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Full Length Research Paper

Fatty acid composition, antioxidant, anti-inflammatory and antibacterial activities of seed oil from *Crotalaria juncea* Linn.

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Chemical characteristics, fatty acid composition, antioxidant, anti-inflammatory and antibacterial activities of *Crotalaria juncea* seed oil (CJSPE) were evaluated in this study. High amount of linoleic acid (62.36%) was found in CJSPE by the gas liquid chromatography (GLC) study. Antioxidant activity of CJSPE was evaluated by *in vitro* assay methods which revealed the 2,2-Diphenyl-1-picrylhydrazyl (DPPH), hydroxyl and superoxide radical scavenging activity of CJSPE; its antioxidant activity was found to be concentration dependent and IC_{50} values were 132.31, 286.409 and 31.254 µg/ml respectively. Moreover, CJSPE has displayed dose dependant, significant inhibition of NO production in the isolated rat peritoneum macrophages; and significant anti-inflammatory activity in carragennan-induced paw edema (64.52 ± 0.053%, p < 0.001, at 6 h, 200 mg/kg oral dose) and cotton pellet-induced granuloma formation (48.55 ± 0.244%, p < 0.001, at 200 mg/kg oral dose) models of inflammation; and its anti-inflammatory effect was comparable to that of diclofenac sodium. However, moderate antibacterial activity of CJSPE was observed. In conclusion, the study demonstrated significant antioxidant and anti-inflammatory activities of CJSPE.

Key words: *Crotalaria juncea*, antibacterial activity, anti-inflammatory, antioxidant activity, linoleic acid.

INTRODUCTION

Plant seeds always remain an important source of proteins and oils for their nutritional, industrial and pharmaceutical applications (Eromosele, 1997). *Crotalaria juncea* Linn. (leguminoceae) is popularly known as sunn hemp and is used for its food, fibre and medicinal values by the ethnic communities. It is widely distributed in the tropical and subtropical region of India, Nepal, Sri Lanka, and Southern Africa. *C. juncea* is used as blood purifier, abortificient, astringent, demulcent, emetic, purgative and in the treatment of anaemia, impetigo, menorrhagia and psoriasis (Chopra et al., 1956; Kirtikar and Basu, 1935; Sharma et al., 2001). *C. juncea* seeds have been reported to possess significant antispermatogenic, anti-ovulatory and contraceptive activities (Prakash, 1985; Rao et al., 1979; Vijaykumar et al., 2004). The anti-inflammatory and anti-ulcerogenic activities of the ethanol extract of leaves of *C. juncea* have also been reported (Ashok et al., 2006). The methylene chloride and methanol extracts of aerial part of *C. juncea* was reported to possess moderate antifungal activity (Goun et al., 2003). Few, but interesting compounds have been isolated from *C. juncea* which include monocrotaline, riddelline, seneciphylline, senecionine, trichodesmine, chodesmine; galactose-specific lectin and cardiogenin 3-*O*-[*β*]-d-xylopyranoside (Adams and Gianturco, 1956; Ersson, 1977; Ji et al., 2005). However, no report on the composition and biological activity of the seed oil of *C. juncea* are available as per our knowledge. Hence, this study was designed to evaluate the chemical characteristics, fatty acid composition, antioxidant, anti-inflammatory and

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antibacterial activities of *C. juncea* seed oil.

MATERIALS AND METHODS

Chemicals

L(+)-ascorbic acid (AA) was purchased from National Chemicals Pvt. Ltd. India. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), nitroblue tetrazolium (NBT), phenazine methosulphate (PMS) were purchased from Sigma Aldrich (India). All other common chemicals and organic solvents were purchased from Merck. Double distilled water was used throughout the experiment.

Animals

Wistar albino rats (120 to 150 g) of either sex were obtained from the central animal house of Institute of Medical Sciences, Banaras Hindu University, Varanasi (Reg. No.542/02/ab/CPCSEA). Rats were acclimatized in the laboratory condition at 12 h light/dark cycle for 15 days. Rats were allowed to have free access of water and standard diet; and were fasted overnight before the experiment. Approval from Institutional Ethical Committee was taken for the commencement of animal experimental study. Guidelines for the care of laboratory animals and the investigation of experimental pain in conscious animals had been followed during the experiment.

Plant materials

The seeds of *C. juncea* were purchased from the local market and were identified morphologically by Prof. N.K. Dubey, Department of Botany, Banaras Hindu University, Varanasi and voucher specimen (PCRL-41) was deposited in the Pharmaceutical Chemistry Research Lab, Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi for the future reference. Seeds were pulverized to course crude powder and were stored in air tight containers at room temperature till the extraction.

Preparation of extract

Pulverized crude powder (2.0 kg) was extracted by soxhlation with the petroleum ether for 36 h. *C. juncea* seeds petroleum ether extract (CJSPE) was then concentrated under vacuum at low temperature to obtain as pale yellow color oil (250 ml).

Chemical composition and gas liquid chromatography (GLC) analysis of seed oil

Qualitative determinations of CJSPE for the chemical constituents were carried out using standard procedures as described by Trease and Evans (1989). The determination of acid value, iodine value and saponification number were carried out according to method as described by Indian pharmacopoeia (2007). For the GLC study, methyl ester of oil was prepared and fatty acid compositions of the methyl esters were analysed by Hawlett Packard instrument (model 26890) supplied with split inlet, flame ionisation detector (FID). A DB 225 column (30 m x 0.25 mm ID, 0.25 µm film) was used. The carrier gas was Nitrogen 1 ml/min column flow and 1: 50 split ratio. Injector and detector temperatures were 230 and 260°C, respectively. Oven temperature was initially set at 90°C for 5 min and then gradually increased to 130°C by the rate of 3°C/min; kept at 130°C for next 12 min. and than temperature was raised up to 230°C at rate of 2°C/min.

DPPH radical scavenging activity

The DPPH radical scavenging activity of CJSPE was evaluated by method as described by Kumaran and Karunakaran (2006) with slight modification. Briefly, 0.5 ml DPPH solution (0.05% w/v in methanol) was mixed with serial dilution of (25 to 200 µg/ml, in methanol) of CJSPE and mixture was incubated for 30 min at room temperature. Absorbance of reaction mixtures were measured at 517 nm against the blank, which contained all reagents except the test compound. DPPH radical scavenging activity was calculated by using following formula:

$$\% \text{ inhibition} = \left(1 - \frac{Ae}{Ac}\right) \times 100 \quad \text{...eq. (1)}$$

Where Ae and Ac are absorbance of CJSPE and control sample respectively. Concentration of CJSPE required for scavenging 50% of DPPH radicals (IC_{50}) was determined by plotting graph between percentage inhibition and concentrations. AA was used as standard and experiment was performed in triplicate.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of CJSPE was evaluated by method as described by Kuda and Ikemori (2009) with slight modification. Briefly, 200 µl 3.75 mM 1,10-phenanthroline solution, 200 µl 3.75 mM $FeSO_4$ and 400 µl 0.05% v/v H_2O_2 were added to test tube containing 400 µl serial dilution of (25 to 200 µg/ml) of CJSPE prepared in pH 7.4 phosphate buffer and mixed well. Mixture was then incubated for 1 h at 37°C and absorbance was measured at 532 nm. Hydroxyl radical scavenging activity of CJSPE was determined using Equation (1) and IC_{50} value was determined. AA was used as standard and experiment was performed in triplicate.

Superoxide radical scavenging activity

Superoxide radicals were generated from a NADH-PMS system and were quantified by measuring reduction of NBT using method as described by Kuda and Ikemori (2009) with slight modification. Briefly, various concentrations of CJSPE (25 to 200 µg/ml) were incubated with PMS (0.1 mM, 0.1 ml), NBT (1 mM, 0.1 ml) and made up to 0.9 ml with KH_2PO_4 buffer (0.05 M, pH 7.4). The reaction mixtures were initiated by the addition of 0.1 ml 2 mM NADH. After incubation at 25°C for 10 min, the absorbance of mixture was measured at 570 nm. Superoxide scavenging activity of CJSPE was determined using equation (1) and the IC_{50} value was determined. AA was used as standard and experiment was performed in triplicate.

Isolation of rat peritoneal macrophages and *in vitro* effect on NO production in activated macrophages by CJSPE

Rats were anaesthetized with diethyl ether and 10 ml of chilled Ca and Mg free-Phosphate buffer saline (PBS, pH 7.4) was injected in the peritoneal cavity and abdomen was massaged for 5 min. The peritoneal fluid was then aspirated out, centrifuged at 1,500 rpm for

10 min and cell pellets were washed three times with PBS. The pellets were suspended in 1 ml RPMI media and viable cells were counted by trypan blue exclusion method using hemocytometer. Macrophages ($1x10^5$ cells/well) were treated with different concentration of CJSPE (100 to 500 µg) and were exposed to lipopolysaccharide (1 µg/ml) in 96 well plate, which was incubated for next 16 h.

Supernatant of culture medium was pipetted out and collected in another plate. Accumulated NO radical in the culture supernatant was estimated by griess reagent (Batkhuu et al., 2002).

Evaluation of anti-inflammatory activity by carrageenan induced rat paw edema model

The anti-inflammatory activity of CJSPE was determined by carrageenan induced rat paw edema model (Singh et al., 2009). Rats were randomly divided into four groups (A-D) containing six rats in each group. Edema was induced by the injection of 0.1 ml carrageenan suspension (in normal saline, 1% w/v) in sub-plantar region of right hind paw of rats.

Group A served as control and received an oral dose of sodium caboxymethylcellulose solution (1 ml, 1% w/v). Group B served as standard and received an oral dose of diclofenac sodium (50 mg/kg). Group C and D served as test groups and were received 100 and 200 mg/kg oral dose of CJSPE respectively. Volume of right hind paw of rats was measured by using plethysmometer before the 30 min and each 1 h interval up to six hour, then at 12- and 24th-h after the carrageenan injection. Anti-inflammatory activity was measured as the efficacy of drug/CJSPE for reduction of edema volume with respect to control.

Evaluation of anti-inflammatory activity by cotton pellet-induced granuloma formation model

Rats were divided into four groups (A-D) containing six rats in each group and were anaesthetized with diethyl ether. Sterile cotton pellet (10 mg) was then implanted in the shaved axilla region of rats using small incision. Group A served as control and received an oral dose of sodium caboxymethylcellulose solution (1 ml, 1% w/v). Group B served as standard and received an oral dose of diclofenac sodium (50 mg/kg). Group C and D served as test groups and received 100 and 200 mg/kg oral dose of CJSPE respectively, for seven consecutive days starting from the day of implantation. Cotton pellets were removed on the eighth day after anaesthetizing rats with diethyl ether and made free from extraneous tissues. Pellets were dried at 60°C for 24 h and were weighed to determine mean weight of granuloma tissue formed (Singh et al., 2009).

Evaluation of antibacterial activity

The antibacterial activity of CJSPE was evaluated by the disc diffusion method and performed according to the guidelines of National Committee for Clinical Laboratory Standards (NCCS). A 24/48 h-old culture of selected bacteria was mixed with sterile physiological saline (0.85% w/v) and turbidity was adjusted to the standard inoculum of MacFarland scale 0.5 (~10^6 colony forming units (CFU)/ml). Petri plates containing 20 ml of Mueller Hinton agar were used for testing antibacterial activity.

The inoculum was spread on the surface of the solidified media and filter paper disc (6 mm diameter, whatman no. 1) impregnated with the CJSPE prepared in DMSO (10 µl/disc; 500 µg extract/disc) was placed on the plates. Plates inoculated with the bacteria were incubated for next 24 h at 37°C. Antibacterial activity of CJSPE was measured as diameter of zone of inhibition in mm. Ciprofloxacin (5 µg/disc) and dimethylsulfoxide (DMSO) impregnated paper disc were used as positive control and negative control respectively. Experiment was performed in triplicate.

Statistical analysis

All the results were expressed as mean ± SEM with one-way analysis of variance (ANOVA), followed by Tukey multiple comparison test. Values were considered statistically significant, if $p<0.05$.

RESULTS AND DISCUSSION

Chemical property and fatty acid composition of seed oil

The pale yellow color oil obtained from the petroleum ether extract of seeds of CJSPE occurs consistently liquid at 25 ± 2°C. Preliminary phytochemical tests revealed the presence of fatty acids, terpenes and sterols in the CJSPE. The chemical characteristics of CJSPE was evaluated by acid value, iodine value and saponification number determination and these values were found as 9.66 ± 0.29, 105.33 ± 0.735 and 192.8 ± 1.858 respectively.

Acid value of oil is an important indicator for the assessment of nutritional and industrial value of oil. The high acid value of *C. juncea* oil makes it unsuitable for the nutritional use, however, can be used for the industrial purpose. The low iodine value of CJSPE suggests the presence of lower number of unsaturated bond and hence categorised as non-drying oil. The high saponification number indicates that the *C. juncea* seed oil is suitable for the soap making purpose (Trease and Evans, 1989). The oil content of *C. juncea* seed (13% w/w) was found to be higher than the *C. striata* seeds (5% w/w) and slightly lower than the *C. retusa* seeds (15% w/w). Interestingly, the iodine value and saponification number of *C. juncea* seed oil was found to be comparable with the values of *C. striata* seeds oil (Hosamani and Ramesh, 2001). However, less iodine value but higher saponification number was found for CJSPE than that of *C. retusa* seeds oil (Umerie et al., 2010). The fatty acid composition is the most valuable feature of oil, commonly employed to determine the identity and purity of oil. The fatty acid composition of CJSPE is presented in Table 1. GLC analysis of CJSPE revealed the presence of higher percentage of unsaturated fatty acids. The major components of fatty acid in *C. juncea* seed oil were found as linoleic acid, palmatic acid, steric acid and oleic acid. Linoleic acid content of CJSPE was found to be much higher than its content in seed oil obtained from *C. striata* (Hosamani and Ramesh, 2001).

Table 1. GLC analysis for the fatty acid composition (%) of oil obtained from C. juncea seed

Compound	Composition (%)
Myristic acid	0.197
Palmatic acid	18.019
Steric acid	10.154
Oleic acid	6.689
Linoleic acid	62.360
Linolenic acid	0.700
Arachidic acid	1.199
Behenic acid	1.369

Assessment of antioxidant activity

Keen interest in the antioxidant activity of foodstuffs and natural drugs are gaining momentum due to the wide recognition of their protective effect during the oxidative stress conditions of the cells. Over production of the free radicals and reactive oxygen species (ROS) during the oxidative stress causes the cellular damage by oxidizing membrane lipids, cellular proteins, enzymes, and DNA; consequently arresting the cellular respiration leading to cell death. The pathology of most diseases/conditions viz. aging, inflammatory disorders, psoriasis, etc. have implicated various free-radical reactions (Moure et al., 2001). Therefore, scavenging of free radicals' viz. hydroxyl radicals and superoxide are of great importance. In this study, we used DPPH, hydroxyl and superoxide radical scavenging assays to evaluate the antioxidant activity of CJSPE.

DPPH occurs as a stable free radical in the methanolic solution and gives purple/violet color with maximum absorption at 517 nm. Its color is bleached by capturing a proton from the chemical-entity like antioxidant(s). This response of DPPH is unaffected by the side reactions viz. chelation of metal ion, enzymatic inhibition, etc. which is advantageous over the other assay methods used for the evaluation of antioxidant activity (Kumaran and Karunakaran, 2006). The reduced absorbance of DPPH solution by the antioxidant(s) indicates its ability to donate proton. The DPPH radical scavenging activity of CJSPE is presented in the Figure (1a) which suggests that its activity was concentration dependant. The IC_{50} value of CJSPE for the DPPH radical scavenging activity was found to be 122.52 µg/ml, while the IC_{50} value of AA was 77.72 µg/ml. Hydroxyl radical-scavenging activity of CJSPE was evaluated by phenanthroline-Fe (II) oxidation assay. Phenthroline-Fe(II) is a common redox indicator, showing maximum absorption at 532 nm in the acidic environment. Phenthroline-Fe(II) is easily oxidized into phenthroline-Fe(III) form by $OH^{\cdot}$ radicals generated in H_2O_2/Fe^{++} reaction mixture. As a result, notable reduction in the absorbance of reaction mixture occurs at 532 nm (Kuda and Ikemori, 2009). Presence of antioxidant(s) in reaction mixture minimizes the change in absorbance, which is directly proportional to the number of hydroxyl radicals scavenged. Figure 1b showed that CJSPE have a concentration dependent hydroxyl radical scavenging activity and the IC_{50} value for the CJSPE was found as 286.409 whereas the IC_{50} value of AA was 180.596 µg/ml.

Superoxide scavenging activity of CJSPE was determined by another experiment based on similar principal, but utilizes PMS-NADH mixture to produce superoxide from the dissolved oxygen, which led to reduction of NBT with maximum absorption at 560 nm (Kuda and Ikemori, 2009). Results showed (Figure 1c) that CJSPE causes concentration dependant reduction in absorption at 560 nm which suggests its strength to scavenge the superoxide radical (IC_{50} = 31.254).

Assessment of anti-inflammatory activity

Anti-inflammatory effect of the CJSPE was assessed by its effect on NO radical production in isolated macrophages from rat peritoneal (*in vitro* method); and using carragennan-induced paw edema rat model and cotton pellet-induced granuloma formation in rat model (*in vivo* method). These models are commonly employed for the evaluation of efficacy of natural products and synthetic drugs to treat acute and chronic inflammation.

The onset and progress of inflammation occurs in biological system through intricate processes involving numerous factors. Macrophages play a crucial role by generating various inflammatory mediators, such as NO, which regulates inflammation. Inducible isoform of NO synthase (iNOS) present in inflammatory macrophages continuously produces large quantities of NO upon the trigger from molecules like LPS and certain cytokines. NO is responsible for the development of non specific immunity of host and is pronounced during the infection, skin disorders, cancer, etc. (Batkhuu et al., 2002). Hence, the measurement of NO level in LPS-activated macrophages can be used for demonstrating the anti-inflammatory activity of CJSPE. It is evident from the study that CJSPE has a dose dependant and direct effect on NO production in the isolated macrophages from rat peritoneal (Figure 2). This result suggests that anti-inflammatory activity of CJSPE is mediated by its inhibitory effect on NO production and its effect on the non-specific immunity of body.

Anti-inflammatory activity of CJSPE on the carragennan-induced paw edema rat model and cotton pellet-induced granuloma formation in rat models were summarized in the Tables 2 and 3, respectively. Study demonstrated a dose dependant reduction of carragennan-induced rat paw edema by the CJSPE. Moreover, significant ($p < 0.001$) anti-inflammatory activity was displayed by CJSPE (dose 200 mg/kg) in the

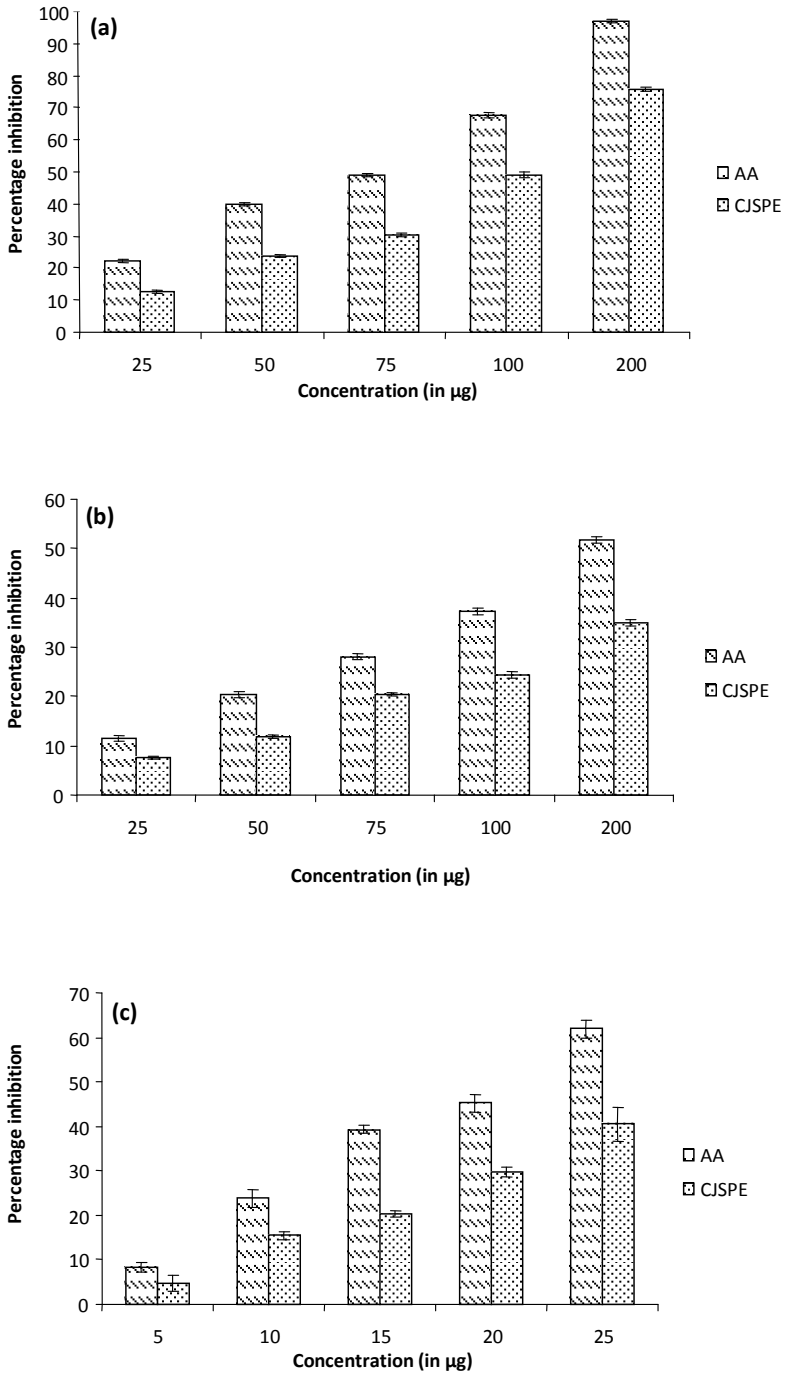

Figure 1. (a) DPPH radical scavenging activity (b) Hydroxyl radical scavenging activity (c) Superoxide radical scavenging activity of CJSPE.

late phase of inflammation; and effect was comparable to that of diclofenac sodium. CJSPE was also found to be effective in the reduction of size (48.55 ± 0.244%) of granuloma formation and effect was nearly equal to that of diclofenac sodium. Inflammatory events occurring in the early hour of carragennan-induced inflammation are mediated by secretion of histamine, 5-hydroxytryptamine, bradykinin. On the other hand, synthesis of prostaglandins (PGs), leucotrienes (LTs), etc. mediates the late phase inflammation (Singh et al., 2009). The late phase of anti-inflammatory activity exhibited by CJSPE suggests that the activity may be due to inhibitory action

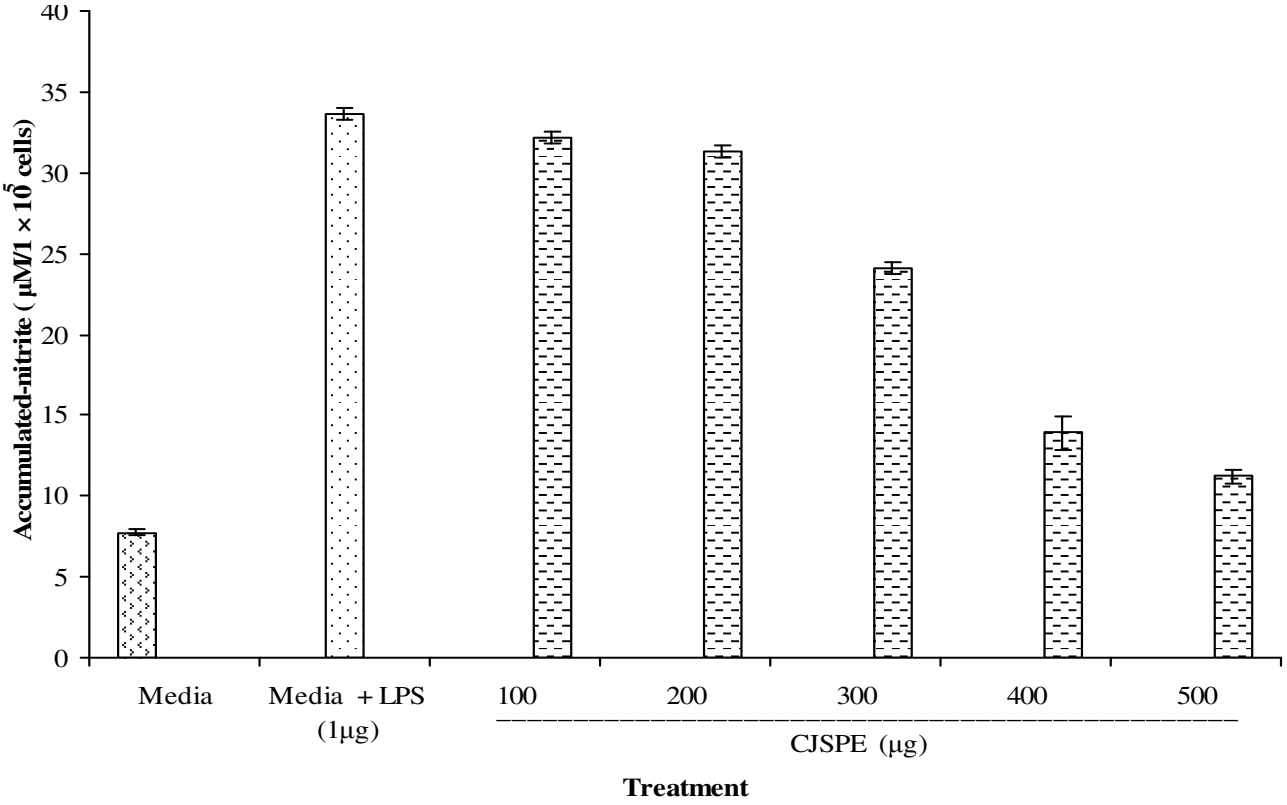

Figure 2. Effect of CJSPE on NO production in isolated macrophages from rat peritoneum.

Table 2. Effect of CJSPE in carrageenan-induced paw edema (volume in ml).

Time interval (h)	Control (Group A)	Diclofenac sodium 50 mg /kg (Group B)	CJSPE	
			100 mg/kg (Group C)	200 mg/kg (Group D)
1	1.28 ± 0.023	0.85 ± 0.013*	1.27 ± 0.020	1.26 ± 0.019
2	1.50 ± 0.020	0.93 ± 0.013*	1.48 ± 0.021	1.38 ± 0.024‡
3	1.59 ± 0.025	0.93 ± 0.013*	1.55 ± 0.025	1.43 ± 0.022†
4	1.62 ± 0.015	0.82 ± 0.010*	1.50 ± 0.011‡	1.19 ± 0.017*
5	1.59 ± 0.012	0.67 ± 0.011*	1.33 ± 0.018*	0.96 ± 0.039*
6	1.55 ± 0.013	0.45 ± 0.012*	0.95 ± 0.016*	0.55 ± 0.053*
12	1.547 ± 0.017	0.28 ± 0.010*	0.85 ± 0.016*	0.36 ± 0.058*
24	1.367 ± 0.017	0.10 ± 0.020*	0.71 ± 0.026*	0.29 ± 0.058*

All the values are expressed as mean ± S.E.M (n=6), * $p < 0.001$, † $p < 0.05$ and ‡ $p < 0.01$ when compared with control group.

on PGs synthesis. The higher fatty acids content (viz. linoleic acid, linolenic acid, oleic acid, steric acid, palmatic acid, etc.) of CJSPE could be responsible for its anti-inflammatory activity. Further, linoleic acid the major constituent (62.36%) of CJSPE might play a pivotal role in its anti-inflammatory effect.

Linoleic acid, a naturally occurring polyunsaturated fatty acid (omega-6) is one of the essential fatty acids. It occurs in isomeric forms (cis, trans and cis-trans) collectively known as conjugated linoleic acid (CLA). Many scientific investigations have found CLA to be beneficial for human health due to its regulation of body fat gain, enhanced immunity, reduced inflammation and minimized adverse reactions which occur with increased body immunity (Pariza, 2004; Calder, 2001; 2006). Likewise, another constituent- linolenic acid was found to exhibit anti-inflammatory activity by metabolic end products viz. 6,9,12,15-octadecatetraaenoic acid, stearadonic acid

Table 3. Effect of CJSPE on cotton pellet-induced granuloma formation.

Treatment groups	Dose (mg/kg)	Dry weight of granuloma (mg)	Inhibition (%)
A	-	73.7 ± 0.384	-
B	50	33.067 ± 0.653*	55.13
C	100	57.95 ± 0.283*	21.37
D	200	37.917 ± 0.244*	48.55

All the values are expressed as mean ± S.E.M (n=6), * $p < 0.001$ when compared with control group.

Table 4. Antimicrobial activity of CJSPE (zone of inhibition in mm).

Microorganism	CJSPE	Ciprofloxacin
E. faecalis	-	19-21
S. aureus	18	25-28
E. coli	17	24-29
K. pneumonia	16	23-26
P. aeruginosa	8	24-27
S. flexneri	16	23-24
S. dysenteriae	-	27-29
V. cholare	14	17-20

and eicosapentaenoic acid; later metabolite is well recognized for its inhibitory action on PGs and LTs, also simultaneously acts as substrate for the formation of PGs and LTs (containing three and five bonds, respectively) of anti-inflammatory nature (Singh et al., 2008).

Free radicals play a vital role in a number of normal regulatory processes; the deregulation of these processes may causes inflammation. Activity of the ROS is increased at the site of inflammation causing a variety of physiological changes viz. deregulation of vascular tone by NO radical, fibroblast proliferation by O_2, increased activity of NFκB transcription factor, neutrophil NADPH oxidase activation, uncoupling of a variety of redox reactions, etc. and leads to tissue destruction and/or inflammation (Winrow et al., 1993). The anti-inflammatory activity of CJSPE may be mediated through its antioxidant property as revealed by its *in vitro* DPPH, $OH^{\cdot}$ and $O^{\cdot\cdot}$ free radicals scavenging activity. CLA might be primarily responsible for antioxidant activity of CJSPE. Earlier studies also supported antioxidant activity of CLA, which showed that linoleic acid react well with thiobarbituric-acid-reactive substances, inhibited the fenton-reaction and lipid oxidation in rat hepatocytes (Mikkelsenaet et al., 1993).

Assessment of antibacterial activity

The antibacterial activity of CJSPE was evaluated against two strains of gram positive and six strains of negative bacteria by the paper disc agar diffusion method. Results of antibacterial activity were summarized in the Table 4. Results showed that CJSPE have good antibacterial activity against the *Staphylococcus aureus, Escherichia coli, Klebsiella Pneumonia* and *Shigella flexneri*. However, the zone of inhibition showed by CJSPE was found less than that of ciprofloxacin (5 µg/disc) used as standard in the experiment.

Conclusion

Seed oils from the plants have been attributed for their nutritional, industrial and medicinal values. This study highlights chemical property, fatty acid composition, antioxidant, anti-inflammatory and antibacterial activities of the seed oil from the *C. juncea.* The high content of linoleic acid present in the *C. juncea* seed oil may be primarily responsible for its significant anti-inflammatory activity and antioxidant activities. However, study also finds moderate antibacterial activity of *C. juncea* seed oil.

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