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Studies of preliminary phytochemical and Anti-arthritic activity of heart wood of *Cedrus deodar* (Roxb.)

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ABSTRACT

The aim of the research was to validate the use of cedrus deodar in external applications in the ayurvedic system of medicine.and conduct a preliminary phytochemical study of the same to help deduce the possible active ingredients.The petroleum ether, chloroform, alcoholic extracts of the heart wood of *Cedrus deodar* were prepared by Soxhlet extractor and examined for its external anti arthritic activity in rats using the freunds adjuvant method. The results of the phytochemical study revealed the presence of alkaloids, flavonoids, glycosides, phenolic compounds, saponins and proteins. Application of all the three extracts exhibited significant inhibition of CFA (Complete Freund's Adjuvant) induced rat paw edema when compared with the arthritic control group. These findings seem to justify the use of the plant in traditional Indian medicine in the treatment of inflammation, including arthritic conditions.

Keywords: Anti- arthritic activity, External application, *Cedrus deodar*, Complete Freund's adjuvant.

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INTRODUCTION

Natural sources of plant origin are still a major part of traditional medicinal systems in developing countries. There is also a resurgence of interest in herbal medicines in western countries as an alternative source of drugs often for intractable diseases such as rheumatoid arthritis .[7] A literature survey on the plant *C. deodara* revealed that there is lack scientific evidence of its usefulness in the external treatment of rheumatoid arthritis. The need for safer and effective analgesic and anti-inflammatory drug and the lack of enough scientific data to support the claims made in ancient literature prompted the present study. (Wealth of India, 1950). Hence the present work was aimed to study the phytochemical properties and anti arthritic activity of different heart wood extracts of *C. Deodara*.

Cedrus deodara (Roxb.), belonging to the family Pinaceae (Hindi-Marathi Deodar;Sanskrit-Devadaru;English-Cedar) is graceful, ornamental evergreen tree growing extensively on the slopes of the Himalayas. C. Deodar forests are common from Kashmir, especially krishnaganga, kishtwar and Jhelum, to Garhwal [1]. The wood of *C. deodara* has been used since ancient days in Ayurvedic medical practice for the treatment of inflammations and rheumatoid arthritis [2]. However, there is not enough scientific data to support the claims made in the ancient literature. During a routine screening of Indian medicinal plants for biological activity, 50% ethanolic extract of the wood of *C. deodara* showed a significant anti-spasmodic activity [3]. Studies were undertaken for the isolation, identification and pharmacological evaluation of the major spasmolytic constituent himachalol [4]. The alcoholic extract of the stem of *C. Deodara* was found to have anti-cancer activity against human epidermoid carcinoma of nasopharynx in tissue culture [3]. The oil of *C. deodara* wood was found to have potent disinfectant [5] and anti-fungal properties [6].

MATERIALS AND METHODS

Extraction

Air dried and coarsely powdered (500 g) of *C. deodar* heart wood were taken. Extraction was carried out in a Soxhlet extractor using petroleum ether (60-80), chloroform and alcohol for 16 hrs. The extracts were then concentrated to dryness under reduced pressure and controlled temperature. Final traces of solvent were removed under pressure by using rotary vacuum flask evaporator and they were preserved in a refrigerator [8, 9].

Chemicals

Complete Freund's Adjuvant (CFA) was purchased from Sigma Aldrich St. Lovis, USA. All other chemicals used were analytical grade and procured from approved chemical supplier.

Animals

Male wistar albino rats weighing between 150-200 g each were used for this experiment. They were procured from Indian Institutes of Sciences, Bangalore, India. They were housed in polypropylene cages and maintained at 27±2°C, relative 65±10% under 12 h light/dark cycles. The animals were allowed to acclimatize to laboratory conditions 48 hrs before the start of the experiment. Groups of 6 rats were used in all sets of experiment. Animals were provided with standard rodent pellet diet (Amrut, India) and the food was withdrawn 18-24 h before the experiment were conducted after obtaining permission from the Institutional Animal Ethics committee (IAEC) of Department of Pharmacognosy, Government College of Pharmacy, Bangalore.

Ointment preparation

The extracts (Petrolium ether, Chloroform, alcohol extracts) were formulated as a 3% ointment using the poly ethylene glycol base.

PHYTOCHEMICAL EVALUATION

The heart wood extracts of *C. deodar* were analysed for the presence of alkaloids, flavonoids, glycosides, phenolic compounds, saponins and proteins [10, 11].

Test for alkaloids

The extracts were stirred separately with few drops of dilute hydrochloric acid and filtered. The filtrate may be tested carefully with various alkaloidal reagents such as Mayer's reagent, Dragendroff's reagent, Hagers's reagent and Wager's reagent. Precipitation in any of the 4 test indicates the presence of alkaloids.

Test for flavonoids

5 ml of dilute ammonia solution were added to a portion of filtrate of plant extract followed by addition of concentrated H2SO4. A yellow coloration absorbed in extract indicated presence of flavonoids. The yellow coloration disappeared on standing.

Test for glycosides (Borntrager's test)

Extract was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. A layer of pink, red or violet colour indicates the presence of glycosides.

Test for tannins and phenolic compounds

Extract of the sample was treated with 5% ferric chloride test solution. The resultant colour was noted. A violet colour indicated the presence of hydrolysable tannin. Or into 1% solution of gelatin containing 10% sodium chloride in a beaker, 0.5 g of extract was added and shaken to dissolve. A white precipitate observed indicates the presence of tannins. Or into 10% lead acetate solution, 0.5 g of the extract was added and shaken to dissolve. A white precipitate observed indicates the presence of tannins and phenolic compounds.

Test for saponin

0.5 g of the plant extract was shaken with water in a test tube. Frothing, which persist on warming was taken as a preliminary evidence for the presence of saponin.

Test for proteins

Dissolve small quantities of various extracts in few ml of water and added with

Million's reagent: Appearance of red colour indicates presence of proteins.

Ninhydrin's reagent: Appearance of purple colour indicates presence of proteins.

Biuret test: Equal volume of 5% solution of sodium hydroxide and 15% solution of copper sulphate were added. Pink colour indicates presence of proteins

Test for lignins

Extracts was treated with alcohol followed by addition of phloroglucinol and hydrochloric acid. Appearance of red colour indicates presence of lignins.

Test for gums and mucillages

About 10 ml of various extracts were treated with absolute alcohol and filtered. Occurrence of precipitation indicates the presence of gums and mucilages.

EVALUATION OF ANTI ARTHRITIC ACTIVITY

Rats were injected sc, 0.05 ml of CFA into planter region of the left hind paw. The changes in the paw volume (left and right) were measured on various days up to 28 days following CFA injection.

Thirty six animals were randomly divided into seven groups as group I, II, III, IV, V and VI containing six animals in each, Group I served as arthritic control by injecting CFA without treatment and maintained on regular rat food and drinking water *ad libitum.* Group II served as control group injecting by CFA and applied PEG base. All remaining groups CFA inducing treatment for 28 days. Group III applied piroxicam gel (Standard) from 1st day to 28th day of CFA

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induction. Group IV, V and VI served as treatment group, received pet ether, chloroform and alcohol extracts ointments respectively. The change in paw volume of edema was measured each time using mercury plethysmograph. The average volume of the edema of treated rats was compared arthritic control. The percentage inhibition of edema for each group was calculated by using the following equation.

$$\% \text{ inhibition of edema} = \frac{N - N1 \text{ X } 100}{N}$$

Where N = Volume of edema in control group
N^1 = Volume of edema in treated group

Statistical Analysis

The results were expressed as mean ± standard error mean (SEM). The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Dunnett's comparison test and $p<0.05$ was considered significant,**$p<0.01$=very significant, *$p<0.05$=significant, number of animals (N) = 6, Comparison are made against Group I arthritic control.

RESULTS AND DISCUSSION

Phytochemical evaluation on *C. deodara* extract revealed the presence of alkaloids, flavonoids, glycosides, phenolic compounds, saponins tannins and proteins. The *C. deodara* did not show the presence of gums and mucilage in any of the extracts that were tested of its presence.

TABLE 1: PHYTOCHEMICAL EVALUATION OF PET ETHER, CHLOROFORM AND ALCOHOLIC EXTRACTS OF *C. DEODARA* HEART WOOD.

Sl.no	Test	Petrolium ether extract	Chloroform extract	Alcoholic extract
1.	Alkaloids	+	+	+
3.	Flavonoids	+	+	+
4.	Glycosides	+	+	+
5.	Phenolic compounds	+	+	+
6.	Saponins	+	+	+
7	Tannins	+	_	+
8.	Gums	-	-	-
9.	Lignin	-	-	-
10.	Proteins	+	+	+

Anti-arthritic effect of petroleum ether, chloroform and alcoholic extracts of *C. deodara* against adjuvant induced polyarthritis is shown in **Table 2.**

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TABLE 2: ANTIARTHRITIC ACTIVITY OF *C. DEODAR* HEART WOOD.

Group no.	Total volume of edema (28 days)	Mean volume ± SEM of edema (28 days)	% of Inhibition
I	96.81	3.45±0.19	-
II	91.49	3.26±0.28	5.50
III	31.92	1.14±0.31**	66.96
IV	52.41	1.87±0.19**	45.79
V	77.65	2.77±0.21	19.71
VI	42.61	1.52±0.24**	55.94

The acute phase of adjuvant induced response measured as the increase in paw thickness of the injected limb over the 28 days after injection of CFA were significantly suppressed by petroleum ether and alcoholic extracts of *C. Deodara* but chloroform extract showed reduction statistically not significant. secondary lesions such as reddening and swelling in forepaws, ears and tails could be graded as moderately severe in the adjuvant treated arthritic control group, and mild in the case of the *C. deodara* extracts applied groups. Secondary lesions were absent in the case of standard group animals applied with piroxicam gel.

CFA induced arthritis is the most widely used chronic test model in which the clinical and pathological changes are comparable to those seen in human rheumatoid arthritis. The effect of *C. deodara* on adjuvant induced arthritis in rats showed that it effectively inhibited the polyarthritis phase as measured by the paw swellings on the injected limbs. It also inhibited the acute phase of CFA induced response (measured by the response every day from the first day 1 following the injection of CFA), confirming its activity against the acute and chronic inflammatory response. It had been considered that CFA induced arthritis is closely related to the formation of antibodies. Thus, from the findings of the test it may be inferred that the *C. deodara* is effective in the delayed immunological response to the constituents of the Mycobacterium which is disseminated after applied to the plantar region. In the test on CFA induced arthritis the *C. deodara* showed less anti-arthritic activity compared to piroxicam gel. Its anti-arthritic activity in adjuvant induced arthritis confirms its effectiveness against arthritic condition.

CONCLUSION

The studies reveal that the petroleum ether is better than that of chloroform and alcoholic extracts of *C. deodara* heart wood in respect to their antiarthritic activity. Phytochemical analysis confirms that the presence of alkaloids, flavonoids, glycosides, phenolic compounds saponins and proteins.

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