Original Article

Formulation And Anti Inflammatory Studies Of Newly Formulated Arishta From Ficus Religiosa Root Bark

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Abstract

Introduction: Ficus religiosa is a traditional medicinal plant used as a remedy for several ailments. Arishta is one of the unique and valuable therapeutic alignment, due to its efficacy, stability and its desirable features.

Methods: This study is to formulate an arishta from hydro alcoholic extract of Ficus religiosa root bark and then formulated arishta was estimated by a validated HPTLC method. Pharmacological evaluation of arishta was also done for evaluating its anti-inflammatory activity. The extraction was done by using different solvents like, hexane, petroleum ether, chloroform, ethanol and water, by soxhlet extraction method. Arishta was prepared as per Ayurvedic pharmacopoeia. HPTLC studies were carried out by using CAMAG HPTLC system and mobile phase used was toluene: ethyl acetate: formic acid: methanol (14:10:2:1). Pharmacological evaluation was done for finding its anti inflammatory activity by SRBC membrane stabilization method and Carrageenan induced paw oedema method.

Results: Arishta was prepared from Ficus religiosa root bark. And HPTLC study helps to identify the marker compounds present in the formulated arishta of Ficus religiosa root bark. The proposed HPTLC method was found to be simple, precise, accurate, robust etc. and is suitable for estimation of herbal formulations. Formulated arishta at a concentration of 800µg/ml shows a good protection activity in SRBC membrane stabilization method and a concentration of 2ml/kg shows anti inflammatory activity in Carrageenan induced paw oedema method.

Conclusion: Arishta was prepared from hydro alcoholic extract of Ficus religiosa root bark, which shows a good anti inflammatory activity compared to NSAIDS, and HPTLC studies showed presence of tannins and phenolic compounds as marker compounds.

Keywords: Ficus religiosa, HPTLC, arishta, anti inflammatory activity, SRBC membrane stabilization method, Carrageenan induced paw oedema method

INTRODUCTION

Phytoconstituents from medicinal plants play a vital role in healthcare sector and the favorable effects of plants are mainly due to the presence of secondary metabolites which provide health promoting properties [1]. A variety of active phytoconstituents were identified from Ficus religiosa which including Flavonoids, polyphenols, tannins, terpenoids, saponins, steroids, glycosides etc., and are found to be safe and effective for the therapeutic activity. Ficus religiosa is one of the important traditional medicines that have been used as a remedy for various diseases.

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Ficus religiosa has many properties includes, antimicrobial, antiulcer, antidiabetics, antiasthmatics, antioxidant, anti-inflammatory, wound healing, hepato protective, memory enhancing and anti arthritic [2-4].

In this present study, various extracts of Ficus religiosa root bark was prepared in order to find out which all the constituents are present and responsible for the various activities. Here decoction of Ficus religiosa root bark was taken to prepare arishta which has been produced in accordance with Ayurvedic pharmacopoeia. From herbal formulation separation, identification and estimation of chemical component is very difficult. Literature survey revealed that marker compounds have serious pharmacological properties. TLC and HPTLC has become the efficient analytical tool for the analysis of phytochemical constituents from herbs. So with the aid of this instrument, the estimation of prepared arishta was carried out and the method has been validated. The experiment also aims to determine the anti inflammatory property of the formulated arishta of Ficus religiosa root bark (L.) The anti inflammatory activity was planned to evaluate by invitro and invivo methods such as membrane stabilization test and Carrageenan induced paw edema methods.

Materials And Methods

Plant profile

Ficus religiosa is a species of fig native to the Indian subcontinent belong to family Moraceae, popularly known as 'bodhi tree' or peepal tree and has got religious and medicinal importance in Indian culture. Because of its contribution in historical events it has an important place in medicinal, mythological and religious systems of India. Almost every part of the tree is rich in phytochemicals and is used in various food and herbal medicinal preparations. Fruits of Ficus religiosa are rich in phytochemicals like flavonoids; terpenoids etc., and used to cure respiratory and digestive disorders. Leaves contain Flavonoids; tannins etc., which effectively cure diseases like vomiting, antivenom, inflammation and the barks are used as antibacterial, astringent, antidiarrhoeal, in the treatment of gonorrhea etc [5-9].

Figure 1: Ficus religiosa

The root bark of the plant Ficus religiosa was collected from Kozhikode, Kerala, India. The plant was authenticated by Dr. A.K. Pradeep, Associate Professor, Dept. of Botany, and Calicut University with specimen No. 88488. The root bark was washed thoroughly with tap water, shade dried and then pulverized to fine powder. It is stored in air tight container for further study. The macroscopic and physiochemical evaluation were carried out to know the shape, size, colour, odour, taste, fracture of the material, ash value, loss on drying, foreign organic matter etc., by using standard procedures.

The root bark was collected, washed, adhering foreign matters were removed. Then root bark was shade dried for 2 weeks. Powdered the crude drug and stored in closed vessel for further use. Accurately weighed 50gms of crude drug were packed in a thimble of soxhlet apparatus and successively extracted by 250ml of solvents in increasing polarity order for 72 hrs by Hexane, Pet. ether, Chloroform, Ethanol and Water. Each extract was concentrated by distilling off the solvent and then evaporating the solvent to dryness to become residue. Then calculate the percentage yield of the extract. Phytochemical screening tests of different extracts were carried out using standard procedures.

Preparation of arishta

About 50gm of dried root bark powder was extracted with 250ml of ethanol- water mixture (70:30) using soxhlet apparatus for 72hrs. Then the extract was concentrated by

evaporating the solvent and the obtained extract was weighed and kept in a desiccator.

The dried extract was mixed with boiled and cooled water for preparing kashaya of extract. This kashaya was strained and poured into a ghee smeared fermentation vessel and 25ml of jaggery syrup was added into the vessel along with 450mlof water. After that, finely powdered Prakshepadravya was added into it. Mixed well and at the end, washed Dhatakipushpa was added. The mouth of the vessel was sealed by clay smeared cloth and wound by air tight lid. The container was kept in a pit in the soil for 30 days in a constant temperature. After the specified period, lid was removed and examined for fermentation. The fluid was first decanted and then strained after 2-3 days. After the settling of fine suspended particles, it was strained again and bottled.

Table 1: Formulation composition

Ingredients	*Qty.*
Ficus religiosa (drug) extract	20g
Guda (Jaggery)	60g
Prakshepadravya	
Marica (black pepper)	1.5g
Nagara (dried ginger)	1.5g
Jeeraka (cumin seeds)	1.5g
DhatakiPushpa (woodfordia flower)	1g
Water	450ml

Validation of arishta using HPTLC

Preparation of working standard solution:

The working standard solution of ellagic acid and catechin (100µg/ml) was prepared by dissolving specified quantity in methanol and adjusted the volume as per standard procedure.

Preparation of sample solution:

Likewise the formulated arishta was also dissolved in methanol and a sample solution also prepared.

HPTLC analysis

With the help of Halmiton syring and CAMAG Linomat 5 instruments, 10µl of test solution was loaded as 5mm band width in 20×10 cm silica gel 60 F 254 TLC plate. And it was kept in TLC twin trough developing chamber and developed in the mobile phase of toluene- ethyl acetate- formic acid-methanol (14:10:2:1), up to 90mm. then dried and kept in photo documentation chamber and captured the image at white light, UV 254nm, UV 366nm. The plate was scanned at UV 254nm before and after derivatization, then the peak table and peak display were noted. Derivatization was done by spraying with 20% sodium carbonate solution and dried by Folin- ciocalteu reagent and dried at 1000C in hot air oven. The developed method was validated as per ICH guidelines for determining its accuracy, precision, LOD, LOQ, robustness, specificity etc.

Pharmacological anti inflammatory activity study

Invitro anti inflammatory study using Sheep Red Blood Cell Membrane Stabilization method (SRBC)

The blood will be collected and mixed with equal volumes of Alsever solution, then centrifuged at 3000 rpm. The packed cells washed with isosaline and 10% suspension of various concentrations of formulation will be prepared. To each concentration 1ml of phosphate buffer, 2ml hyposaline, 0.5 ml HRBC suspension will be added and incubated at 37ºC for 30 minutes and centrifuged for 20 minutes. The haemoglobin content of supernatant solution will be estimated spectrophotometrically at 560 nm by taking Diclofenac as reference standard.

Invivo anti inflammatory study by Carrageenan Induced Paw Oedema in Rats

The animal's experimental protocol has been approved by Institutional Animal Ethics Committee (IAEC) registration no: DAMCOP/IAEC/035.

The acute oral toxicity study was carried out on Swiss Albino mice as per the guidelines No: 425 given by the organization for Economic Co-operations and Development (OECD 425, 1988). A limit test at one dose level of 2 ml/kg. body weight was carried out with six animals (Three animals per step) and they were fasted overnight. Animals were observed individually after dosing at least once in the first 30 minutes periodically during the first 24 hrs, with special attention given during first 4 hrs and daily thereafter for 14 days.

Divide the Wistar rats into four groups having six animals in each group. Treat the animals of group-1 (control group) with normal saline solution, group-2 (standard drug treated group) with Indomethacin (10 mg/kg, p. o.), and group-3 with hydro alcoholic extract of Ficus religiosa root (250mg/kg) bark and group-4 with test sample (2ml/kg p. o.). Group II and Group IV animals was injected 0.1 ml of 1% Carrageenan solution in 9% saline, 30 minutes after the drug is given. The paw volume was measured using digital vernier calipers at 15, 30, 60, 120 and 180 minutes after Carrageenan administration. The difference in the left and right paw thickness indicates the degree of inflammation and percent inhibition is calculated.

Results And Discussion

Results

We have conducted the study on macroscopic and physiochemical evaluation of powdered Ficus religiosa root bark and found to be within the limit. Ficus religiosa root bark was extracted using hexane, pet. ether, chloroform, ethanol and water, to find out the presence of valuable phytochemical constituents present in it by soxhlet extraction procedure. Out of these five extracts, ethanolic extract showed the presence of alkaloids, flavonoids, phenols, tannins, carbohydrates, and glycosides etc., which are at maximum.

Table 2: Physiochemical parameters

Parameter	*Value:*	*Limits:*
Foreign organic matter	1.2%	NMT 2%
Loss on drying	0.1%	
Total ash	4.5%	NMT 7%
Acid insoluble ash	0.17mg	NMT 0.3mg
Water insoluble ash	0.14mg	NMT 50mg
Sulphated ash	0.25mg	NMT 0.5 mg
Alcohol soluble extractives	6.2%	NMT 8%
Water soluble extractive	6.8%	NMT 9%

Table 3: Phytochemical screening of extracts

Phytoconstituents	*H.E*	*P.E*	*C.E*	*E.E*	*A.E.*
Alkaloids	-	-	-	+	+
Flavanoids	-	-	-	+	-
Phenols	-	-	-	+	+
Saponins	-	-	-	-	+
Tannins	-	-	-	+	+
Glycosides	-	-	-	+	-
Carbohydrates	-	-	+	+	+

H.E.- Hexane extract P.E. – Petroleum ether extract C.E. – Chloroform extract E.E.- Ethanol extract A.E. – Aqueous extract
+ :- Present - :- Absent

The work attempts to optimize the simultaneous HPTLC profiles of secondary metabolites in newly formulated arishta of Ficus religiosa root bark. The presence of active ingredients responsible for biological activities are identified by determining the Rf values using HPTLC (Table 4, 5) and was detected at 254nm.

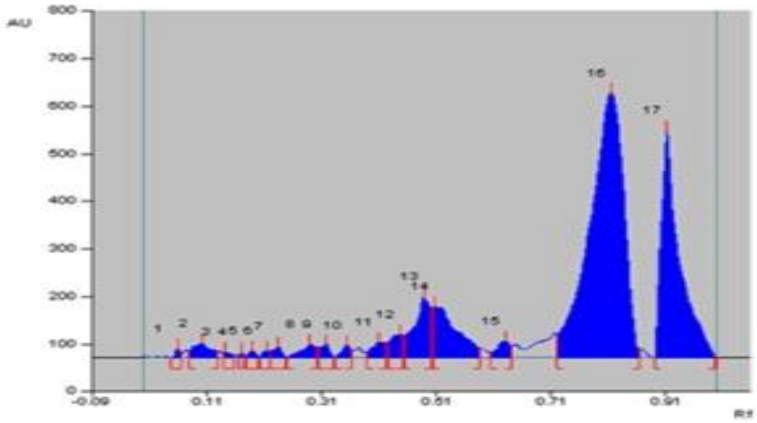

Figure 2: HPTLC Chromatogram

The newly formulated arishta evidenced 17 spots with corresponding Rf values ranging from 0.01-0.82. The bands with Rf values 0.11, 0.29, 0.43, 0.50 and 0.76 revealed the presence of phenolic compounds. Rf value 0.50 showed the presence of catechin in Ficus religiosa root bark and different types of tannins have been observed at Rf values 0.14, 0.33 and 0.53 and peak at 0.82 revealed the presence of gallic acid derivative. Rf value 0.80 +- 0.04 validated the presence of ellagic acid which is a dimeric derivative of gallic acid. In the same way Rf values ranging 0.33 and 0.92 showed the presence of saponins, 0.23 showed the presence of steroids and 0.93 indicated the presence of alkaloids in the formulated arishta.

Table 4: Method validation of HPTLC fingerprinting technique using standard ellagic acid and catechin

Method property	*Ellagic acid*	*Catechin*
Rf	0.79	0.48
Accuracy/ Recovery study	0.5132	0.4122
Intraday precision (RSD [%] n = 6)	0.032	1.19
Interday precision (RSD [%] n = 6)	0.048	0.052
Correlation coefficient, r	0.996	0.993
Calibration range [ng]	300–700	300–800
LOD	300ng/ml	300ng/ml
LOQ	900ng/ml	900ng/ml
Specificity	Specific	Specific
Robustness	Robust	Robust

method

The developed HPTLC method was validated as per ICH guidelines. In this, we have validated various parameters like accuracy, precision; LOD, LOQ, Specificity, robustness etc., and the reported values are found to be within the limit.

Evaluation of anti inflammatory activity

Invitro method – SRBC Membrane Stabilization

Formulated arishta at different concentrations (200, 400, 600 and 800 µg/ml) shows significant stabilization compared with same concentration of reference drug Diclofenac sodium. At maximum concentration of Arishta showed less heamolytic activity and a good protection activity, which showed the anti inflammatory activity of formulated Arishta (Table 5 and 6)

Table 5: % of Haemolytic activity

	% anti inflammatory activity			
	200 µg/ml	400µg/ml	600µg/ml	800µg/ml
Arishta	54. 83	49.32	41.15	36.71
Diclofenac sodium	15.41	12.33	09.52	03.17

Table 6: % of Protection activity

	% anti inflammatory activity			
	200µg/ml	400µg/ml	600µg/ml	800µg/ml
Arishta	48.65	53.02	61.15	69.71
Diclofenac sodium	69.14	74.33	83.52	91.11

Invivo method- Carrageenan Induced Paw Edema in Rats

The paw thickness of the animal was measured at different time intervals after treatment with formulation (Table 7). The difference in the paw thickness was calculated and decrease in paw thickness was found with increase in time. Group IV (which was treated with formulation) showed greater decrease in paw thickness than that of group treated with extract alone. Mean of paw difference was calculated using Oneway ANOVA method. Group treated with standard drug (positive control) showed extreme significance from 60 minutes, group IV showed at 120 minutes and group III (treated with extract alone) showed only at 180 minutes. All the comparison was done with negative control group with is Carrageenan induced untreated group. Percentage inhibition in paw thickness of group IV treated with formulation was greater than that of group III treated with extract alone (Table 8).

Table 7: Mean of difference in paw thickness at different time interval

Groups	*15 minute*	*30 minute*	*60 minute*	*120 minute*	*180 minute*
Group 1 Negative control	1.56±0.01	1.57±0.10	1.31±0.04	1.31±0.03	1.19±0.01
Group 2 Positive control	1.55±0.04 ns	1.29±0.01*	0.61±0.03***	0.23±0.04***	0.1±0.01***
Group 3 Extract	1.42±0.08 ns	1.37±0.02 ns	1.15±0.03*	1.1±0.03**	0.77±0.03***
Group 4 Formulation	1.34±0.18 ns	1.25±0.09*	1.07±0.03**	0.91±0.02***	0.61±0.02***

Values are mean ± S.E.M; Oneway ANOVA followed by Dunnett t test: *p<0.05, **p<0.01, ***p<0.001, ns – non significant. Groups compared with negative control

Table 8: Percentage inhibition of paw oedema at different time interval

Groups	*15 minute*	*30 minute*	*60 minute*	*120 minute*	*180 minute*
Group I	-	-	-	-	-
Group II	0.076%	17.83%	53.16%	82.21%	91.54%
Group III	9.03%	12.46%	19.26%	47.02%	55.58%
Group IV	13.83%	20.19%	49.65%	66.61%	68.94%

The formulation showed extreme significance in decrease in paw thickness at 120 minutes on comparison with negative control. Whereas the extract alone showed extreme significance only at 180 minutes. This reveals that formulated arishta had anti-inflammatory action than hydro alcoholic extract, and this may be due to the presence of Tannins (Figure 3).

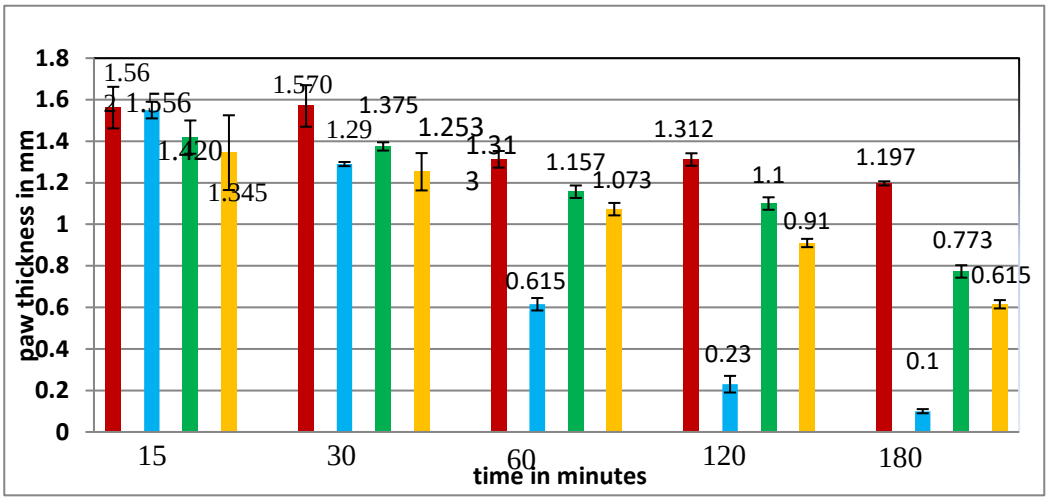

Figure 3: Paw thicknesses Vs Time

Negative control Positive control Extract250 mg /kg Formulation 2ml/kg

Discussion

Ficus religiosa belongs to the family Moraceae and it found throughout tropic and subtropic regions in India. The various parts of the plant have claimed to have several traditional medicinal properties. In this research work, we have found that this statement is very correct and this is correlated with the results that we have gathered.

As a preliminary study, we have conducted a study on various physiochemical parameters of Ficus religiosa root bark powder which includes loss on drying, foreign organic matter, acid insoluble substance and water insoluble substance etc. and found that none of these properties crosses the normal limits prescribed by the official formularies.

The qualitative analysis were carried out to identify the presence of various phytoconstituents in various extracts and the results showed that ethanolic and aqueous extracts consists of maximum and favorable constituents like alkaloids, flavonoids, phenols, tannins, carbohydrates, glycosides. So for the further development of the study, we have selected hydro alcoholic extract.

Arishta is extensively used as a herbal formulation to promote health, immunity and long activity and may have enormous concentration of tannins such as ellagic acid and catechin and it is pertinent to maintain their quality for safety and efficacy. In this research work, we have formulated arishta from the hydro alcoholic extract of Ficus religiosa root bark as per in Ayurvedic Pharmacopoeia.

HPTLC chromatogram of the formulated arishta reveals that the presence of catechin, ellagic acid (gallic acid derivative), steroids and alkaloids, as these constituents are very much responsible for the treatment of various diseases and disorders.

Anti inflammatory activities of formulated arishta were conducted by both invivo and invitro methods. SRBC membrane stabilization method was selected for invitro evaluation of anti inflammatory property of formulated arishta, because erythrocyte membrane was analogous to lysosomal membrane and its stabilization implies that the formulation can stabilize lysosomal membranes. For limiting the inflammatory response, stabilization of lysosomal membrane by preventing the release of lysosomal constituents like bactericidal enzymes, proteases etc were

important. These might cause further tissue inflammation and damage of extra cellular release. The invitro anti inflammatory study results reveals that formulated arishta of Ficus religiosa root bark at various concentrations has significant anti inflammatory activity.

The acute oral toxicity study of formulated arishta was carried out as per the OECD guidelines 425 and it revealed that the arishta was safe up to the dose level of 2ml/kg body weight of animals, and no mortality was observed among the animals used. Dose selection of formulation was made on the basis of acute oral toxicity study as per OECD guideline. The study of invivo anti inflammatory activity was done by Carrageenan induced paw edema method. Indomethazine was taken as standard and the result were compared with it. The result showed that the formulated arishta shows better anti inflammatory activity with high dose than the extract.

Conclusion

In the present study, extraction and its phytochemical screening of Ficus religiosa root bark powder were done and found out maximum phytoconstituents in ethanolic and aqueous extracts. Further one arishta was prepared from hydro alcoholic extract of Ficus religiosa root bark and that was estimated by HPTLC finger printing technique. It was identified that, a number of marker compounds like phenolic, tannins, saponins etc., present in the HPTLC chromatogram of formulated arishta. The proposed HPTLC method was found to be accurate, precise, robust, specific etc. Pharmacological studies were done to find out the therapeutic activity of the formulated arishta. Both invivo and invitro study results revealed that the formulated arishta possess good anti inflammatory activity compared with available NSAIDs.

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