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Research Article

EVALUATION OF ANTIMICROBIAL AND TOPICAL ANTI-INFLAMMATORY ACTIVITY OF EXTRACTS AND FORMULATIONS OF CASSIA TORA LEAVES

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ABSTRACT

Objective: The aim of the present study was to evaluate the antimicrobial and anti-inflammatory activity of Aqueous, alcoholic and hydroalcoholic extracts of *Cassia tora linn.* leaves and their topical ointment preparations.

Methods: Anti inflammatory effect of the leaf extracts and their respective ointment preparations was evaluated topically in rat models using croton-oil-induced inflammation or oedema of the rat ear. Antimicrobial testing of herbal extracts and their formulations was performed for antibacterial and antifungal activities by cup-plate method as per the standard procedure in Indian Pharmacopoeia.

Results: Maximum anti-inflammatory activity was seen in Alcoholic and hydroalcoholic extracts and their ointments. The Aqueous extract was found to show maximum antibacterial action against gram Positive bacteria and good antifungal action. However, they show less antibacterial action on gram negative bacteria

Conclusion: The results obtained from this study indicate that the tested extracts and their ointments have potential anti-inflammatory activity. The aqueous extract and its ointment was found to possess good antibacterial action against gram positive bacteria and good antifungal activity as well.

Keywords: Extracts *Cassia tora linn.* leaves, Formulation, Anti-inflammatory, Antimicrobial activity.

INTRODUCTION

Many Ayurvedic preparations use medicinal plants traditionally for thousands of years now, and are known for their interesting multilevel activities. Amongst the medicinal plants used in Ayurvedic preparations for their therapeutic action, some have been thoroughly investigated and some are still to be explored. Several pharmacopoeias now include monographs of the plant materials. Hence the modern methods of preparing dosage forms require us to not only confirm the medicinal activities of the plant material but also confirm the effectiveness of the modern dosage forms prepared from these plant materials. Also, the WHO has emphasized the need to ensure the quality of medicinal plant products using modern controlled techniques and their comparison with suitable standards [1][2].

Cassia tora Linn. is a well known oriental herb used in traditional medicine which grows up to 1-2 m in height and is found as a weed throughout India[3]. It is commonly known as Chakunda in hindi and bengali, Chakramarda in Sanskrit, and Foetid cassia in English. In Unani, it is known as Sanjisboya. It is found up to the height of 1400 m in Himachal Pradesh and mainly in wastelands, on roadsides, field borders etc. It grows abundantly during rainy season, in dry soil throughout the tropical parts. Flowering/ fruiting of this plant occurs in months of August-October[4]. The leaves of *Cassia tora* linn. contain several anthraquinone glycosides which are well known for their therapeutic anti-inflammatory action. The extracts of *Cassia tora* leaves are also reported to possess purgative action and significant antifungal activity. The plant is also reported to have a significant hepato-protective effect against the toxicity of carbon tetrachloride in rats. The plant has been used as laxative and in treatment of skin disorder [5].

The proposed work aims to study the anti-inflammatory activity and the antimicrobial activity of the leaf extracts and their topical formulation.

MATERIALS AND METHODS

Collection and authentification of plant material [6]

The fresh leaves of *Cassia tora*Linn. were collected in the month of August-September from Marunje village, Tal.-Mulshi, Dist. Pune, Maharashtra, India. These were identified, confirmed and authenticated by DR. P.G. Diwakar, Joint Director of Botanical Survey of India, Pune. The Authentification No. for the plant is (BSI/WRC/Tech/2011/667). Collected fresh leaves were washed, dried, pulverized and used for further studies.

Physicochemical Evaluation [7, 8, 9, 10]

The powder of dried leaves was used for the determination of physicochemical evaluation such as Total Ash value, water-soluble ash value, acid-insoluble ash value, water soluble and alcohol soluble extractive values, loss on drying, which were determined as per Indian Pharmacopoeia and WHO guidelines. The results are reported in Table No 1.

Table 1: Physicochemical Evaluation of Powder of Dried Leaves of *Cassia tora Linn*

S. No.	Physicochemical Parameters	Results
1.	Description	Green colored powder
2.	Ash Values	
	a) Total ash	19% w/w
	b) Water soluble ash value	13%w/w
	c) Acid insoluble ash value	6% w/w
3.	Extractive Values	
	a) Water Soluble Extractive	16.8% w/w
	b) Alcohol Soluble Extractive	4% w/w
4.	Loss on drying	0.513% w/w

Extraction of *Cassia tora* linn. Leaves [11]

The dried leaves powder was extracted by continuous hot extraction method by using Soxhlet apparatus. The solvent selected for the extraction were water, methanol and water methanol mixture (1:1). The dried material (20g) extracted with water, ethanol and water ethanol mixture (1:1) in a Soxhlet apparatus for 3 hours. The extracts were collected and evaporated on water bath and then dried in desiccators. The extracts were labelled as AQE, ALE and HAE for aqueous, alcoholic and hydroalcoholic extracts respectively. The yield obtained for each type of extract is reported in Table No 2.

Table 2: Extracts obtained from different solvents

S. No.	Type of Extract	% Yield of Extract
1.	Aqueous Extract	12.5% w/w
2.	Alcoholic Extract	9.0% w/w
3.	Hydro-alcoholic Extract	9.5% w/w

Formulation of Topical ointment [12]

Chemicals and extracts used for the Formulation

Extract (Aqueous, Alcoholic, Hydro-alcoholic), Emulsifying Wax (Fine Chemicals) Liq. Paraffin White(Fine Chemicals) soft Paraffin (Fine Chemicals).

Preparation method of topical Ointments

Herbal topical Ointments of all three extracts were prepared separately. Trial batches were prepared by using aqueous, alcoholic and hydroalcoholic extracts of *Cassia tora* Linn leaves. The ointments were prepared by fusion method. In this method the constituents of the base were placed together in a melting pan and allowed to melt together at 70°C. After melting, the ingredients were stirred gently maintaining temperature of 70°C for about 5 minutes and then cooled to 45°C and extracts were added and stirred well. The prepared herbal ointments were put in ointment jars, labeled, stored at room temperature and were used for further studies. The formula for the preparation of ointment is given in Table No 3. The formulations were labeled as AQF, ALF and HAF for aqueous, alcoholic and hydroalcoholic formulation.

Table 3: Herbal Topical Ointment Formulation

S. No.	Ingredients	Quantity
1.	Extract (Aqueous/ Alcoholic/ Hydro-alcoholic)	1 g
2.	Emulsifying Wax	30 g
3.	Liq. Paraffin	20g
4.	White soft Paraffin q.s.	100g

Topical Anti-Inflammatory Study

Chemicals

Croton oil and standard drug Hydrocortisone was procured from Sigma Aldrich (St.louis, MO, USA).

Experimental Animals

Male Sprague-Dawley rats with a weight range of 120-150 gm [14] were procured from National Institute of Biosciences, Pune, India. The rats were divided into groups having six rats per group and housed in polypropylene cages at a temperature of 24 ± 1 °C with 12h:12h dark–light cycle, with free access to standard pellet feed (Pranav Agro Industries Ltd., Sangli, India) and purified water. All experiments were carried out between 09:00 h and 17:00 h in a quiet laboratory. The research protocol was approved (No: ACP/IAEC/II/2011/09) by Institutional Animal Ethics Committee (IAEC) and as per Indian norms laid down by Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), New Delhi.

Croton oil-induced rat ear oedema[13]

Male Sprague Dawley rats were used for the study and divided into 8 groups of 6 rats each for getting results with 95% confidence level.

Group 1: Control group, applied with Croton Oil Solution.

Group 2: Standard Drug Hydrocortisone + Croton Oil Solution.

Group 3: Aqueous extract + Croton Oil solution

Group 4:Hydroalcoholic Extract + Croton Oil Solution

Group 5: Alcoholic Extract + Croton Oil Solution

Group 6: Aqueous extract Formulation + Croton Oil solution

Group 7:Hydroalcoholic Extract Formulation + Croton Oil Solution

Group 8: Alcoholic Extract Formulation + Croton Oil Solution

For tests in rats the following croton oil solution was prepared (v/v): 4 parts Croton oil, 10 parts ethanol, 20 parts pyridine, 66 parts ethyl ether. The standard drug and the test extract were dissolved in this solution[14]. Formulation of each extract was applied after application of croton oil irritant solution. Male Sprague-Dawley rats with a weight of 120-150 gm were used. The test compounds were dissolved in a concentration of 10 mg/ml in the irritant solution and the standard drug hydrocortisone was dissolved in a concentration of 1mg/ml in the irritant solution. Irritant solution (0.02 ml) was then applied on both sides of the right ear. Controls received only the irritant solution. The left ear remained untreated. The irritant solution was applied under ether anaesthesia. The animals were sacrificed under anaesthesia after four hours of application. Both ears were removed and discs of 8 mm diameter were punched. The discs were then weighed immediately and the difference in weight between the treated and untreated discs were recorded indicating the degree of inflammatory oedema.

The anti-inflammatory effect has been determined by expressing the change in weight of the treated ear as compared to the untreated ear and also the control group. The discs were weighed and the difference between the right and left ears were determined for each group. Percentage inhibition of ear oedema was calculated relative to the untreated ear as follows.[15]

$$\text{Inhibition (\%)} = \frac{Wc - Wt \times 100}{Wc}$$

Wc = mean of the difference in ear disc weight of control mice.

Wt = mean of the difference in ear disc weight of treated mice.

The difference between both ears or excised discs have been calculated as the average values for treated and control groups and the effect has been evaluated by statistical methods.

Study of antimicrobial activity. [16, 18, 19]

Material

Soyabean casein agar (Himedia), Sabouraud dextrose Agar (Hi Media), Staphylococcus aureus ATCC 6538. *Pseudomonas aeruginosa ATCC 9027, Bacillus Subtilis ATCC 6633, Candida Albicans ATCC 10231, Aspergillus niger ATCC 16404* purchased from National Chemical Laboratory, Pune.

Procedure

Samples of three extracts and their respective ointments were studied for their anti- microbial activity as per standard method in Indian Pharmacopoeia.

Antimicrobial testing of herbal extracts and their formulations was performed for antibacterial and antifungal activities by cup-plate method. For forming inoculum, bacterial Strains were incubated in Soyabean casein digest broth at 30^0C for 24 hrs while fungal Strains (*Candida Albicans ATCC 10231, Aspergillus niger ATCC 16404)* were incubated separately on Sabouraud dextrose agar medium at 25^0C for 48 hrs.

Stock solution of herbal extract and formulation was prepared to get a concentration equivalent to 100 mg herbal extract/ml. Serial dilutions of all extracts (alcoholic, hydro-alcoholic & aqueous) were prepared as 10^{-1}, 10^{-2}, 10^{-3}. Plates with test microorganism were prepared by pour plate method. Bores were prepared by borer no.4. Respective concentration samples were placed in respective cups and the plates were incubated at 25^0C for 24 hours for antibacterial action and 48 hours for testing antifungal action. Zone of inhibition for each extract was observed after incubation period and recorded.

Statistical analysis of anti-inflammatory Activity

Data obtained were expressed as mean ± standard error (SEM). One way ANOVA followed by Dunnett's test was used for statistical analysis. Change in ear weight was considered significant at p-value < 0.05 when compared to control.

RESULTS AND DISCUSSION

Topically applied croton oil is responsible for activation of various pro inflammatory mediators like arachidonic acid metabolites, cytokine, other inflammatory mediators and promoted the manifestation of inflammatory oedema[15, 17]. As shown in the table 4, the alcoholic and hydroalcoholic extracts of *Cassia tora linn* leaves were capable to inhibit one important event, i.e. oedema of inflammation. The standard drug hydrocortisone shows 80%

inhibition of oedema, while aqueous, alcoholic and hydroalcoholic extracts show 27%, 38% and 33 % inhibition and their respective formulations show 30%, 68% and 55% inhibition of oedema, as given in table 4 below. The results show that the anti-inflammatory action of the extracts is less prominent than their respective formulations. This could be due to the fact that as per standard procedures referred to, the extracts have been mixed in the croton oil solution and then applied, while in case of formulations, the croton oil solution was first applied and formulation was applied to the ear immediately thereafter. This indicates that the extracts in formulation get absorbed better resulting in its higher effectiveness.

The Aqueous extract was found to show maximum antibacterial action against gram Positive bacteria and less action on gram negative bacteria. It also shows good antifungal activity as shown in table No.5. The antibacterial action of aqueous extract against *S. aureus* indicates usefulness of the extract for topical applications.

Table 4: Anti-inflammatory activity of extracts and formulations of *Cassia tora linn*. Leaves in croton oil-induced ear oedema in rats.

Treatment	Croton oil-induced ear edema in mice (n=6)		
	Dosemg/ear	Change in ear weight in mg(Mean±SEM)	% inhibition
Control (Croton Oil)	-	9±0.77	-
Hydrocortisone	0.02	1.8±0.31**	80
Aqueous Extract	0.2	6.6±0.92	27
Alcoholic Extract	0.2	5.6±0.67**	38
HydroAcoholic Extract	0.2	6.0±0.36*	33
Aqueous ExtractFormulation	0.2	6.3±0.33*	30
Alcoholic Extract Formulation	0.2	2.8±0.48**	68
HydroAlcoholic Extract Formulation	0.2	4.0±0.86**	55

Values are Mean ± SEM (n=6 in each group) one-way ANOVA followed by Dunnett's t-test: Compare all vs. Control group (reference drug): *P<0.05; **P<0.01 compared with control.

Table 5: Antimicrobial Activities of Extracts and Formulations.

S. No.	Sample Type	For Gram +ve(*S. aureus*)	For Gram -ve(*E. coli)*	For Fungi (*C.albicans*)
1	Aqueous Extract	+++	+	++
2	Aqueous Extract Formulation	++	+	+
3	Hydro-alcoholic Extract	+	-	+
4	Hydro-alcoholic ExtractFormulation	+	-	+
5.	Alcoholic Extract	-	-	-
6	Alcoholic Extract Formulation	-	-	-

CONCLUSION

This study of anti-inflammatory activity and antimicrobial activity of *Cassia tora linn.* leaf extracts and their respective formulations shows that all three extracts, i..e. aqueous, alcoholic and hydroalcoholic posses anti-inflammatory activity. However, the alcoholic and hydroalcoholic extracts and their formulations show more anti-inflammatory activity as compared to the aqueous extract. On the other hand, the aqueous, hydroalcoholic extracts and their formulations show significant antimicrobial activity against fungi and gram positive bacteria, which is useful for topical preparations. Thus, this indicates further scope for study of hydroalcoholic extract in topical preparation in appropriately increased concentrations for increased therapeutic effect.

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