

Determination of Antipyretic and Antioxidant Activity of *Cassia occidentalis* Linn Methanolic Seed Extract

Vijay Vikram Singh^{1*}, Jainendra Jain¹, Arun Kumar Mishra²

ABSTRACT

Objective: To perform the pharmacognostical screening and determination of antipyretic and antioxidant activity of *Cassia occidentalis* L. Methanolic seed extract by different in-vitro models. **Methods:** The different pharmacognostical parameters were evaluated as per standard procedure. Finally, antipyretic (Brewer's Yeast Pyrexia model) and antioxidant activity (DPPH and Hydrogen Peroxide Method) was evaluated by adopting different methods. **Results:** The extracts showed a marked antipyretic effect (Figure 1) by causing a reduction in yeast-induced fever. Methanolic extract (400 mg/kg) showed the effect to the same degree as paracetamol (20 mg/kg, i.p.). The experimentally induced laboratory model was employed in evaluating the antipyretic activities of methanolic extracts of *Cassia occidentalis* L. The extract caused a better hypothermal activity against yeast-induced pyrexia in rats. Free radical scavenging activity of *Cassia occidentalis* L. was found because polyphenolic compounds present in plant contribute significantly to the total antioxidant capacity of the seed. Flavonoids play some important pharmacological roles against diseases, such as cardiovascular diseases, cancer, inflammation and allergy. **Conclusion:** The results of the study indicate that the methanolic seed extract of *Cassia occidentalis* L. possesses strong antipyretic and antioxidant activity. This study described many pharmacognostical features and antioxidant activity of seeds of *Cassia occidentalis* L. which will give a new direction for the future scientific research.

Key word: *Cassia occidentalis* L., Phytochemical, Pharmacological, Antipyretic, Antioxidant.

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INTRODUCTION

Cassia occidentalis L. is medicinal plant used as a traditional medicine for the treatment of various diseases. These plants extracts are known to have several activities like anti-inflammatory activity, antibacterial activity, antioxidant, hepatoprotective and Immunosuppression activity. Phytochemical constituents include achrosin, aloemodin, emodin, anthraquinones, anthrones, apigenin, aurantiobtusin, campesterol, cassiollin, chryso-obtusin, chrysophanic acid, chrysarobin, chrysophanol, chrysoeriol etc. have been investigated in *Cassia occidentalis* L. This review summarizes the ethnopharmacological, phytochemical, bioactivity studies of *C. occidentalis* L. plant.

Cassia is a large genus of around 500 species of flowering plants in the family Fabaceae. *Cassia occidentalis* Linn is commonly known as kasaundi, kasamarda in India. It is an ayurvedic plant with important medical values. It is known by various names, e.g. Coffee Senna, Fedegoso, and Negro coffee. It is common weed scattered from Himalayas to the Western Bengal, South India, Burma and Ceylon. The main plant constituents in *Cassia occidentalis* L. include: achrosin, aloemodin, emodin, anthraquinones, anthrones, apigenin, aurantiobtusin, campesterol, cassiollin, chrysophanol, chrysoeriol, emodin, physicon,

quercetin, rhamnosides, rhein, sitosterols, tannins, and xanthorine are presents. The plant is bitter, sweet, thermogenic, purgative, expectorant, antipyretic, antiepilepsy and anticonvulsions. The roots are useful in conditions of inflammation, diabetes, elephantiasis, ring worm, flatulence, epilepsy and convulsions. The leaves useful in conditions of kapha, leprosy, erysipelas, pruritus, wounds and ulcers, cough, bronchitis, asthma, pharyngodynia, fever and hydrophobia. The seeds are useful in leprosy, erysipelas, ulcers, cough, bronchitis and Constipation.¹

Classification

Kingdom :	Plantae
Subkingdom:	Tracheobionta
Division :	Tracheophyta
Class :	Magnoliopsida
Subclass :	Rosidae
Order :	Fabales
Family :	Fabaceae
Genus :	<i>Cassia</i>
Species :	<i>occidentalis</i>

The cotyledons are smooth, round, about 1 cm long and usually less than 1 cm wide with 3 distinct veins

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in the upper surface. The stems have visible hairs just above and below the cotyledons.

Coffee Senna is a smooth annual that can be 2 m tall. The leaves are compound. The leaflets are in 4-6 pairs and have a sharp leaf apex. These leaflets are 2-9 cm long and 2-3 cm wide with a distinct gland 3-5 mm from the base of the stalk. Flowering occurs in the leaf axils. The sepals are green and 6-9 mm long. The petals are yellow and 1-2 cm long. The 6-7 stamens are of two different lengths. The seed pods are dark brown, 8 to 12 cm long, 7-10 mm wide and curve slightly upward. The seeds are dull brown, 4-5 mm long and flattened on both ends (Figure 3). *Senna* is an ancient Arabic name for these plants. The Latin word *occidentalis* means western, and refers to the origin. *S. occidentalis* is widespread in warm areas of the world except for Australasia. On two different soil types growth was greater the higher the pH, 4.7-6.3. The seeds are known to be weakly toxic to various stock animals. Animals normally avoid ingesting these seeds. Increased germination is obtained by seed scarification.²

MATERIAL AND METHOD

Plant material

Collection and authentication of plant materials

The seeds of *Cassia occidentalis* L. belonging to the family Fabaceae were collected in the month of July 2015 from the Herbal garden area of Ram-Eesh Institute of Vocational and Technical Education, Greater Noida, District Gautam Budhha Nagar U.P., India. The plant was identified and authenticated by Dr. Sunita Garg, Chief Scientist, National Institute of Science Communication and Information Resources (NISCAIR) New Delhi. A herbarium is kept in Deptt. of Pharmacy, Ram-Eesh Institute of Vocational and Technical Education for future reference.

Preparation of extracts

Preparation of methanolic extract of *Cassia occidentalis* L. seeds

The seeds of *Cassia occidentalis* L. were shade dried and reduced to coarse powder. The standardized coarse powder was evenly packed in the Soxhlet apparatus and subjected to defatting. The powdered seed was defatted by Petroleum Ether until the color has been changed from dark yellow to colorless.³ Then the powder was subjected to the methanolic extraction. The extract was filtered and filtrate was concentrated by vacuum distillation. Percentage yield of methanolic extract was found to be 13.8%.⁴

Ethical approval

The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC), Ram-Eesh Institute of Vocational and Technical Education, Greater Noida and all the experiments were conducted according to the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). The room temperature of animal house was 25±4°C.

Antipyretic activity

Mice were divided into four groups each group containing six animals. The test performed in rats by injecting 10 ml/kg subcutaneous of 15% aqueous solution of Brewer's yeast to induce pyrexia. Rectal temperature of each animal was taken after the yeast injection using digital clinical thermometer. Animal that did not show a minimum increase of 0.7°C in temperature 24 hrs after yeast injection were discarded. The selected animals were divided into 4 groups and treated as follows:⁵

- Group I (Control) : Vehicle (3ml/kg, p.o.), 1% suspension of Tween-80
 Group II (Standard): Paracetamol (20mg/kg, i.p.)

- Group III (Test-1) : Methanolic extract (500 mg/kg PO)
 Group IV (Test-2) : Methanolic extract (250mg/kg PO)

In-Vitro methods to assess antioxidant activity

Determination of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

DPPH[•] is a stable free radical at room temperature which when accepts an electron or hydrogen radical becomes a stable diamagnetic molecule. The reduction capability of the DPPH radical is determined by the decrease in its absorbance at 517nm, induced by antioxidants. The absorption maximum of a stable DPPH radical in methanol was at 517nm. On reaction with antioxidant or free radical there is decrease in absorbance of DPPH radical because of scavenging of the radical by hydrogen donation. There is change in color from purple to yellow which is visually noticeable. Hence, DPPH is usually used as a substrate to evaluate the antioxidative property.⁵ 0.1 ml solution of DPPH in methanol was prepared and 1.0mL of this solution was added to 3.0ml of extract solution in water at different concentrations (5-160µg/mL). It was incubated at room temperature for 45 min. and absorbance was measured at 517nm against the corresponding blank solution. The assay was performed in triplicates. Ascorbic acid was taken as reference standard. Percentage inhibition of DPPH free radical was calculated based on the control reading, which contain DPPH and distilled water without extract using the following equation:

$$\text{DPPH Scavenged (\%)} = (A_{\text{cont}} - A_{\text{test}}) / A_{\text{cont}} \times 100$$

Where; A_{cont} is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample of the extracts.⁶

Sample Preparation: 50 mg of extract was weighed and dissolved the extract in 50 ml of methanol in 50 ml volumetric flask. Afterwards, 0.1 to 0.4 ml content was pipette out in four 10 ml volumetric flask and volume was made upto the mark. These solutions of concentrations 10,20,30,40 µg/ml were prepared.⁷

Determination of Hydrogen Peroxide scavenging activity

Hydrogen peroxide scavenging activity of *Cassia occidentalis* L. methanolic extract was estimated by replacement titration. Aliquot of 1.0 ml of 0.1 mM H₂O₂ and 1.0 ml of various concentrations of extracts (10-40 µg / ml) were mixed, in this, 2 drops of 3% ammonium molybdate, 10 ml of 2 M H₂SO₄ and 7.0 ml of 1.8 M KI were added. The mixed solution was titrated with 5.09 mM NaS₂O₃ until yellow color disappeared. Percentage of scavenging of hydrogen peroxide was calculated as:

$$\% \text{Inhibition} = (A_0 - A_1) / A_0 \times 100.$$

Where A₀ was the absorbance of the control (blank, without extract) and A₁ was the absorbance in the presence of the extract.⁸

RESULT AND DISCUSSION

The extracts showed a marked antipyretic effect (Table 1) by causing a reduction in yeast-induced fever. Methanolic extract (400mg/kg) showed the effect to the same degree as paracetamol (20 mg/kg, i.p.). The experimentally induced laboratory model was employed in evaluating the antipyretic activities of methanolic extracts of *Cassia occidentalis* L. The extract caused a better hypothermic activity against yeast-induced pyrexia in rats.⁹

Free radical scavenging activity of *Cassia occidentalis* L. expressed in Table 2. Polyphenolic compounds present in plant contribute significantly to the total antioxidant capacity of the seed. Flavonoids play some important pharmacological roles against diseases, such as cardiovascular diseases, cancer, inflammation and allergy. In the present study, reduction of the DPPH radicals was found in concentration- dependent manner.¹⁰ The *Cassia occidentalis* L. methanolic extract reduced the stable

Table 1: Effect of methanolic extract of *Cassia occidentalis* L. seed on yeast induced pyrexia in rats

Group	Treatment	Rectal Temperature(°C)				
		0 Min	30 Min	60 Min	90 Min	120 Min
Group-I	Control 3ml/kg, p.o.	37.7±.05	38.3±.03	38.8±.03	37.6±.1	37.5±.05
GroupII	Paracetamol20mg/kg	36.4±.05	35.6±.08	35.6±.1	35.2±.06	34.6±.1
GroupIII	Extract 400mg/kg, p.o.	39.5±.1	37.3±.05	36.5±.05	36.2±.1	36.5±.1
GroupIV	Extract 200mg/kg, p.o.	37.6±.1	36.2±.1	36.2±.05	36.1±.1	36.3±.08

Table 2: In-vitro Antioxidant effect of methanolic extract of *Cassia occidentalis* L. seed

TEST	10µg/ml extract	10µg/ml AA	20 µg/ml extract	20 µg/ml AA	30 µg/ml extract	30 µg/ml AA	40 µg/ml extract	40 µg/ml AA
DPPH	6.7±.1	10.6±.05	13.3±.1	20.6±.25	19.6±.1	31.8±.4	25.9±.1	43.2±.1
H ₂ O ₂	8.3±.2	10.1±.5	20.3±.5	23.6±.8	36.2±.9	38.6±.8	48.2±.5	48.7±.4

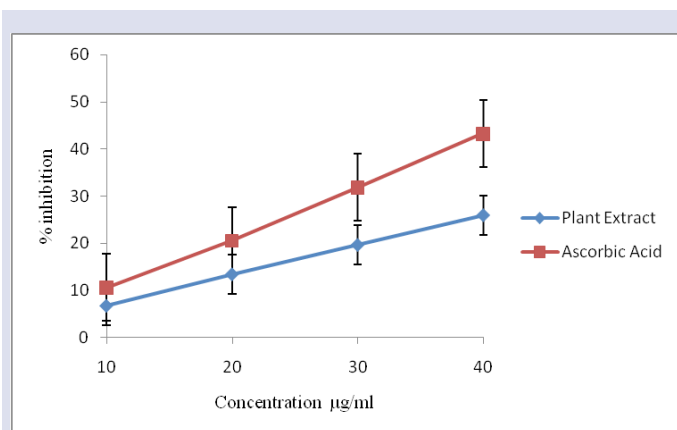


Figure 1: DPPH scavenging activity of *Cassia occidentalis* L. methanolic extract at different concentrations.

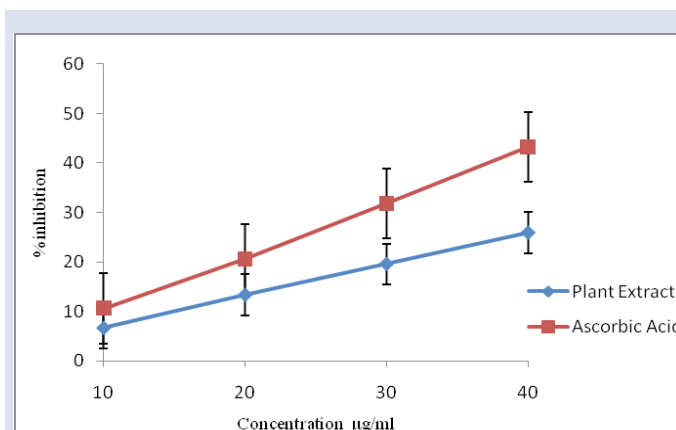


Figure 2: Hydrogen Peroxide scavenging activity of *Cassia occidentalis* L. methanolic extract at different concentrations.



Figure 3: Twig of *S. occidentalis*.

DPPH radical to yellow colored unstable compound. However, ascorbic acid displays significant scavenging activity over the *Cassia occidentalis* L. methanolic extract. This might be due to the presence of methoxy group which increases the accessibility of radical center of DPPH to ascorbic acid.¹¹

CONCLUSION

From the result as in Figure 1, we conclude that the antipyretic and as in Figure 2 antioxidant property in the methanolic extract of *Cassia occidentalis* L. is present. These results clearly indicate that the methanolic extract of *Cassia occidentalis* L. is effective against free radical mediated disease. The seed would be useful as an antipyretic, antioxidant and free radical scavenging agent and it helps in treatment of many diseases that was mediated by reactive oxygen species. Accordingly, in this study, a significant and linear relationship was found between the antioxidant activity and phenolic content, indicating that phenolic compounds could be major contributors to antioxidant activity. Thus, it can be concluded that methanolic extract of *Cassia occidentalis* L. seeds can be used as an accessible source of natural antipyretic and antioxidants with consequent health benefits. Further studies should be undertaken to elucidate the mechanism of action through which the extract exerts the antipyretic and antioxidant activity.¹²

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CONFLICT OF INTEREST

The authors are declared no conflict of interest.

ABBREVIATION USED

C Occidentalis: *Cassia occidentalis*; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **PO:** Per Oral.

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SUMMARY

- In order to ensure the authenticity of seed, the seed was authenticated by National Institute of Science Communication and Information Resources (NISCAIR) New Delhi.
- Antipyretic and Antioxidant properties were studied. It was seen that the *Cassia occidentalis* L seed shows a good properties in the treatment of pyrexia and free radical.
- Further, the antipyretic activity seen against the standard drug Paracetamol and antioxidant activity seen against DPPH and Hydrogen Peroxide.

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