Research Article

Anti-inflammatory and antiradical potential of methanolic extract of *Cajanus cajan*

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Abstract

Objective: The present study was carried out to investigate the anti-inflammatory and antioxidant activity of methanolic extract of *Cajanus cajan*. **Materials and Methods:** The extract was evaluated for its *in vivo* anti-inflammatory activity by carrageenan induced paw oedema model using indomethacin as standard and *in vitro* anti-inflammatory activity by protein denaturation method using diclofenac sodium as standard. The extract was evaluated for its antioxidant activity by reducing power assay and hydrogen peroxide assay using ascorbic acid as standard. **Results:** The methanolic root extract of *Cajanus cajan* (MECC) significantly ($p<0.05$) inhibited the paw oedema volume. The extract significantly ($p<0.05$) protected protein membranes from denaturation. The extract also significantly scavenged the free radicals. The lupeol present in the extract targeted inflammatory signalling pathways there by exerting its anti-inflammatory activity. The phenolic constituents present in the extract might be responsible for its antioxidant activity as phenolics possess strong ability to inhibit oxidants and free radicals. **Conclusion:** From the above it is clear that MECC possess anti-inflammatory and antiradical activities.

***Keywords*:** *Cajanus cajan*, anti-inflammatory, antiradical activities, diclofenac sodium

Introduction

Inflammation and oxidative stress play an important role in various diseases. Inflammation is an immunological defence mechanism elicited in response to mechanical injuries, burns, microbial infections, allergens and other noxious stimulus (Menichini et al., 2009; Mueller et al., 2010). Use of anti-inflammatory agents may therefore, be helpful in the treatment of inflammatory disorders (Sosa et al., 2002). Non-steroidal anti-inflammatory drugs (NSAID) are commonly used to treat different inflammatory diseases. The adverse effects of the currently available anti-inflammatory drugs however, pose a major problem in their clinical use therefore; naturally, originated agents with very little side effects are desirable to substitute chemical therapeutics. There has been a growing interest in phenolic components of fruits and vegetables, which may promote human health or lower the risk of disease. Recent studies have focused on health functions of phenolics including flavonoids from fruit and vegetables (Chen et al., 2006). In search for sources of natural antioxidants, some medicinal plants have been extensively studied for their antioxidant activity and radical scavenging activity (Schinella et al., 2002).

C. cajan being a forage crop has been utilized as an important remedy for various ailments. Chemical constituent investigations have indicated that *C. cajan* leaves are rich in flavonoids and stilbenes. They also contain saponins, conspicuous amount of tannins, and moderate quantities of reducing sugars, resins and terpenoids. Chemical studies reveal 2'-2' methyl cajanone, 2-hydroxy genistein, isoflavones, cajanin cahanones etc., which impart antioxidant properties (Kong et al., 2010). Roots are also found to possess genistein and genistin. It also contains hexadecanoic acid, α amyrin, β-sitosterol, Pinostrobin, longistylin A and longistylin C which impart anticancer activity. Herbal formulations are preferred due to lesser side effects and their low cost. Therefore, in the present study, an attempt has been made to explore anti-inflammatory and antiradical activities of *Cajanus cajan* root extract.

Materials and methods

Collection, authentication of plant material

The roots of *Cajanus cajan* were collected from Ranga Reddy District, Telangana in the month of January. The plant

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was identified and authenticated by a botanist. The roots were dried under shade and pulverized in the laboratory.

Preparation of the extract

The root powder of *Cajanus cajan* was dried. Then the powder was extracted with methanol by simple soxhlet extraction technique.

Preliminary phytochemical screening

The extract was subjected to preliminary phytochemical investigation for plant secondary metabolites by utilizing standard methods (Khandelwal KR, 2005).

Experimental animals

Wistar rats (180-200 g) of either sex approximately the same age, procured from listed suppliers of Albino labs, Hyderabad, India were used for the study. All the experimental works with the animals were carried out after obtaining approval from the Institutional Animal Ethics Committee (Reg. No. 1175/PO/Ere/S/08/CPCSEA).

Acute toxicity studies

An acute oral toxicity study was performed as per Organization for Economic Co-operation and Development 423 guidelines (acute toxic class method). Wistar rats (n=6) of either sex selected by random sampling technique were used for the study.

Anti-inflammatory activity

Carrageenan induced rat paw oedema

Male Wistar rats (180-200 g) divided into 6 animals in each groups used for study, induced by subcutaneous injection of a 0.1 ml of 1% freshly prepared carrageenan in saline in the right hind paw of rats and sub-plantar injection of histamine at dose of 0.1 ml of 0.1%). Carrageenan solution (0.1 ml/ paw) was injected subcutaneously into planter surface of the right hind paw of the rats (Sugishita et al., 1981). The paw volume of rats in control, standard and test groups was measured with the help of the plethysmometer during the interval of 30, 60, 120, 180, 240 min after Carrageenan administration.

Inhibition of protein denaturation

The reaction mixture consists of 1 ml of different concentrations of extracts ranging from 20-100 µg/ml and diclofenac sodium and 3 ml of phosphate buffered saline (pH-6.4) was mixed with 1 ml of egg albumin solution (1%), the reaction mixture without plant extracts taken as control and incubated at 37 °C for 20 minutes. Denaturation was induced by keeping the reaction mixture at 90 °C in a water bath for two minutes. After cooling the turbidity was measured spectrophotometrically at 660 nm. Diclofenac sodium was used as standard. Percentage inhibition of denaturation was calculated by using the following formula (Mizushima and Kobayashi, 1968).

$$Inhibition\% = \frac{A_t - A_c}{A_c} X\ 100$$

Where,

A_c= Absorbance of control,

A_t= absorbance of test sample

***In vitro* Antioxidant Activity**

Determination of Reducing Power

The reducing power of samples was measured according to the previous method (Allouche et al., 2010) with a slight modification. An aliquot of samples (1 mL), with different concentrations, was mixed with 200 mM phosphate buffer (2.5 mL, pH 6.6) followed by of 1% potassium ferricyanide [K3Fe(CN) 6, 2.5 mL]. The mixture was incubated for 20 min in a water bath at 50 °C. After incubation, 10% TCA (1 mL) was added, followed by centrifugation at 3000 rpm for 10 min. The supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and 0.1% ferric chloride (0.5 mL). Then the Abs was measured at 700 nm against a blank.

Hydroxyl radical scavenging activity

The scavenging activity of different extracts of *Cajanus cajan* root (10, 20, 30, 40 and 50 µg/ml) on hydroxyl radical activity was measured according to the previously described method (Duh et al., 1999). The intensity of the colour formed was measured spectroscopic ally at 412 nm against reagent blank. The hydroxyl radical scavenging activity of the sample extracts was evaluated as % of antioxidant activity.

Statistical analysis

The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Dunnetts Test by using Graph Pad Prism. The values were expressed as mean ± Standard Error Mean (SEM) for six rats in each group.

Results

Preliminary Phytochemical analysis

The preliminary phytochemical investigation of methanolic root extract of *Cajanus cajan* showed the presence of steroids, flavonoids, triterpenoids, tannins & phenolic compounds, saponins, glycosides, carbohydrates and proteins. Methanolic root extract of *Cajanus cajan* was tested on female mice upto a dose of 2000 mg/kg bd. wt. *p.o.* All animals were safe even after 14 days of observation. Hence the extract is found to be safe even upto 2000 mg/kg bd. wt.

***In vivo* anti-inflammatory activity**

Carrageenan induced rat hind paw oedema method

Table 1. Anti-inflammatory activity for methanolic root extract of *Cajanus cajan* on Carrageenan induced paw oedema model

Groups	Treatment	Initial Paw volume (mL)	Paw volume (mL)				
			1h	2h	3h	4h	5h
I	Control	1.4±0.32.	1.5±0.13	1.4±0.18	1.4±0.16	1.4±0.52	1.5±0.25
II	Negative control	1.5±0.25	1.8±0.12 c	2.2±0.23 b	2.7±0.50 a	2.9±0.33 a	3.2±0.23 a
III	MECC200 mg/kg	1.4±0.15	1.9±0.12 *C	2.1±0.25 **B	2.2±0.23 ***B	1.9±0.25 ***,A	1.6±0.16 ***A
IV	MECC400 mg/kg	1.4±0.20	1.9±0.20 **C	2.0±0.20 ***	1.9±0.20 ***B	1.6±0.16 ***A	1.4±0.33 ***,A
V	Indomethacin5 mg/kg	1.4±0.25	1.9±0.18 **,b	2.6±0.25 ***,b	2.1±0.16 ***,a	1.9±0.18 ***,a	1.6±0.18 a***

Values were expressed as mean ±SEM (n=6). Statistical analysis was performed by using ANOVA followed by Dunnet's t-test by comparing with normal control, carrageenan induced and standard. Significant values were expressed as control group (a=p<0.001, b=p<0.01, c=p<0.05), negative control (***=p<0.001, **=p<0.01, *=p<0.05) and standard (A=p<0.001, B=p<0.01, C=p<0.05).

In normal control group, which received saline the average initial paw volume of rats was found to be 1.4±0.32 and the paw volume after 5 h of drug administration was found to be 1.5±0.25. In carrageen control group the initial and final paw volume were found to be 1.5±0.25 and 3.2±0.23. The MECC at a dose of 200 and 400 mg/kg bd wt, the initial and final paw volume after 5 h were 1.4±0.15, 1.4±0.20, and 1.6±0.16, 1.4±0.33. The initial and final paw volume of indomethacin treated group was 1.4±0.25 and 1.6±0.18.

In vitro anti-inflammatory activity

Inhibition of protein denaturation

The MECC exhibited maximum inhibition of protein denaturation at (520 µg/ml) and its effect was compared with the standard anti-inflammatory drug, Diclofenac showed the maximum inhibition (422 µg/ml) at the same concentration.

Table 2. *In vitro* protein denaturation of methanolic root extract of *Cajanus cajan*.

Compounds	Concentrations (µg/mL)	%	IC_{50} Value
MECC	50	3.26± 1.21	520
	100	20.55±1.04	
	200	39.15±1.04	
	400	41.2 ±0.99	
	600	46.3±2.04	
	800	51.5±0.99	
Diclofenac Sodium	50	4.12±1.02	422
	100	8.24±1.03	
	200	22.65±1.04	
	400	44.75±1.54	
	600	71.05±2.04	
	800	83.45±2.04	
	1000	96.85±1.04	

Values are expressed as mean ± SEM.

In vitro antioxidant assays

The methanolic root extract of *Cajanus cajan* was subjected to *in vitro* antioxidant activity. *In vitro* anti-oxidant activity was performed using reducing power assay & hydrogen peroxide scavenging assay.

a) Reducing power assay

In reducing power assay, the methanolic root extract was tested at different concentrations like 10, 20, 30, 40, and 50µg/mL. The lowest concentration of 10µg/mL showed a percentage inhibition of 26.49±0.16 whereas the highest concentration of 50 µg/mL showed a percentage inhibition of 64.49±0.83. The IC_{50} value for the methanolic root extract of *Cajanus cajan* was found to be 35 µg/mL which is compared with standard ascorbic acid having IC_{50} value of 31 µg/mL respectively.

Table 3. Anti-oxidant activity for methanolic root extract of *Cajanus cajan* by reducing power assay method

Compounds	Concentration (µg/ mL)	% inhibition	IC_{50} value (µg/ mL)
MECC	10	26.49±0.16	
	20	40.83±0.50	
	30	46.66±0.33	35
	40	54.32±0.66	
	50	64.49±0.83	
Ascorbic acid	10	29.49±0.83	
	20	45.49±0.50	
	30	49.66±0.67g	31
	40	62.32±0.33	
	50	67.16±0.17	

Values are expressed as mean ± SEM

Table 4. Anti-oxidant activity of methanolic extract of *Cajanus cajan* by H_2O_2 radical scavenging activity

Compounds	Concentration (µg/ mL)	% inhibition	IC_{50} value (µg/ mL)
MECC	10	28.99±1.0	40
	20	34.24±0.25	
	30	41.54±0.55	
	40	50.24±0.74	
	50	64.04±0.45	
Ascorbic acid	10	31.74±0.75	35
	20	35.99±0.50	
	30	44.04±0.95	
	40	56.99±1.5	
	50	68.99±1.0	

b) Hydrogen peroxide Scavenging assay

In hydrogen peroxide scavenging assay, the methanolic root extract was tested at different concentrations like 10, 20, 30, 40, and 50µg/mL. The lowest conc of 10µg/mL showed a percentage inhibition of 28.99±1.0 whereas the highest concentration of 50 µg/mL showed a percentage inhibition of 64.04±0.45. The IC_{50} value for the methanolic root extract of *Cajanus cajan* was found to be 40 µg/mL which is compared with standard ascorbic acid having IC_{50} value of 35 µg/mL respectively. From the above results it is clear that the MECC showed scavenging activity against hydrogen peroxide radicals.

Discussion

The preliminary phytochemical studies of the methanolic root extract of the *Cajanus cajan* showed the presence of phenolic compounds like simple phenolic acids, and polyphenolic compounds like flavonoids especially flavonol, flavones, isoflavones flavonones and isoflavanone.

Carrageenan- induced rat paw oedema is ubiquitously used test to determine anti-inflammatory activity and constitutes a simple and routine animal model for evaluation of pain at the site of inflammation without any injury or damage to the inflamed paw (Sugishitha et al., 1981).

Several studies have been reported that flavonoids inhibit pro-inflammatory enzymes, such as cyclooxygenase-2, lipooxygenase and inducible NO synthase, inhibition of NF-Kb and activating protein-1 (AP-1) and activation of MAPK, protein kinase C (Welton et al., 1986). Saponins have been reported to possess a wide range of biological activities including anti-inflammatory activity (Capra, 1972; Singh et al., 1992). These saponins might act through inhibiting COX-2 and inducible nitric oxide synthase (iNOS), nitric oxide, PGE2 and tumor necrosis factor (TNF-α) (Yaun et al., 2006). These phytochemical constituents in MECC might be responsible for its anti-inflammatory activity.

Protein denaturation is a process in which proteins lose their tertiary and secondary structure by application of external stress or compounds such as strong acid or base a concentrated inorganic salt, an organic solvent or heat. Denaturation of protein is a well-documented cause of inflammation (Leelaprakash and Mohan Dass, 2010). In the present study, the *in vitro* anti-inflammatory activity of MECC can be attributed to its poly phenol contents, flavonoids and saponins. These have the ability to bind cations and able to protect the protein membrane from denaturation.

In reducing power assay, the methanolic root extract was tested at different concentrations. The reducing ability of a compound generally depends on the electron donating capacity and the reducing agent transfers electrons to another substance and thus itself oxidized forming Fe^{3+}-Fe^{2+} complex. The reducing power activity of MECC might be due to presence of various active constituents like phenolics, triterpenoids and flavonoids with adequate number of hydroxyl groups. Several studies reported the anti-oxidant activity of flavonoids which act by blocking the generation of free radicals in chain reaction (Allouche et al., 2010).

MECC showed the presence of phenolic compounds like simple phenolic acids, and polyphenolic compounds like flavonoids especially flavonol, flavones, isoflavones flavonones and isoflavanone. Scavenging of hydrogen peroxide by the extract may be attributed to their phenolic nature, which can donate electrons to H_2O_2, thus neutralizing it to water (Duh et al., 1999).

From the above MECC have shown reducing power ability and inhibition of hydrogen peroxide which was comparable to standard ascorbic acid. Although the active principles responsible for the antioxidant activity of MECC have not yet been identified, we suggest that these extracts could be a good source to obtain compounds that would help to increase the overall antioxidant capacity of an organism and protect it against inflammation induced by oxidative stress. Therefore it is suggested that further work could be done on the isolation and identification of antioxidative components in methanolic extract of *Cajanus cajan*.

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References

Allouche Y, Beltrán G, Gaforio JJ, Uceda M, Mesa MD. 2010. Antioxidant and antiatherogenic activities of pentacyclic triterpenic diols and acids. Food Chemistry and Toxicology, 48:2885–2890.

Capra C. 1972. Anti-inflammatory activity of the saponins from *Ruscus aculeatus*. Fitoterapia, 43(4):99–113.

Chen PN, Chu SC, Chiou HL, Kuo WH, Chiang CL, Hsieh YS. 2006. Mulberry anthocyanins, cyaniding 3-rutinoside and cyaniding-3-glucoside, exhibited and inhibitory effect on the migration and invasion of a human lung cancer cell line. Cancer Letters, 28:248-259.

Duh PD, Tu YY, Yen GC. 1999. Antioxidant activity of aqueous extract of harn jyur (*Chyrsanthemum morifolium* Ramat). Lebensmittel-Wissenschaft und Technologie, 32:269–277.

Khandelwal KR. 2005. Practical Pharmacognosy Techniques and Experiments, 13th Ed, Nirali Prakashan,

Kong Y, Fu YJ, Zu YG, Chang FR, Chen YH, Liu XL. 2010. Cajanuslactone a new coumarin with Antibacterial activity from pigeon pea leaves. Food Chemistry, 121:1150–5.

Leelaprakash G, Dass SM. 2011. *In vitro* Anti-inflammatory activity of Methanol extract of *Enicostemma axillare*. International Journal of Drug Development & Research, 3(3):189-196.

Menichini F, Tundis R, Bonesi M, Loizzo MR, Conforti F, Statti G, De Cindio B, Houghton PJ, Menichini F. 2009. The influence of fruit ripening on the phytochemical content and biological activity of *Capsicum chinense* Jacq. cv Habanero Food Chemistry, 114:553-560.

Mizushima Y, Kobayashi M. 1968. Interaction of Anti-inflammatory drugs with serum preoteins, especially with some biologically active proteins. Journal of Pharmacy and Pharmacology, 20:169-173.

Mueller M, Hobiger S, Jungbauer A**.** 2010. Anti-inflammatory activity of extracts from fruits, herbs and spices. Food Chemistry, 122:987-996.

Schinella GR, Tournier HA, Prieto JM, Mordujovich de Buschiazzo P, Rios JL. 2002. Antioxidant activity of anti-inflammatory plant extracts. Life Sciences, 70:1023-1033.

Singh GB, Singh S, Bani S, Gupta BD, Banerjee SK. 1992. Anti-inflammatory activity of oleanolic acid in rats and mice. Journal of Pharmacy and Pharmacology, 44:456–458.

Sosa S, Balic MJ, Arvigo R, Esposito RG, Pizza C, Altinier G, Tubaro A. 2002. Screening of the topical anti-inflammatory activity of some Central American plants Journal of Ethnopharmacology, 81:211-215.

Sugishita E, Amagaya S, Ogihara Y. 1981. Anti-inflammatory testing methods: comparative evaluation of mice and rats. Journal of Pharmaco-biodynamics, 8:565–575.

Welton AF, Tobias LD, Fiedler-Nagy C, Anderson W, Hope W, Meyers K, Coffey JW. 1986. Effect of flavonoids on arachidonic acid metabolism. Progress in Clinical and Biological Research, 213:231- 242.

Winter CA, Risley EA, Nuss GW. 1962. Carrageenan-induced oedema in the hind paw of rat as an assay for Anti-inflammatory activity. Proceedings of the Society for Experimental Biology and Medicine, 111:544-547.

Yaun G. Wahlqvist ML, He G, Yang M, Li D. 2006. Natural products and anti-inflammatory activity: Asia Pacific Journal of Clinical Nutrition, 15(2):143-152.