the absence of glycosides.

6. Test for Cardiac Glycoside

- **Baljet's test**

The extract was mixed with picric acid or sodium picrate. No orange colour was formed.

- **Legal's test**

To the alcoholic solution of extract, 1ml pyridine and 1ml sodium nitro-prusside solution, No blood red colour was observed shows the absence of cardiac glycoside.

- **Keller-Killiani Test**

To 2ml of the extract, 3ml of glacial acetic acid and 1 drop of 5% ferric chloride were added, this solution was carefully transferred to the surface of 2ml concentrated H_2SO_4 and the observation was noted down.

7. Test for Antraquinone Glycosides

- **Borndrager's test**

The test material was boiled with 1ml of H_2SO_4 in a test tube for five minutes. It was filtered while hot, the filterate was cooled and shaken with equal volume of dichloromethane or chloroform. The lower layer of dichloromethane or chloroform was separated and shaken it with half of its volume of dilute ammonia. No rose pink to red colour was produced in the ammonical layer.

- **Modified Borndrager's test**

200mg of the leaves were boiled with 2ml dilute H_2SO_4. It was treated with 2ml of 5% aqueous ferric chloride solution for 5 minutes, it was shaken with equal volume of chloroform. The organic solvent layers were separated and an equal volume of dilute ammonia were added, ammonia layer does not show pinkish red colour.

8. Test for Flavonoids

- **Shinoda's test**

To the extract solution few magnesium turnings were added and conc. HCl was added drop wise, pink scarlet, red appeared after a few minutes which indicates the presence of flavonoids.

- To small quantity of residue lead acetate solution was added & the colour changes was observed.

9. Test for Fats and Oils

- **Solubility test**

1. To 2-3ml of the hydro alcoholic extract solution, few ml of chloroform was added and solubility was observed.
2. To 2-3ml of the hydro alcoholic extract solution few ml of 90% ethanol were added and the solubility was observed.

10. Test for Tannins and Phenolic Compounds

To 2-3ml of the aqueous solution of extract, few drops of following reagents were added.

- **Ferric chloride test**

To 2-3ml of aqueous extract solution few drops of 5%w/v ferric chloride solution was added and there was no colour change.

- **Lead acetate test**

To 2-3ml of aqueous extract solution add few drops of lead acetate solution and no colour change.

11. Test for Alkaloids

The extract is evaporated separately. To the residue dilute HCl was added and it was shaken well and filtered. The Following tests were performed.

- **Dragendorff's reagent**

To 2-3ml of filtrate few drops of dragendorff's reagent were added and no precipitate was observed. It indicates the absence of alkaloid.

- **Mayer's test**

To 2-3ml of filtrate few drops of Mayer's reagent were added and no precipitate were observed. It indicates the absence of alkaloid.

- **Hager's Test**

To 2-3ml of filtrate few drops of Hager's reagent (saturated solution of picric acid) were added and no precipitate was observed. It indicates the absence of alkaloid.

- **Wagner's Test**

To 2-3ml of filtrate few drops of Wagner's reagent were added and no precipitate was formed. It indicates the absence of alkaloid.

12. Test for Amino Acids

- **Ninhydrin Test**

3ml of the test solution was heated with 3 drops of 5% ninhydrin solution in a boiling water bath for 10 minutes and then the colour was observed and shows the presence of Amino acids.

- **Millon's Test**

To the test solution about 2ml of Millon's reagent was added. A white precipitate was obtained indicating the presence of amino acids.

Pharmacological Action

Diuretic Activity

Diuretic activity was determined by the following methods of Kau *et al.*, with minor modifications. The rats were randomly divided into four groups of six animals each as follows:

Group-I Control – given 5 ml/kg body weight of de-ionized water;

Group-II Aqueous extract – 200 mg/kg body weight;

Group-III Aqueous extract – 400 mg/kg body weight; and

Group-IV Furosemide – 100 mg/kg body weight.

Determination of Diuretic Activity

Lipschitz Method

Wistar albino strain rats of either sex weighing 100 - 150gm were maintained under standard condition of temperature and humidity. 3 groups of six rats in each were fasted and deprived of water for eighteen hours prior to the experiment. The first group of animals serving as control received normal saline (5ml/Kg), the second and third group received extract at the doses of

200mg/kg and 400 mg/Kg body weight respectively in normal saline route and the fourth group received furosemide (100mg/Kg) in saline. The urine was collected in measuring cylinders up to 5 hrs after dosing. During this period, no food or water was given to animals. The parameters was taken for individual rat per body weight before and after test period. Total concentrations of Na^+, K^+, and Cl^- in the urine sample were measured by Flame photometry.

Measurement of Urine Output and Analysis of Electrolytes

Na^+, K^+ and Cl^- concentrations are measured using flame photometer. The instrument was calibrated with standard solutions containing different concentrations of Na^+, K^+ and Cl^-

RESULT AND DISCUSSION

Pharmacognostic Studies

Preliminary Phytochemical Screening

The plant extracts were tested for the presence of various phytochemical components which were reported to possess distinctive medicinal properties. The plant extracts contains proteins, carbohydrates, lipids, steroids, etc. Thus proves the existence of phytochemical constituents in the extracts.

Statistical Analysis

The results are expressed as mean values ± S.E.M. (standard error of mean). Statistical comparison was carried out by Analysis of Variance (ANOVA). The difference between the means of treated groups and the non-treated control group was evaluated by the Tukey-kramer multiple comparison test. The results were considered statistically significant when *P < 0.001.

Results of the Phytochemical constituents of leaves of *Ficus Religiosa* Linn.

S.No	Constituent	Aqueous Extract Of *Ficus Religiosa* Linn
1.	CARBOHYDRATES	+ ve
2.	FATS AND OILS	-ve
3.	PROTEIN	+ ve
4.	SAPONINS	+ ve
5.	STEROIDS	+ ve
6.	ALKALOIDS	+ve
7.	GLYCOSIDES	-ve
8.	FLAVONOIDS	+ ve
9.	TANNINS AND PHENOLIC	+ ve
10.	AMINO ACID	+ ve
11.	TERPENOIDS AND ESSENTIAL OILS	+ ve

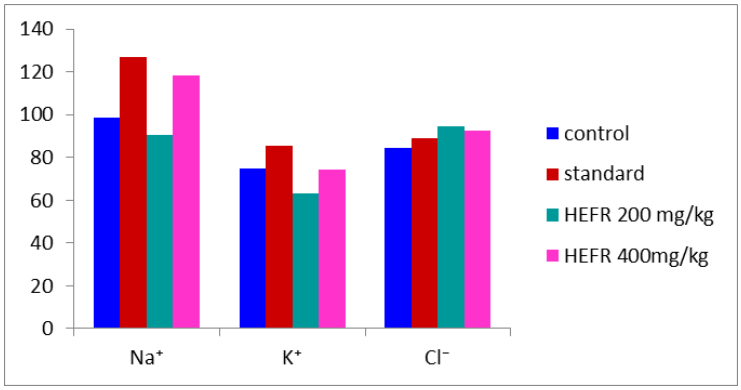
+ ve = Presence;
- ve = Absence

Result For Diuretic Activity

S.NO	Urine Sample	Volume of urine in ml	Na^+ Level µequ/L	K^+ Level µequ/L	Cl^- Level µequ/L
1.	Control (Normal saline)	2.1±0.412	98.41±0.21	74.64±1.43	84.59±2.0
2.	Standard (100mg/kg b.w) (Furosemide)	3.19±0.45	127.1±0.58*	85.31±0.72*	89.22±0.46
3.	Low Dose (200mg/kg b.w) (Aqueous Extract)	1.60±0.313	90.45±3.45	63.22±1.32	94.65±0.72
4.	High Dose (400mg/kg b.w) (Aqueous extract)	2.50±0.321	118.45±0.95*	74.43±0.71*	92.63±0.73

Each value represents the mean ± SEM of six rats *P<0.001. The difference between the means of treated groups and the non-treated control group was evaluated by the Tukey-kramer multiple comparison test.

Effect of Hydroalcoholic extract of *Ficus Religiosa* leaves, and Furosemide on excretion of Na^+, K^+ and Cl^- ions in urine.



HEFR= Hydroalcoholic Extract of leaves of *Ficus Religiosa* Linn

SUMMARY AND CONCLUSION

The leaves of *Ficus Religiosa* Linn was selected, collected, authenticated, shade dried, granulated and extracted with mixture of distilled water and ethanol (1:1), as per the procedure.The hydroalcoholic extract was subjected to preliminary phytochemical analysis and it reveals the presence of Carbohydrates, Terpenoids, Proteins, Saponins, Steroids, Flavonoids, Tannins and Phenolic, Amino acids, Essential oils.

The hydroalcoholic extract of leaves of *Ficus Religiosa* Linn was subjected to diuretic activity at the dose of 200 and 400mg/kg body weight. In Both doses, *Ficus religiosa* exhibits the diuretic activity in a dose dependent manner. The diuretic activity produced by the hydroalcoholic extract 400mg/kg body weight is compared with the standard dose of furosemide (100mg/kg)

It is evident that, the hydroalcoholic extract of *Ficus Religiosa* Linn exhibits the diuretic activity and the results were compared to that of standard drug, Furosemide. The diuretic activity of aqueous extract of *Ficus Religiosa* Linn may be due to the presence of flavonoids.

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