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EXTRACTION, PHYTOCHEMICAL SCREENING AND *IN VIVO* ANTIINFLLAMATRY ACTIVITY OF HYDROALCOHOLIC EXTRACT OF WHITE CATECHU

Sugam Pathak*, Harish Pandey, Prabhakar Budholiya

College of Pharmacy, Sri Satya Sai University of Technology and Medical Sciences, Sehore (M. P.)

*Corresponding Author's E mail: Sagarpathak2008@gmail.com

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ABSTRACT

Acacia catechu willdis an indigenous plant having tremendous medicinal properties and well-reviewed in Indian literature. *Acacia catechu* bark and heartwood has already been reported to possess manypharmacological activities such as astringent, antioxidant, treating fever, leucorrhoea, piles, erysipelas, haemoptysis, gonorrhea, hepatoprotective, antipyretic and chest infection ect.The anti-inflammatory activity was evaluated by carrageenan-induced rat paw edema method. Acute toxicity of the extract (2000 mg/kg) was examined in wistar rats for 14 days. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenolics and flavonoids were determined by the well-known test protocol available in the literature. Quantitative analysis of phenolic and flavonoids was carried out by FolinsCiocalteau reagent and aluminium chloride method respectively. Phytochemical analysis revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids. The total phenolics content of *A. catechu* extract was (1.984mg/100mg), followed by flavonoids (0.865mg/100mg) respectively. Hydroalcoholic extract up to 2000 mg/kg did not produce any toxic effects. The hydroalcoholic extract of *A. catechu* (100 and 200 mg/kg) inhibited the inflammation induced by carrageenan in rats in a dose dependent manner. The hydroalcoholic extract of *A. catechu* possesses a strong anti-inflammatory activity and may be considered an interesting source of effective anti-inflammatory compounds.

Keywords: *Acacia catechu* willd, Acute toxicity, Anti-inflammatory effect, Phytochemical screening, FolinsCiocalteau reagent.

INTRODUCTION:

India is a rich source of medicinal plants and a number of plantderived extracts are used against diseases in various systems ofmedicine such as Ayurveda, Siddha and Unani. Only a few of themhave been scientifically explored. Plant derived natural productssuch as alkaloids, tannins, terpenes and flavonoids[1]have received considerable attention in recent yearsdue to their diverse pharmacological properties including analgesic,inflammatory and antioxidant activities.Pain is a pathophysiological response of living tissue to undesirablestimuli. The pharmacology of pain has become a complexfield. More recently, completely synthetic compounds based onnatural pharmacophores have been introduced into the marketbut, research and medical fields still struggle with side-effect profiles from these anti-inflammatory substances that are undesirable. Therefore, development of newer and more substantial anti-inflammatory drugs with lesser side-effects is necessary. *Acacia catechu* Willdis an evergreen tree,

which belongs to the family *fabacaae*, and sub family *mimosiaceae*[2]. It possesses various pharmacological actions. The bark, heartwood, leaves of the plant exhibits antioxidant, hypotensive, and antimicrobial, hepato-protective, anti-cancer, antiviral, gastro protective activity[3-7].The extract of this plant is used to treat sore throat, diarrhea, dysentery, colitis, gastric problems, bronchial asthma, cough, leucorrhoea and leprosy[8]. It is used as mouthwash for mouth, gum, sore throat, and gingivitis, dental and oral infections. *A. catechu* leaf, bark and heartwood extract are active against various oro dental pathogens that are responsible for causing dental caries/plaque[9, 10]. Therefore, the present study was designed to investigate anti-inflammatory activities of hydroalcoholic extract of twigs of *A. catechu* by using carrageenan-induced rat paw edema model.

Materials and methods

Plant material

Barksof *A. catechu*were collected from area adjoining forests of Bhopal in the month of March.

Chemical reagents

Diclofenac sodium (Themis Pharmaceuticals, Mumbai), Carrageenin (Sigma Chemical Co, St Louis, MO, USA) were used in present study.All otherchemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), SigmaAldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade.

Extraction by maceration process

50 gm of *A. catechu* dried bark were exhaustively extracted with hydroalcoholic extract solvent (methanol 70%) and using drug-solvent ratios (1:2) using hot maceration process (10hrs.).The extracts were evaporated above their boiling points. Finally, the percentage yields were calculated of the dried extracts.

Phytochemical screening

Phytochemical screening to detect the presence of bioactive agents was performed by standard procedures[11, 12]. After the addition of specific reagents to the solution, the tests were detected by visual observation of color change or by precipitate formation.

Total phenolic contents

The total phenolic content was determined using the method of Olufunmiso et al [13]. A volume of 1 ml of *A. catechu*bark extracts or standard was mixed with 1ml of FolinCiocalteau reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (75g/l) of sodium carbonate. The mixture was allowed to stand for 15 min under room temperature. The blue colour developed was read at 765 nm using UV/visible

spectrophotometer. The total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as gallic acid equivalent (mg/g).

Total flavonoid contents

The total flavonoid content was determined using the method of Olufunmiso et al [13]. 1 ml of 2% AlCl3 methanolic solution was added to 3 ml of extract or standard and allowed to stand for 60 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/g).

Animals

In the present investigation the Wistar rats (150-200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Acute oral toxicity

Acute oral toxicity was performed according to Organization for Economic Co-operation and Development (OECD) guideline No. 420(OECD 420, 2011). Wistar rats fasted overnight, accessing water *ad libitum* were used in this study. The extract was administered orally at a dose of 2000 mg/kg body weight and the animals were observed for mortality or any abnormal behavior for first 24 h, then for next 14 days. Further behavioral responses, neurological responses as well as autonomic responses were observed[14].

Carrageenan induced hind paw oedema

The animals were divided into four groups of six animals each and were fasted for a period of 24 h prior to the study. Group 1 was treated as control (0.1 ml of 1% (w/v) of carrageenin subcutaneously), Group 2 was received diclofenac sodium 30mg/kg, p.o. Group 3 and Group 4 were treated with 100 and 200 mg/kg/p.o. Oedema was induced by injecting 0.1 ml. of a 1% solution of carrageenan in saline into the sub plantar region of the right hind paw of the rats. The volumes of oedema of the injected and the contra lateral paws were measured at time interval after the induction of inflammation using a plethysomgraph to calculate the percentage of paw oedema inhibition.

$$\text{Percentage Inhibition} = \frac{Vc - Vt}{Vc} \times 100$$

Where, Vc- Edema volume of control group, Vt- Edema volume of test group

Statistical Analysis

All analysis was performed using graph pad prism for windows. All statistical analysis is expressed as mean ± standard error of the mean (SEM). Data were analyzed by one way ANOVA, where applicable $p<0.05$ was considered statistically significant, compared with vehicle followed by Dunnett's test.

Results and discussion

The crude extracts so obtained after hot maceration extraction process was concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extract. The yield of extracts obtained from the leaves of the plants using hydroalcoholic (Water: Methanol 30:70) as solvents are depicted in the Table 1. The results of qualitative phytochemical analysis of the crude powder of barks of *A. catechu*are shown in Table 2. The determination of the total phenolic content, expressed as mg gallic acid equivalents and per 100 mg dry weight of sample. TPC of hydroalcoholic extracts of barks of *A. catechu* showed the content values of 1.984. The total flavonoids content of the extracts was expressed as percentage of quercetin equivalent per 100 mg dry weight of sample. The total flavonoids estimation of hydroalcoholic extracts of barks of *A. catechu* showed the content values of 0.865Table 3& Fig. 1 and 2.No adverse changes and mortality were observed in animals, which orally received hydroalcoholic extract (2000 mg/kg) of *A. catechu*. This indicates that 2000 mg/kg is maximum safe dose. So1/20th and 1/10th*i.e.*100 and 200 mg/kg of body weight of the maximum safe dose were selected for studying *in vivo* anti-inflammatoryeffects.Carrageenan-induced acute inflammation is one of the most suitable test procedures to screen anti-inflammatory agents. The time course of edema development in carrageenan-induced paw oedema model in rats is generally represented by a biphasic curve [15]. The first phase of inflammation occurs within an hour of carrageenan injection and is partly due to the trauma of injection and also due to histamine and serotonin component. Table 4 & Fig 3 shows the effect of hydro-alcoholic extract of *A. catechu*and diclofenac sodium (standard drug) as compared to carrageenan control.

Table 1 Percentageyield of extract by maceration (%w/w)

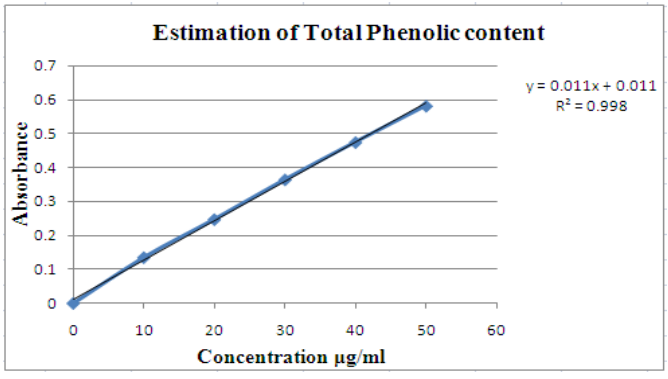
S.No.	Ratios	Hydroalcoholic
1.	1:2	4.98

Table 2 Phytochemical screening of extract of *A. catechu*

S. no.	Constituents	Hydro alcoholic extracts
1.	**Alkaloids**	
	Dragendroff's test	-ve
	Wagner's test	-ve
	Mayer's test	-ve
	Hager's test	-ve
2.	**Glycosides**	
	Generalglycosides test	-ve
3.	**Flavonoids**	
	Lead acetate test	+ve
	Shinoda test	+ve
5.	**Tannins and Phenolics**	
	5% $fecl_3$ test	+ve
6.	**Amino acids**	
	Ninhydrin test	-ve
7.	**Cabohydrates**	
	Molichs test	+ve
8.	**Diterpines**	-ve

Table 3Estimation of total phenolics and total flavonoids content

Extracts	Total phenolic content (mg/100mg of dried powder)	Total flavonoids (mg/ 100 mg of dried extract)
Acacia catechu	1.984	0.865



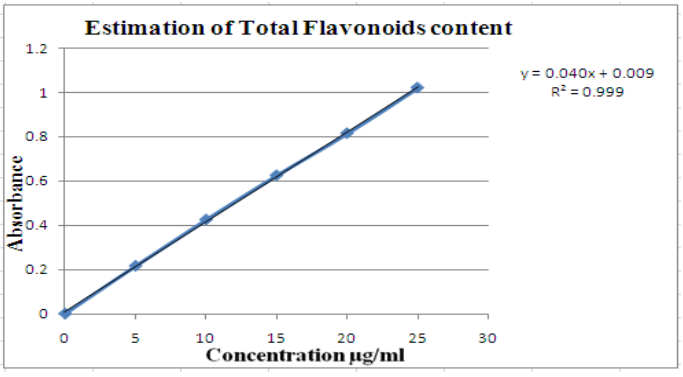
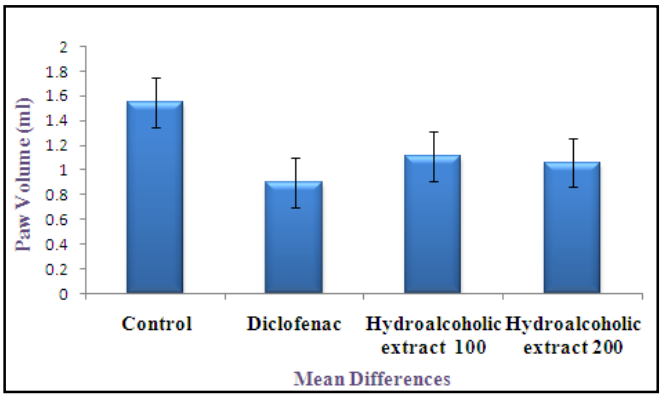


Fig1 Graph of estimation of total phenolic content **Fig2 Graph of estimation of total flavonoids content**

Table 4*In vivo* anti-inflammatory activity by using Carrageenan-induced paw edema model

Treatment	Dose (mg/kg)	Mean differences in Paw Volume (ml)	Percentage of Inhibition (%)
Control	0.1 ml of 1% (w/v)	1.55 ± 0.08	--
Diclofenac	30	0.90 ± 0.36*	96.93
Hydroalcoholic extract	100	1.11 ± 0.12	83.38
Hydroalcoholic extract	200	1.06 ± 0.09*	86.61

Each data suggests Mean ± SEM (n=6). One-way ANOVA using Dunnett's test is applied for statistical analysis, Treatment groups compared with Control group.Significant at* $p < 0.01$, compared to control group.



Each data suggests Mean ± SEM (n=6). One-way ANOVA using Dunnett's test is applied for statistical analysis, Treatment groups compared with Control group. Significant at $p < 0.01$, compared to control group.

Fig 3 Effect of different compounds on paw edema induced by carrageenan in rats

Conclusion

Altogether, the present study results confirmed that *A. catechu*possess significant anti-inflammatory activity, which may be devoted to major secondary active metabolite present in it. In conclusion we suggest that the future studies on*A. catechu*could be useful for the management of inflammatory diseases and oxidative stress.

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