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ANTI MICROBIAL, ANALGESIC ANTI INFLAMMATORY ACTIVITY AND ANTI OXIDANT ACTIVITY OF LEAVES EXTRACT OF *CASSIA AURICULATA*

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ABSTRACT
Cassia auriculata is traditionally used for the treatment of body pains, chest complaints, cough, diabetes, diarrhoea, headaches, heart diseases and toothaches. The present study anti microbial activity was performed on gram positive and gram negative organism using disc diffusion, analgesic and anti inflammatory activity using tail flick, paw oedema volume method, Anti oxidant activity of leaves extract of *Cassia auriculata* using DPPH Assay method compare the results with the standard ascorbic acid.

KEYWORDS: *Cassia auriculata* anti oxidant activity, DPPH activity.

INTRODUCTION
Cassia auriculata Thunb. (*Cassia auriculata*) is one of the traditional medicines (TM) widely utilized in the tropics for various human diseases and ailments. Due to its popularity as TM in southern Africa, the roots of Cassia auriculata are sold as herbal medicines in informal herbal medicine or Muthi shops in the Gauteng province in South Africa.[1] Traditional medicine is defined as the indigenous knowledge, skill, cultural beliefs and practices that are used to manage, treat, prevent, and diagnose illnesses.[2] The term TM is sometimes used interchangeably with "complementary medicine" or "alternative medicine" in some countries. The term CAM, that is, complementary and alternative medicine is used to refer to any other healthcare therapies that are different from conventional medical treatments.[2] Traditional medicines are important sources of natural products which serve as sources of pharmaceutical drugs and other health products.[3] According to Yeh et al.[4] current research is focussing on the use of TM and CAM mainly because these strategies are widely used in improving primary health care of local communities. This is particularly important in sub-Saharan Africa where rural communities and those people living in marginalized areas are reliant on TM as their basic source of health care.[5] Research by Mander et al. revealed that there is growing demand for consumers of TM in South Africa where about three quarters (72%) of the Black Africans are reliant on herbal medicines, accounting for 27 million people in the country.[6] Research by Mander et al. revealed that 97% of traditional healers' patients in South Africa prefer TMs.[6] Research done by van Wyk et al. revealed that 50% of pharmaceutical drugs and health products are derived from natural products isolated from plants.[3]

MATERIALS AND METHODS[4]
The leaves and roots of *Cassia auriculata (Fabaceae) was* collected locally. The identities of the medicinal plants were verified local botanical guardian and from internet source. The plant materials were allowed to dry completely at room temperature and later ground to a fine powder. The powdered materials were extracted in separate aliquots with solvents of varying polarities viz., *n*-hexane, dichloromethane, acetone and methanol by shaking for 24 hours at room temperature. The supernatants were filtered into pre-weighed beakers using 15.0 cm Whatman filter papers (Whatman). Supernatants were concentrated by drying under cold air using a fan; the residues were weighed daily until a constant mass was obtained.

Preliminary Phytochemical Analysis[5-8]
Test for Tannins
1. A small portion of extract was treated with 5% ferric chloride solution. Appearance of green to blue color was taken as a positive test for tannins.
2. Small portion of extract was treated with lead acetate. Appearance of creamy precipitate was considered as a positive test for tannins.

Test for Alkaloids

1. **Mayer's Test:** The Extract to be tested is treated with few drops of dilute 2N HCL and 0.5 ml Mayer's reagent. White precipitate was obtained which confirm the presence of alkaloids.
2. **Wagner's Test:** The extract is treated with few drops of 2N HCL and 0.5 ml Wagner's reagent. Brown flocculent precipitate was obtained which confirm the presence of alkaloids.
3. **Hager's Test**: The extract is treated with few drops of dilute 2N HCL and 0.5 ml Hager's reagent. Yellow colored precipitate was obtained which confirms the presence of alkaloids.

Test for Steroids

1. **Salkowski reaction:** To 2ml. of extract, add 2ml of chloroform and 2ml.conc. H_2SO_4. Shake well, Chloroform layer appears red and acid layer shows greenish yellow fluorescence.
2. **Liebermann-Burchard reaction:** Mix 2 ml extract with chloroform. Add 1-2ml. aceteic anhydride and 2 drops of conc. H_2SO_4 from the side of test tube.
3. **Libermann's reaction:** mix 3ml.extract with 3ml. acetic anhydride. Heat and cool. Add few drops conc. H_2SO_4. Blue color appears.

Tests for Glycosides

1. **Borntrager's test:** About 50mg of extract was hydrolysed with 2ml of concentrated HCl for 2hrs on water bath and filtered. To 2ml of filtrate hydrolysate, 3ml of $CHCl_3$ was added and shaken. $CHCl_3$ layer was separated and 10% NH_3 solution was added. Formation of pink colour indicates the presence of anthraquinone glycosides.
2. **Baljet's test:** The alcoholic or aqueous extract test solution is treated with sodium picrate. Appearance of yellow to orange colour indicates the presence of glycosides.
3. **Keller-Kiliani test:** About 2ml of test solution is treated with few drops of ferric chloride solution and mixed and then sulphuric acid containing ferric chloride solution is added, it forms two layers. Appearance of lower layer in reddish brown and upper layer in bluish green indicates the presence of glycosides**.**

Test for Saponins

Foam's test: A small amount of dry extract was boiled with water and allowed to cool. It was then shaken vigorously for a minute. The formation of persistent honey comb like froth was considered as a positive test for saponins.

Test for Sugars

1. **Molisch's test:** It was performed for the presence of carbohydrates. 1 ml of 10% alcoholic solution of α-napthol was added to the extract and mixed. Then 1ml of concentrated sulphuric acid was carefully poured along the sides of the test tube violet ring formed at the junction which is considered positive test for carbohydrates.
2. **Fehling's test:** 5ml of solution of extract was heated with equal volumes of Fehling's solution A & B. Transition of color from blue through green to reddish orange confirms the presence of reducing sugars.
3. **Benedict's test:** 5 ml of solution of the extract was heated with 5 ml of Benedict's reagent. A green, yellow or orange red precipitate was considered as a positive test for reducing sugars.

Test for Proteins

1. **Biuret test:** A small portion of extract was treated with Biuret reagent.
2. **Xanthoprotein test:** Mix 3ml. T.S. with 1ml.conc. H_2SO_4. White precipitate is formed. Boil. Solution turns black or brownish due to Lead sulphide formation.

Pharmacological evolution

Anti microbial evolution 9

Test Organisms Bacterial strains were obtained from National Chemical Laboratories (NCL), Pune and Microbial Type Culture Collection (MTCC), Chandigarh. The strains used for the present study were Staphycococcus aureus (NCIM 2079), Bacillus subtilis (NCIM 2063), Escherichia coli (NCIM 2931, Proteus vulgaris (NCIM 2027).

Procedure

The antimicrobial activity of the extract was assessed by disc diffusion method. Nutrient agar medium was prepared and sterilized by an autoclave. In an aseptic room, they were poured into a petridishes to a uniform depth of 4 mm and then allowed to solidify at room temperature. After solidification, the test organisms, Escherichia coli, Bacillus subtilis, Staphylococcus aureus, and Proteus vulgaris were spread over the media with the help of a sterile swab socked in bacterium and is used for antibacterial study. The ethanolic extract residues were dissolved in dimethyl sulfoxide (DMSO) to produce a concentration of 100, 250,500 µg/disc and used for the study. Ofloxacin 5 µg/disc was used as the standard. Then the sterile filter paper discs (6mm) having a capacity to hold 10 µl of extracts were immersed in definite concentration of plant extracts and placed over the solidified agar in such a way that there is no overlapping of the zone of inhibition. Plates were kept at room temperature for half an hour for the diffusion of the sample into the agar media. The organism inoculated petridishes were incubated at 37 °C for 24 hours. After the incubation period is over, the zone of inhibition produced by the samples and standard were measured. All tests were performed in triplicate.

Analgesic and Anti inflamamtory activity

Analgesic activity

Tail-Fick Test

The basal reaction time of each mouse was determined using tail-withdrawal response when one-third of the tail was immersed in water bath at 51˚C.[11] The cutoff time for immersion was 180 s. The reaction time was evaluated 30, 60, 90, 120 and 240 min after oral administration of extracts, distilled water or acetylsalicylic acid.

Formalin Test

The method used in our study was similar to that described previously.[12] Twenty microliter of 5% formalin was injected subcutaneously into the right hind paw of mice. The time (in seconds) spent in licking and biting responses of the injected paw was taken as an indicator of pain response. Responses were measured for 5 min after formalin injection (early phase) and 20–30 min after formalin injection (late phase). Swertia Chirata root M extracts (0.5 and 1.0 g/kg, i.p.) were administered 60 min before formalin injection. Indomethacin (10 mg/kg, i.p.) was administered 30 min before formalin injection. Control group received the same volume of saline by oral administration.

Anti-inflammatory activity

Carrageenan induced hind paw edema in rats Paw edema was produced in rats by carrageenan following the methods of Winter et al. (1962) respectively.[13] Male rats eighing 100–120 g were divided into groups of six animals. A volume of 0.05 ml of 1% carrageenan in normal saline solution (NSS) in 0.2M carbonate buffer was injected intradermally into the plantar side of the right hind paw of the rat. Test drugs and vehicle were given 1 h prior to carrageenan injection. Paw volumes were measured using a plethysmometer (model 7150, Ugo Basile, Italy) before as well as 1, 3 and 5 h after carrageenan, injection. Results obtained were compared with those obtained from there.

Antioxidant activity by DPPH method[10]

Antioxidant behaviour of the extracted compound was measured *in vitro* by the inhibition of generated stable 2,2-diphenyl- 1-picrylhydrazyl (DPPH) free radical. Methods vary greatly as to the generated radical, the reproducibility of the generation process, and the end point that is used for the determination. The DPPH solution was prepared by dissolving accurately weighed 22 mg of DPPH in 100 ml of ethanol. From this stock solution, 18 ml was diluted to 100 ml with ethanol to obtain 100 µM DPPH solutions. The sample solution was prepared by accurately weighed 2.1 mg of each of the compounds and dissolved in 1 ml of freshly distilled DMSO separately to obtain solutions of 2.1 mg/ml concentration and the standard solution of was prepared by accurately weighed 10.5 mg of α-Tocopherol and dissolved in 1 ml of freshly distilled DMSO to get 10.5 mg/ml concentration.

A different concentration of extract was prepared by the addition of ethanolic solution of DPPH radical. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm against the corresponding blank solution. The final concentration of the samples and standard α-Tocopherol solutions used is 100 µg/ml. The percentage scavenging DPPH radical inhibitions were calculated by using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$$

Where, Abs control was the absorbance of DPPH radical and ethanol, Abs sample was the absorbance of DPPH radical and sample/standard.

The scavenging activity was expressed in terms of IC50, the concentration of the samples required to give a 50% reduction in the intensity of the signal of the DPPH radical. The results were done at least in triplicate.

RESULTS

Table 1: Results of Preliminary Phytochemical Screening of ERD.

S. No	Name of the Test	Result
1.	Flavonoids	++
2.	Phenols	++
3.	Alkaloids	++
4.	Saponins	++
5.	Carbohydrates	++
6.	Proteins & amino acids	++
7.	Tannins	++
8.	Cardiac glycosides	++

Anti microbial activity

	Alcoholic extract of *Cassia auriculata*				Ofloxacin		
	Zone of inhibition in mm						
		100µg/ml	250mg/ml	500µg/ml	100µg/ml	250mg/ml	500µg/ml
Name of the organisms	*Staphylococcus aureus*	6	8	12	12	20	26
	Bacillus subtilis	4	10	14	14	18	24
	Escherichia coli	8	10	22	12	16	25
	Proteus vulgaris	8	10	16	18	14	22
Control	DMSO	-	-	-	-	-	-

Analgesic activity

Table 1: Writhing test.

Group	Traetment	N	Route of administration	Dose mg/kg	No.fo writches	Inhibition writhing response
1	Control	6		-	49.06±4.08	
2	Aspirin	6	300	i.p	14.05±2.14	89
3	Flower of Cassia auriculata	6	50	i.p	22.65±2.10	42
4	Flower of Cassia auriculata	6	100	i.p	20.85±1.78	66
5	Flower of Cassia auriculata	6	150	i.p	9.68±1.14	88

Mean = S.E.M. of 6 animals. ** = P≤0.001= highly significant. Group II,III,IV, and V compared with Group I.

Anti-inflammatory activity

Table 2: Carrageenan –induced paw edema method.

Group	n	Dose (mg/kg)	Pawvolume increase(ml)			Inhibition (%)		
			1hr	3hr	5hr	1hr	3hr	5hr
Control	6		0.35±0.07	0.68±0.05	0.81±0.03			
Aspirin	6	300	0.11±0.02**	0.22±0.02**	0.28±0.03**	74	72	68
Cassia auriculata	6	100	0.24±0.04*	0.48±0.01*	0.54±0.02*	40	33	39
Cassia auriculata	6	150	0.14±0.03*	0.44±0.01*	0.46±0.04*	51	44	50
Cassia auriculata	6	200	0.07±0.01**	0.26±0.02**	0.36±0.02**	82	66	58

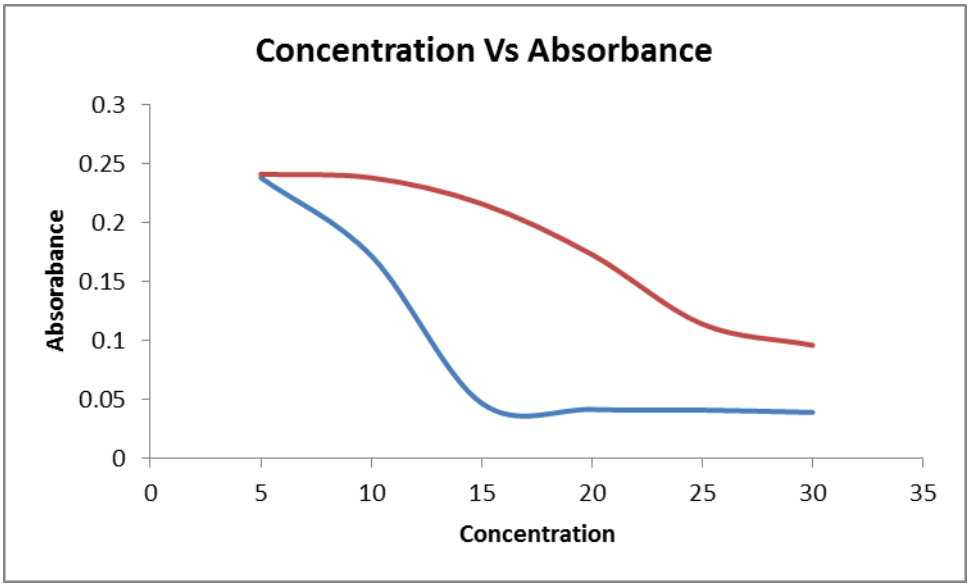
n = 6 animals in each group. * = p≤0.01 (significant).** = p≤0.01(highly significant) control groups, which received vehicle only

Table 2: Anti oxidant activity of alcoholic extract of *Cassia auriculata.*

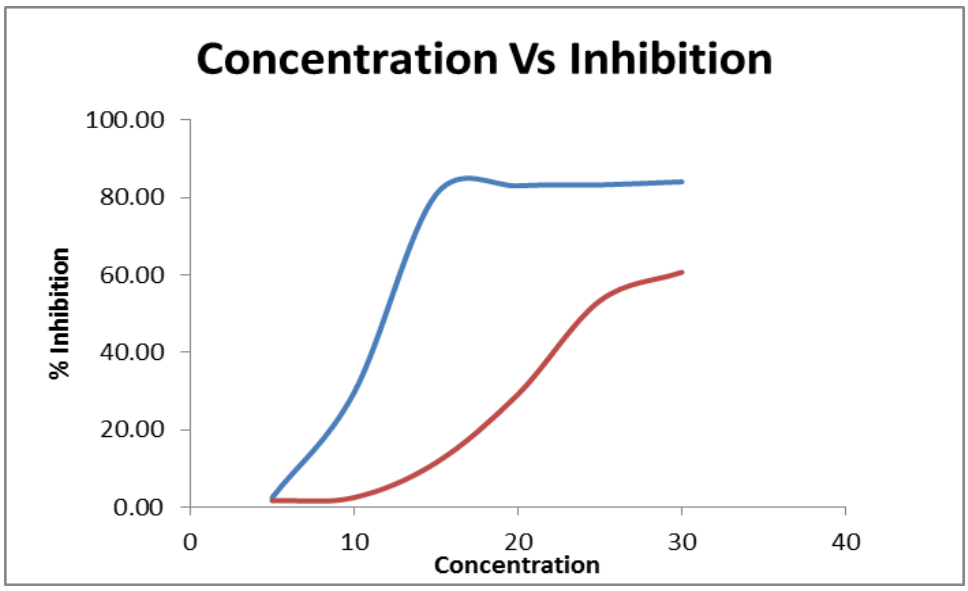
Concentration (µg/ml))	Ascorbic acid (Abs)	Alcoholic extract of *Cassia auriculata* (Abs)
5	0.238	0.241
10	0.1719	0.238
15	0.0469	0.216
20	0.0415	0.173
25	0.041	0.114
30	0.039	0.096
Control	0.2444	

Table 3: % inhibition of alcoholic extract of *Cassia auriculata* with ascorbic acid.

Concentration (µg/ml))	Ascorbic acid (% Inhibition)	Alcoholic extract of *Cassia auriculata* (% Inhibition)
5	2.459016	1.229508
10	29.54918	2.459016
15	80.77869	11.47541
20	82.9918	29.09836
25	83.19672	53.27869
30	84.01639	60.65574



Graph-1: Concentration Vs Absorbance.



Graph-2: concentrations Vs % Inhibition.

DISCUSSION

The present results reveals that the alcoholic extract shows the activity less than the standard. The extract was diluted with concentration of 100 µg/ml, 250µg/ml, 500µg/ml. In that the extract with concentration of 500µg/ml shows the significance activity than the remaining concentrations.

Analgesic activity of administration of Cassia auriculata Flower ethanolic extract at the dose level of 100, 150 and 200 mg./kg b. wt. to the rats produced weak effect on the writhing induced by the injection of 0.6% acetic acid when compared with the aspirin (300mg/kg) by 79% while the treated group with Cassia auriculata Flower ethanolic extracts inhibited the writhing by 40% ,65%, 85% respectively(table 1). The ethanolic extract of Cassia auriculata Flower (100- 200 mg/kg) produced inhibition of formalin induce biphasic pain response (neurogenic and inflammatory pain) in rats. The analgesic effect of this fraction occurred predominately during the II phase; 200 mg dose level was more efficient in the late phase. Anti-inflammatory activity of Cassia auriculata root, The inhibitory activity on carrageenan induced rat hind paw edema, caused by the subplanatar administration of Cassia auriculata Flower ethanolic extract, at various assessment times after carrageenan injection are tabulated. the standard drug aspirin, a cyclooxygenase inhibitor, at the dose of 300mg/kg body weight exhibited significant ($p \leq 0.01$) edema inhibition.

Cassia auriculata Flower ethanolic extract at doses of 50,100,150 mg/kg boy weight also possessed significant ($p \leq 0.001$) inhibitory effect on carrageenan induced paw edema at all recorded times. This increase was observed

at 1 hr. and was maximum at 5hr. after administration of carrageenan in the vehicle group.

The Alcoholic extract of *Cassia auriculata* tested for antioxidant activity by using DPPH Assay method. Here the results were compared with the standard Ascorbic acid. The result reveals that the extract shows results less than the standard. The concentration of the extract was taken in to 5-30 µg/ml. The % of inhibition shows that the up to 30µg/ml. The % inhibition is therefore it shows more activity than compare with other concentrations.

CONCLUSION

Based on the above phyto chemical investigation of extract identified that the compound containing the Flavonoids, Phenols, Alkaloids, Saponins, Carbohydrates, Proteins & amino acids, Tannins, Cardiac glycosides. The anti microbial, analgesic and anti inflammatory activity of extract at higher concentration shows better activity than other concentrations when compare with the ofloxacin, Aspirin. The anti oxidant activity of extract was performed by using DPPH assay Method using the standard ascorbic acid with a concentration of 5,10,15,20,25, 30 µg/ml. here the concentration at 30 µg/ml shows better anti oxidant activity than the other concentrations.

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