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Phytochemical Investigation of *Caesalpinia crista* Seed Extract for their Therapeutic Potential

¹Naresh Singh Gill, ¹Ramandeep Kaur, ¹Rashmi Arora and ²Manoj Bali

¹Rayat School of Pharmacy, Railmajra, S.B.S. Nagar, Punjab-143533, India

²Quest Group of Institutes, Punjab Technical University, Jhanjeri, Mohali, India

Corresponding Author: Naresh Singh Gill, Rayat School of Pharmacy, Punjab Technical University, S.B.S. Nagar, Ropar-143533, India Tel: +918146991679

ABSTRACT

Use of plants for health benefits is widespread in India. The present study was to investigate ethanolic seed extract of *Caesalpinia crista* for antioxidant activity by 1,1-diphenyl-2-picryl hydrazyl and hydrogen peroxide methods and anti inflammatory by Carrageenan induced paw edema and analgesic activity by writhing reflexes and by tail immersion method in mice. The extract showed potent antioxidant activity i.e., 73.9 and 77.7% at 300 $\mu\text{g mL}^{-1}$ by 1,1-diphenyl-2-picryl hydrazyl and hydrogen peroxide method as compared to the standard (ascorbic acid). Further, the extract was evaluated for anti inflammatory activity and the extract showed maximum inhibition of 74.2% at 300 mg kg^{-1} by Carrageenan induced paw edema method as compared to standard (diclofenac). Furthermore, the extract was evaluated for analgesic activity the extract showed potent analgesic activity i.e., 71% at 300 $\mu\text{g mL}^{-1}$ by writhing reflexes in mice and the tail withdrawal latency of mice was 5.30 ± 0.05 sec at 300 $\mu\text{g mL}^{-1}$ by tail immersion method. The present study concludes that *Caesalpinia crista* seeds have potent antioxidant, anti inflammatory and analgesic activity it can be used for therapeutic potential.

Key words: *Caesalpinia crista*, antioxidant, anti-inflammatory, analgesic activity, therapeutic potential

INTRODUCTION

A complete storehouse of remedies has been provided by nature to cure ailment of mankind (Kumar and Chandrashekar, 2011). Medicinal plants have always been the principle sources of medicine in India and all over the world (Kaul and Dwivedi, 2010). Medicinal plant is any plant from which valuable drugs can be synthesized as it contains substances that can be used for medicinal purposes (Karim *et al.*, 2011). Drugs obtained from medicinal plants contribute a lot in the development of new compounds (Samy *et al.*, 2008). It is a fact that the use of medicinal plants and traditional health care is affordable, familiar and is available at the local level (Danquah *et al.*, 2011). Plant material containing phenolic compounds found to be effective antioxidants by various previous researchers. The most potent phenolic antioxidants occur in berries, fruits, vegetables, tea leaves and herbs (Ali, 2011). Plant products today symbolise safety in contrast to synthetic drugs (Gill *et al.*, 2011). Therefore, discovery of new anti-inflammatory and analgesic drugs without any adverse effects are being carried out all over the world. During this process, a great attention has been paid in the screening of plant-based drugs which are used in the traditional system of medicine. Also according to WHO, still about 80% of the world population rely mainly on plant-based drugs (Kumara, 2001).

Caesalpinia crista Linn. is an important plant that belongs to the family Caesalpinaceae. It is a popular traditional medicinal plant which is widely distributed throughout the tropical and subtropical regions of Southeast Asia (Das *et al.*, 2010). In hindi, it is known as karanjwa. Seed kernels of this plant have been used as an antimalarial and anthelmintic (Jabbar *et al.*, 2007). Plants of this family have been reported for various activities, e.g., *Caesalpinia crista* is used as tonic for the treatment of rheumatism and backache while *Caesalpinia pulcherrima* is applied as abortifacient and emmenagogue (Linn *et al.*, 2005; Eisai, 1986). The active constituents of these two species are mainly diterpenoids, flavonoids and peltogynoids (Kalauni *et al.*, 2005a; McPherson *et al.*, 1983, 1985; Srinivas *et al.*, 2003). Some of the constituents of this species are known to possess antitumor, antimicrobial and antimalarial properties (Che *et al.*, 1986; Patil *et al.*, 1997; Ragasa *et al.*, 2002). It is also used as an anthelmintic (Yadav *et al.*, 2009). Ten new diterpenes from the *Caesalpinia crista* from Indonesia and twenty new diterpenes from Myanmar have been isolated (Banskota *et al.*, 2003; Lee *et al.*, 2003; Kalauni *et al.*, 2004; Kalauni *et al.*, 2005b).

There is an emerging interest in the use of naturally occurring antioxidants for the management of a number of pathophysiological conditions, most of which involve free radical damage (Gill *et al.*, 2010). The purpose of the study was to explore *Caesalpinia crista* seeds as antioxidant, anti-inflammatory and analgesic agent for therapeutic potential.

MATERIALS AND METHODS

Chemicals used: Hydrogen peroxide was obtained from E-Merck Ltd., Mumbai. 1,1-Diphenyl-2-picrylhydrazyl was obtained from Sigma Chemicals Co., USA. Carrageenan and ascorbic acid were obtained from Central Drug House Pvt. Ltd., Mumbai, India. Acetyl salicylic acid and Diclofenac were received from Jackson Laboratories Pvt. Ltd., Amritsar. Acetic acid was procured from Loba chemicals. All other chemical reagents used were of analytical grade, procured from different companies (Loba Chem., Mumbai; Merck Limited, Mumbai).

Collection of plant part: The seeds of *Caesalpinia crista* were procured from local market of Ropar, Punjab in the month of August, 2010. Seeds were authenticated and voucher specimen number 0395/herb was deposited in Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar for further reference.

Preparation of extract: Dried seed kernels of *Caesalpinia crista* Linn. were taken. Seed coat was broken and testa was separated. The kernel was powdered and passed through sieve No. 40 and stored in an airtight container for the extraction. It was extracted with ethanol for 16 h in soxhlet assembly. The ethanolic extract was then concentrated on rotary evaporator. The residue was reddish brown sticky mass. The LD₅₀ determination of *Caesalpinia crista* seed extract was reported by various researchers (Kshirsagar, 2011).

Animals: Wistar albino rats (120-150 g) of either sex were divided into four groups of six rats each (24 rats) and Swiss albino mice (25-30 g) were divided into four groups of six mice each (24 mice). Animals were housed under standardised animal house conditions like 12 h light/dark cycle, temperature (24±1°C), relative humidity (55-65%) in all the experiments. They had free access to pelleted food and water *ad libitum*. The animals were acclimatized to laboratory conditions for 1 week prior to experimentation. All the animal experiments were carried out in accordance with

the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) and study was approved by Institutional Animal Ethical Committee with registration no. 874/ac/05/CPCSEA. The animals were assigned to different groups to be treated in experiments according to their weight range.

Phytochemical screening: The phytochemical screening of ethanolic seed extract of *Caesalpinia crista* was carried out to check the presence of chemical constituents like flavonoids, alkaloids, tannins, triterpenoids, coumarin glycosides and proteins using standard procedures of analysis (Harborne, 1973).

Antioxidant activity

Determination of antioxidant by 1,1-diphenyl-2-picryl hydrazyl method: The antioxidant activity was measured by 1,1-diphenyl-2-picryl hydrazyl and hydrogen peroxide method (Gill *et al.*, 2010). This is the most reliable method and widely used for the determination of antioxidant activity.

Determination of antioxidant by Hydrogen peroxide: Hydrogen peroxide (0.6 mL) was added in 1 mL of ethanolic seed extract (50-300 $\mu\text{g mL}^{-1}$) followed by 2.4 mL of 0.1 M phosphate buffer whose pH was adjusted to 7.4. This solution was kept for 10 min. Absorbance was recorded at 230 nm against blank. Percentage scavenging of H_2O_2 was calculated using following formula:

$$\text{IC}\% = 100(A_0 - A_s) / A_0$$

where, IC is inhibition concentration, A_0 is absorbance of control and A_s is absorbance of sample (Sood *et al.*, 2009).

Anti inflammatory activity

Carrageenan induced paw edema: Rats were divided into four groups each containing six. Group I served as control and received vehicle (10 mL kg^{-1}) whereas group II received diclofenac 12 mg kg^{-1} and served as standard. Group III and group IV served as test drug in the dose 100 and 300 mg kg^{-1} , p.o. Drugs were administered to the respective animals on the day of evaluation. One hour after this; the sub plantar injection of 0.1 mL of Carrageenan (1% w/v) in right hind paw was given. The paw volume was measured as a displacement of triton mixed saline solution at 0, 0.5, 1, 1.5 and 2 h using Plethysmometer (Gill *et al.*, 2010).

Analgesic activity

Acetic acid induced writhing reflexes: The pre-screened animals were divided into four groups each containing six. Acetyl salicylic acid in dose of 25 mg kg^{-1} , suspended in carboxy methylcellulose was used as the standard drug. The drugs were autoclaved at 12°C for 30 min (the compounds were assumed to be heat stable) and administered per oral. Writhing was induced 30 min later by intraperitoneal injection of 10 mL kg^{-1} of 0.6% acetic acid in distilled water. The number of writhes was counted for 30 min immediately after the acetic acid injection (Saluja *et al.*, 2010).

The percentage protection was calculated as:

$$\frac{X_1 - X_2}{X_1} \times 1000$$

Where:

X₁ = No. of writhings in control group

X₂ = No. of writhings in treated group

Tail immersion method: The animals were screened for the sensitivity test by immersing the tail of the mice gently in hot water maintained at 55-55.5°C. The animal withdrawing his tail from hot water within 5 sec were selected for the study. The rats were divided into four groups of six rats each. Group I received 1% carboxy methylcellulose (0.1 mL kg⁻¹) in distilled water. Group II received Acetyl salicylic acid (12 mg kg⁻¹) and Group III and Group IV received extract in 1% carboxy methylcellulose in distilled water intraperitoneally at a dose of 100 and 300 mg kg⁻¹, respectively. After administration of the drugs, the response time was measured at 0, 15, 30, 45 and 60 min (Gill *et al.*, 2010).

Statistical analysis: All values are represented as Mean±SEM. Statistical analysis was performed using one way analysis of variance (ANOVA) followed by tukey's test using Sigma stat Version-2.0 Software. p<0.05 was considered statistically significant.

RESULT AND DISCUSSION

Phytochemical screening of *Caesalpinia crista* ethanolic seed extract showed the presence of secondary metabolites such as flavonoids, triterpenoids, tannins and alkaloids as shown in Table 1. These components may be responsible for various therapeutic activities. Phytochemical screening of *Caesalpinia crista* flowers showed the presence of flavonoids (Satnami and Yadava, 2011).

Ethanolic seed extract of *Caesalpinia crista* showed the highest antioxidant activity of i.e., 73.9±0.84% as compared to the standard (ascorbic acid) i.e., 86.6±0.52% by DPPH method as shown in Table 2. The extract showed highest antioxidant activity i.e., 77.7±0.05% at 300 µg mL⁻¹ as compared to the standard i.e., 79.6±0.02% by hydrogen peroxide method as shown in Table 3. Antioxidant activity on the leaves of *Caesalpinia crista* have been carried out (Mandal *et al.*, 2010). Antioxidant activity has also been carried out on *Caesalpinia sappan*

Table 1: Phytochemical screening

Chemical constituents	Results
Flavonoids	++
Tannins	+
Sterols	-
Alkaloids	+
Triterpenoids	++
Anthraquinones	-
Proteins	++

++: Maximum presence of chemical constituent, +: Presence of chemical constituent, -: Absence of chemical constituents

Table 2: DPPH radical scavenging activity

Conc. of extract ($\mu\text{g mL}^{-1}$)	Percentage scavenging of DPPH radical	
	Ethanollic extract	Ascorbic acid
50	22.9 \pm 0.26	48.3 \pm 0.62
100	27.9 \pm 0.58 ^a	63.8 \pm 0.87 ^a
200	52.0 \pm 0.76 ^{ab}	75.1 \pm 0.74 ^{ab}
300	73.9 \pm 0.84 ^{ab}	86.6 \pm 0.52 ^{ab}

Values are average of triplicate experiments and represented as Mean \pm SEM. ^ap<0.05 compared with control group, ^bp<0.05 compared with standard group

Table 3: Hydrogen peroxide radical scavenging activity

Conc. of extract ($\mu\text{g mL}^{-1}$)	Percentage scavenging of H ₂ O ₂ radical	
	Ethanollic extract	Ascorbic acid
50	19.7 \pm 0.02	43.9 \pm 0.01
100	20.8 \pm 0.01 ^a	54.3 \pm 0.04 ^a
200	56.0 \pm 0.04 ^{ab}	66.7 \pm 0.01 ^{ab}
300	77.7 \pm 0.05 ^{ab}	79.6 \pm 0.02 ^{ab}

Values are average of triplicate experiments and represented as mean \pm SEM. ^ap<0.05 compared with control group, ^bp<0.05 compared with standard group

Table 4: Anti inflammatory activity by carrageenan induced paw edema

Group	Dose (mg kg^{-1})	Mean paw volume (mL)			Percentage inhibition
		1 h	2 h	3 h	
Control (1% CMC)	0.1	1.050 \pm 1.010	1.000 \pm 0.057	0.9050 \pm 0.0693	0.0
Standard (Diclofenac)	12.5	1.067 \pm 0.088 ^a	0.750 \pm 0.076 ^a	0.1117 \pm 0.0678 ^a	87.6
Ethanollic extract	100	1.200 \pm 0.057 ^{ab}	0.650 \pm 0.050 ^{ab}	0.3667 \pm 0.0494 ^{ab}	59.9
Ethanollic extract	300	0.840 \pm 0.041 ^{ab}	0.5917 \pm 0.042 ^{ab}	0.2333 \pm 0.0666 ^{ab}	74.2

Values are represented as mean \pm SEM of 6 animals. ^ap<0.05 compared with control group. ^bp<0.05 compared with standard group

(heartwood) and *Caesalpinia bonducella* which belongs to the same family (Wetwitayaklung *et al.*, 2005; Shukla *et al.*, 2009). The principle of antioxidant activity is the availability of electrons to neutralize free radicals (Coulidiati *et al.*, 2011). The most reliable methods to estimate antioxidant activity are DPPH and Hydrogen peroxide method which was used in the present study. DPPH is a stable free radical that can accept an electron or hydrogen radical to become a stable molecule (Chanda *et al.*, 2011).

Further, the extract was evaluated for anti inflammatory activity by carrageenan induced rat paw edema method. The extract showed potent anti inflammatory effect i.e., 74.2% at 300 mg mL⁻¹ after 3 h which was comparable to standard (diclofenac) i.e., 87.6% as shown in Table 4. The same activity has been carried out on *Caesalpinia bonducella* seed oil, *C. Pulcherrima* flowers and *C. ferrea* fruit (Shukla *et al.*, 2010; Patel *et al.*, 2010; Carvalho *et al.*, 1996). Carrageenan is a strong chemical responsible for the release of inflammation mediators like histamine, prostaglandin, serotonin, leukotriene etc. (Sood *et al.*, 2009).

Furthermore, the extract was evaluated for Analgesic activity. The extract showed maximal analgesic effect i.e., 71% at 300 mg kg⁻¹, which was comparable to standard i.e., 80.34% by writhing reflexes in mice as shown in Table 5. This activity was also evaluated by tail immersion method. The extract showed significant analgesic activity at 60 min (5.30 \pm 0.05 sec) as compared

Table 5: Analgesic activity by writhing reflexes method in mice

Group	Treatment	Dose	Average No. of writhings	Percentage inhibition
I	Control	1% CMC	50.00±0.7303	0.00
II	Standard	10 mg kg ⁻¹	9.83±0.047 ^a	80.34
III	Ethanollic extract	100 mg kg ⁻¹	27.50±0.056 ^{ab}	45.00
IV	Ethanollic extract	300 mg kg ⁻¹	14.50±0.042 ^{ab}	71.00

Values are represented as Mean±SEM of 6 animals. ^ap<0.05 compared with control group, ^bp<0.05 compared with standard group

Table 6: Analgesic activity by tail immersion method

Group	Treatment	Dose (mL kg ⁻¹)	Tail withdrawal latency (time in sec)			
			15	30	45	60
I	Control (1% CMC)	0.1	2.56±0.01	2.54±0.05	2.60±0.18	2.58±0.03
II	Standard (Aspirin)	12	3.86±0.02 ^a	4.93±0.18 ^a	5.68±0.02 ^a	6.32±0.01 ^a
III	Ethanollic extract	100	3.13±0.08 ^{ab}	3.85±0.05 ^{ab}	4.21±0.05 ^{ab}	4.98±0.06 ^{ab}
IV	Ethanollic extract	300	3.37±0.01 ^{ab}	4.20±0.01 ^{ab}	4.88±0.22 ^{ab}	5.30±0.05 ^{ab}

Values are represented as Mean±SEM of 6 animals. ^ap<0.05 compared with control group, ^bp<0.05 compared with standard group

to standard (6.32±0.01 sec) at 300 mg kg⁻¹ as shown in Table 6. Ethanollic seed extract of *Caesalpinia crista* showed dose dependent analgesic activity. The same activity has been carried out on leaves of *Caesalpinia bonducella* and *Caesalpinia pulcherrima* (Gupta *et al.*, 2003; Patel *et al.*, 2010). Thus, all the results justifies that the present study done is in the support of previous study done on the other plants of same family.

CONCLUSION

The present study conclude that ethanollic seed extract of *Caesalpinia crista* is potent antioxidant, anti inflammatory as well as analgesic agent and It can be used for the benefits of human wealth.

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