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Phytochemical Studies and *In-vitro* Anti-Oxidant Activity of *Cassia Occidentalis* Linn. Leaves (Caesalpiniaceae)

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A B S T R A C T
Cassia occidentalis Linn. is an important member of plant family Leguminosae. Commonly known as kasundi or Negro coffee, it is generally found growing in India, Burma, Sri Lanka, Australia, United States of America; and many African countries. Its roots, leaves, flowers, and pods contain anthraquinones either in the free form or as glycosides. Pharmacological investigations have revealed the presence of several activities - antioxidant, analgesic, antipyretic, anti-inflammatory, hepatoprotective, antimalarial, antidiabetic, anticancer and antidepressant activities. This plant is also an ingredient of a commercially available formulation (Liv-52 produced by Himalaya Drugs, India) and used in treatment of liver disorders. This article is an attempt to present the overview of pharmacognostical, phytochemical, pharmacological and antimicrobial studies reported on *C. occidentalis*.

Keywords: *Cassia occidentalis* Linn., Leguminosae, Antioxidant, Hepatoprotective, Anthraquinones.

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

Cassia Linn. is an important genus of Leguminosae. There are more than 500 species in this genus. Medicinally important species in this genus are *Cassia angustifolia, C. acutifolia, C. occidentalis, C. javanica, C. tora, C. biflora, C. fistula, C. sophera* etc. These species are rich in phytoconstituents particularly phenolics exemplified by flavonoids and anthraquinones. Leaves and pods of several species possess laxative and purgative action apart from other effects on gastro- intestinal tract. These species possess antioxidant, analgesic, antipyretic, anti-inflammatory, hepatoprotective, antidepressant, muscle relaxant, immunosuppressant and anticancer activities[1]. *Cassia occidentalis* Linn. belongs to the family Leguminosae[2]. The taxonomic status of the plant has been well defined[1]

- Botanical Synonyms[3]:
- *Senna occidentalis* Roxb.
- *Senna occidentalis* (L.)
- *Cassia foetida* Pers.Common Names

The plant has a number of common names in Englishlanguage[4]. Some of them are as follows:

- Coffee senna
- Negro coffee
- Rubbish cassia
- Stinking weed
- Foetid cassia.

Regional & Vernacular Names: The plant is known by several regional and vernacular names[4] (Tables 2-3).

Plant part used: Leaves Geographical sources: *C. occidentalis* grows throughout the tropical and subtropical United States (from Texas to Iowa eastward), Africa, Asia and Australia. It is a common weed found throughout the India[5].

Morphological Description:

C. occidentalis is an annual herb or under shrub. It reaches 60-150 cm in height. It grows at altitudes till 1,500 m. It bears compound leaves - 15-20 cm long and lanceolate or ovate-lanceolate in shape. Each compound leaf has 3 pairs of leaflets. The leaflets are membranous and ovate-lanceolate. The plant bears yellow flowers in short racemes. The mature plants produce characteristic pods. Pods are glabrous and recurved. They are 10-13 cm long and 0.8 cm wide. Pods carry numerous dark olive green-coloured seeds. The seeds are up to 6mm long and 4mm wide. They are hard in texture and have lustrous appearance[4].

Microscopy: The diagnostic microscopical characters of this plant[6] are as follows:

Leaves

Trichomes:Glandular, non-glandular trichomes towards the leaflet margin.

Midrib: Midrib prominent. Collenchyma adjoining the lower epidermis.

Crystals: Prismatic and rosette calcium oxalates in palisade and parenchyma.

Stem.

Endodermis: Distinct young and mature.

Epidermis: Ruptured off in mature stem.

Cork: Present.

Cortex: Inner layers of parenchyma and 1-2 inner collenchyma layers.

Pericycle: Ring of fiber and stone cells.

2. Materials and methods

Collection and identification of plant materials

The leaves of Cassia occidentalis Linn was collected from Madurai district, Tamil Nadu and authenticated by taxonomist Dr. K. Madhava Chetty, M.Sc., Ph.D., Assistant Professor, Department of Botany, S V U Colege of Sciences, S V University, Tirupati.

Preparation of hydroalcoholic extract of Cassia occidentalis Linn (HAECO)

Extraction of leaf powder was carried out by the maceration process. In this method, the shade dried and coarsely powdered leaves of Cassia occidentalis Linn were extracted with 70% hydro alcoholic solvent until the complete exhaustion of the material and filtered. The extract was concentrated under reduced pressure to obtain a semi-solid residue (dark green) 6. The above extract was subjected to physical analysis such as colour, consistency, weight/ml, refractive index.

Qualitative Analysis

Preliminary phytochemical screening

Hydro-alcoholic extract of Cassia occidentalis Linn (Leaf) was subjected to qualitative chemical analysis. The various chemical tests were performed on this extract and aqueous extract for the identification of flavonoids, phenolic compounds, alkaloids, glycosides, carbohydrates, carotenoids, proteins, tannin, aminoacids, sterols 7.

In-vitro studies

Determination of total antioxidant activity

Principle

The total antioxidant activity of the extract is evaluated by phosphomolybdenum method. The assay is based on the reduction of Mo (VI) to Mo (V) by the sample and by the subsequent formation of green phosphate Mo (V) complex at acidic pH which has a maximum absorption at 695 nm. This method is routinely used to determine total antioxidant activity of samples 8.

Procedure

An aliquot of 0.3mL of different concentrations of sample is treated with 2.7mL of the reagent (H2SO4, sodium phosphate and ammonium molybdate). In case of blank, 0.3 mL of methanol is used in place of sample. The tubes are incubated in a boiling water bath at 95°C for 90 min. The samples are cooled to room temperature, the absorbance of the aqueous solution of each concentration is measured at 695 nm against blank. The standard vitamin C is treated in a similar manner. The antioxidant activity is expressed as equivalents of Vitamin C (µg/mL)9.

Determination of scavenging activity against hydrogen peroxide:

Procedure

To 1mL of test solutions of different concentrations, 3.8 mL of 0.1 M phosphate buffer solution (pH 7.4) and then 0.2 mL of hydrogen peroxide solution are added. The absorbance of the reaction mixture is measured at 230 nm after 10 min. The reaction mixture without sample is used as blank. Sample blank is also prepared without reagents. Ascorbic acid is used as standard. The percentage inhibition of hydrogen peroxide is calculated using the formula,

% Inhibition = [(Control–Test) / Control] × 100

The concentration of the sample required for 50 % reduction in absorbance (IC50) is calculated using linear regression analysis 10.

Determination of reducing power assay

Principle

Reducing power assay is a spectrophotometric method and is based on the principle that increases absorbance of the reaction mixture indicates the increase in the reducing power of the sample. Antioxidant activity may be due to a variety of mechanism viz., the prevention of chain initiation, the binding of transition metal ion catalysts, decomposition of peroxides, the reducing capacity and free radical scavenging. The assay is based on the reduction of ferric in potassium ferricyanide to ferrous to form potassium ferrocyanide by the sample and the subsequent formation of Prussian blue colour with ferric chloride. The absorbance of the blue complex is measured at 700 nm 11.

Procedure: The reducing power ability of plant extracts is screened by assessing the ability of the test extract to reduce FeCl3 solution as mentioned by Oyaizu et al., (1986). 0.1 to 0.5mL of plant extract solution (1 mg/mL) is mixed with 0.75mL of phosphate buffer and 0.75mL of 1 % potassium ferricyanide [K3Fe(CN6)] and incubated at 50°C for 20min. About 0.75mL of 10 % trichloro acetic acid is added to the mixture and allowed to stand for 10min. The whole mixture is then centrifuged at 3000 rpm for 10min. Finally 1.5 mL of the supernatant is removed and mixed with 1.5mL of

distilled water and 0.1mL of 0.1 % ferric chloride solution and the absorbance is measured at 700 nm in UV-Visible Spectrophotometer. Ascorbic acid is used as standard and phosphate buffer is used as blank solution 12-15.

3. Results and Discussion

Qualitative phytochemical test

Hydro-alcoholic extract of *cassia occidentalis l.* (leaf) was subjected to qualitative chemical analysis. The various chemical tests were performed on this extract for the identification of phytochemicals, secondary metabolites and the results were displayed in **table: 1**

Table: 1 Preliminary phyto-chemical screening of hydro–alcoholic extract of C*assia occidentalis* l. (leaf)

S.NO	Test	Hydro-alcoholic extract of *Cassia occidentalis* L. (Leaf)
1	**Alkaloids**	
	Mayer' Test	Negative
	Dragendorff's Reagent	Negative
	Hager's Reagent	Negative
	Wagner's Reagent	Negative
2	**Carbohydrates**	
	Benedict's Test	Positive
	Fehling's Test	Positive
	Molisch's Test	Positive
3	**Anthraquinone Glycoside**	
	Borntrager's Test	Positive
	Modified Borntrager's Test	Positive
4	**Cardiac Glycosides**	
	Keller killiani Test	Negative
	Legal Test	Negative
5	**Sterols**	
	Salkowski's Test	Positive
	Libbermann-Burchard's Test	Positive
6	**Saponins**	Positive
7	**Tannins and Phenolic compounds**	
	Folin ciocalteu's phenol Reagent	Positive
	$FeCl_3$ Test	Positive
8	**Flavonoids**	
	Shinoda Test	Positive
	Lead Acetate Test	Positive
	Acid Test	Positive
	Alkali Test	Positive
9	**Protein and Free Amino Acids**	
	Biuret Test	Positive
	Ninhydrin Test	Positive
	Sulphur containing Amino Acid	Positive
10	Terpenoids	Positive
11	Emodin	Positive
12	Volatile oil	Negative
13	Mucilage	Negative
14	Resin	Negative
15	Fixed oil	Negative
16	Gum	Negative

The Preliminary phytochemical screening procedure of the 70% Ethanolic extract of *Cassia occidentalis* Linn leaves showed the presence of Carbohydrates, Anthroquinone glycosides, Sterols, Protein and Amino acids, Flavonoids, Phenolic compounds, Tannins, Saponins, Terpenoids, Emodin and the absence of Alkaloids, Volatile oil, Resin and Cardiac glycosides, Mucilage ,Gum and fixed oil.

Pharmacological studies

In-vitro studies

Hydro-alcoholic extract was subjected to in-vitro antioxidant studies. It includes hydrogen peroxide scavenging activity, total antioxidant capacity and reducing power assay.

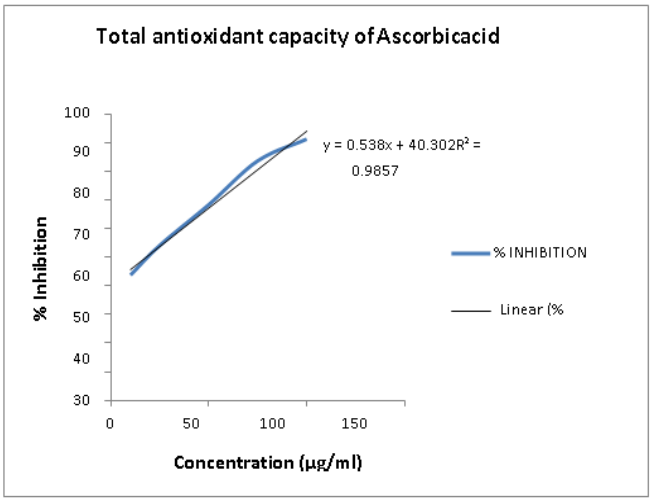

Figure: 1 Determination of Total antioxidant activity of *Cassia occidentalis L.* (leaf) (HAECO)

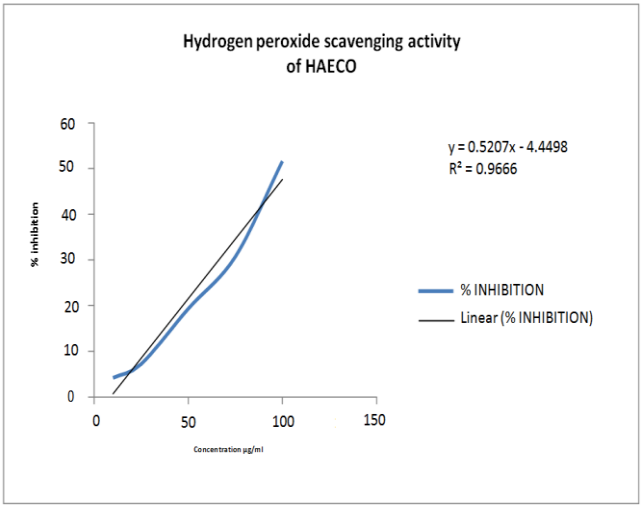

Figure: 4 Determination of reducing power assay of *Cassia occidentalis* L. Leaves (HAECO)

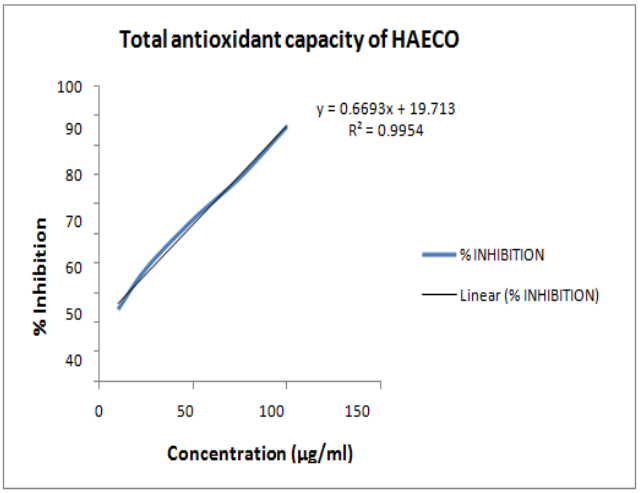

Table 2 Determination of Total antioxidant capacity of *Cassia occidentalis* L. (leaf) (HAECO)

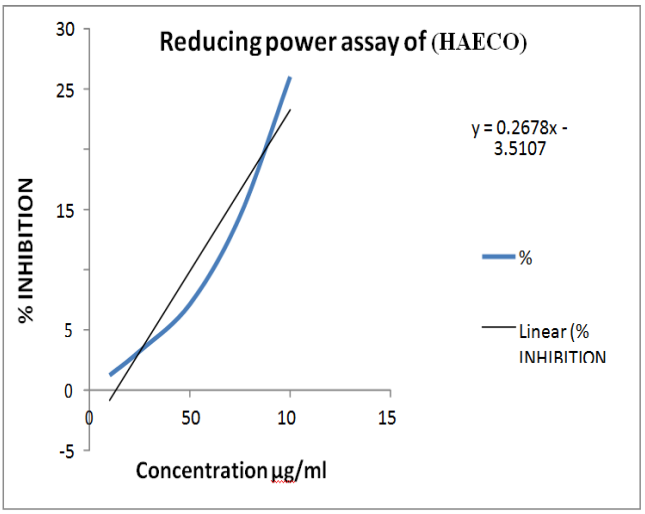

Figure: 5 Determination of reducing power assay of *Cassia occidentalis* L. Leaves (HAECO)

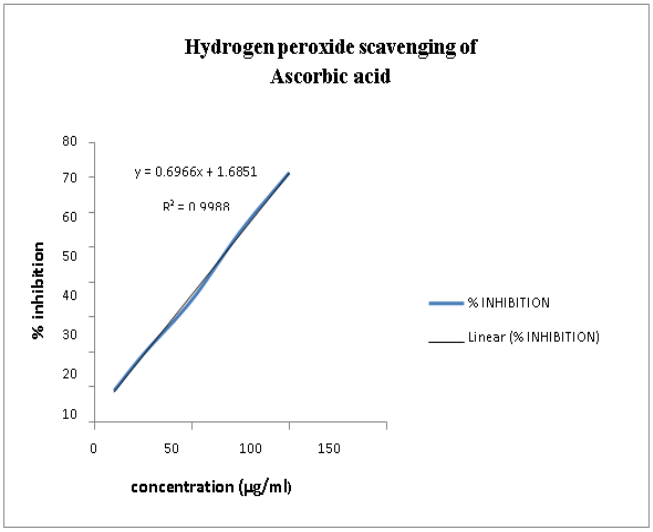

Figure:3 Determination of hydrogen peroxide scavenging activity of *Cassia occidentalis* L. (leaf) (HAECO)

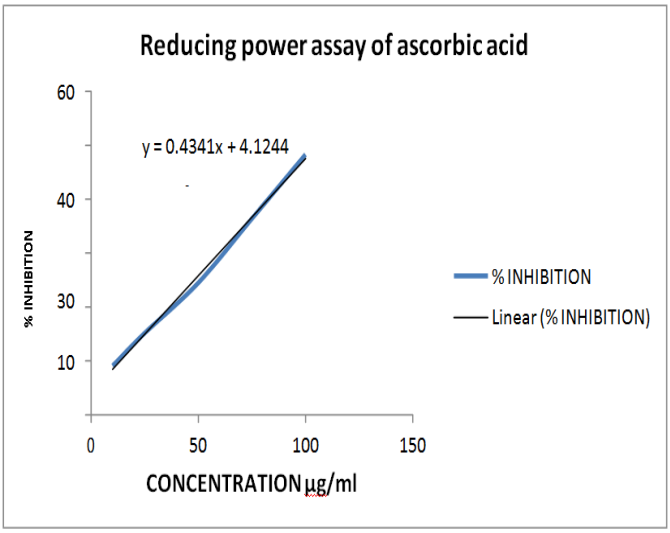

Figure: 6 Determination of reducing power assay of *ascorbic acid*

Table: 2 Determination of Total antioxidant capacity of *Cassia occidentalis* L.(leaf) (HAECO)

S.No.	Concentration of Ascorbic acid / HAECO(µg/ml)	Percentage inhibitionof Ascorbic acid	Percentage inhibition ofHAECO
1	10	43.8	24.3
2	25	54.2	38.2
3	50	68.4	54.8
4	75	83.7	68.98
5	100	91.3	86.3
	IC_{50}	18.03	45.28

The inhibitory concentration (IC50) of *Cassia occidentalis* L. (leaf) against total antioxidant capacity was determined in comparison with ascorbic acid used as a standard. The total antioxidant capacity is found to be 45.28µg/ml in comparison with ascorbic acid 18.03µg/ml.

Table: 3 Determination of hydrogen peroxide scavenging activity of *Cassiaoccidentalis* L. (leaf) (HAECO)

S.No.	Concentration ofAscorbic acid / HAECO (µg/ml)	Percentage inhibitionof Ascorbic acid	Percentage inhibition of HAECO
1	10	9.02	4.2
2	25	19.43	7.23
3	50	34.98	19.34
4	75	54.67	30.72
5	100	71.43	51.65
	IC_{50}	69.42	104.71

The inhibitory concentration (IC50) of *Cassia occidentalis* L. (leaf) against Hydrogen peroxide scavenging was determined in comparison with ascorbic acid used as a standard. The total antioxidant capacity is found to be 104.71µg/ml in comparison with ascorbic acid 69.42µg/ml.

Table: 4 Determination of reducing power assay of *Cassia occidentalis* L. leaf(HAESN)

S.No.	Concentration of Ascorbic acid / HAECO (µg/ml)	Percentage inhibitionof Ascorbic acid	Percentage inhibition of HAECO
1	10	9.1	1.24
2	25	15.2	3.24
3	50	24.5	7.13
4	75	36.4	14.45
5	100	48.3	26.92
	IC_{50}	105.71	200.41

The inhibitory concentration (IC50) of *Cassia occidentalis* L. (leaf) against Reducing power assay was determined in comparison with ascorbic acid used as a standard. The total antioxidant capacity is found to be 200.41µg/ml in comparison with ascorbic acid 105.71µg/ml.

4. Conclusion

The preliminary phyto chemical screening reveals the presence of carbohydrates, anthraquinone glycosides, proteins and amino acids, sterols, flavanoids, phenolic components tannins, saponins, terpenoids, emodin and the absence of alkaloid, volatile oil, resin, cardiac glycoside, mucilage gum and fixed oil. In vitro studies –The hydro alcoholic extract of cassia occidentalis was subjected to anti oxidant studies such as hydrogen peroxide scavenging activity, total anti oxidant activity and reducing power assay. The inhibitory concentration (IC 50) of cassia occidentalis against total anti oxidant activity, hydrogen peroxide scavenging activity and reducing power assay was found to be 45.28µg/ml, 104.71µg/ml, 200.41g/ml respectively. In comparison with ascorbic acid 18.03µg/ml, 69.42µg/ml,105.71 µg/ml respectively. The effect of hydroalcoholic extract of cassia occidentalis on the levels of non-enzymatic anti-oxidants-glutathione, vitamin C, vitamin E showed increase in the levels in dose dependent manner.

Future scope

The future research studies may be extended to discover the new formulation for the potent delivery of the plant drug and the isolation of potent phytoconstituents of this plant *Cassia occidentalis* L.

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