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Investigation on *in-vitro* Antioxidant activity of whole plant of *Cassia occidentalis* Linn. (Caesalpiniaceae)

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Abstract: The present work is carried out to evaluate the antioxidant potential of different fractions of whole plant of *Cassia occidentalis* Linn. (Caesalpiniaceae) using various *in vitro* assay including 1, 1- Diphenyl-2-Picrylhydrazyl (DPPH), nitric oxide scavenging activity, hydrogen peroxide scavenging activity, reducing power assay. In addition, total phenolic content and total antioxidant capacity was also estimated. The various antioxidant activities were compared with ascorbic acid and gallic acid as standard antioxidant. Results showed that ethyl acetate fraction of whole plant of *Cassia occidentalis* Linn. possess significant antioxidant activity than benzene fraction and methanol fraction.

Key words: *Cassia occidentalis,* antioxidant, radical scavenging, total phenolic content, total antioxidant capacity.

INTRODUCTION

Antioxidants are any substance that delay or inhibits oxidative damage to a target molecule. At a time one antioxidant molecule can react with single free radicals and are capable to neutralize free radicals by donating one of their own electrons, ending the carbon-stealing reaction. Antioxidants prevent the cell and tissue damage as they act as scavenger. Free radicals are responsible for causing a wide number of health problems which include cancer, aging, heart diseases and gastric problems etc. Antioxidants cause protective effect by neutralizing free radicals, which are toxic by product of natural cell metabolism. Increasing the antioxidants intake can prevent diseases and lower the health problems[1].

Cassia is a large genus of around 500 species of flowering plants in the family Leguminosae[2]. *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 (English)[3]. It is common weed scattered from Himalayas to the Western Bengal, South India, Burma, and Ceylon[4]. The main plant constituents in *Cassia occidentalis* include: achrosin, aloe-emodin, emodin, anthraquinones, anthrones, apigenin, aurantiobtusin, campesterol, cassiollin, chrysophanol, chrysoeriol, emodin, physicon, quarcetin, rhamnosides, rhein, sitosterols, tannins, and xanthorine are presents[5].

The plant is bitter, sweet, thermogenic, purgative, expectorant, fever, epilepsy and convulsions. The roots are useful in vitiated conditions of vata, inflammation, diadetes, elephantiasis, ring worm, flatulence, epilepsy and convulsions. The leaves useful in vitiated conditions of vata and kapha, leprosy, erysipelas, pruritus, wounds and ulcers, cough, bronchitis, asthma, pharyngodynia, fever and hydrophobia. The seeds are useful in leprosy, erysipelas, ulcers, strangury, cough, bronchitis and constipation[6].

MATERIAL AND METHOD

Collection of plant material

The whole plants of *Cassia occidentalis* was collected from local region Chopda, Dist- Jalgaon, Maharashtra, India during August 2010. Plant material was authenticated by Botanical Survey of India (BSI), Pune, Maharashtra, India. A voucher specimen (No. CASOJA2) was deposited at B.S.I. Pune, India.

Preparation of plant extract

The plant material was washed thoroughly in tap water then dried and powdered. The plant material (750 gm) was extracted with ethanol by using soxhlet apparatus for 72 hours at temperature not exceeding the boiling point of respective solvent. Solvent was distilled off using the distillation assembly of the extracted material. It is further dried, by heating on the water bath, and then fractionated with different solvent like benzene, ethyl acetate, and methanol. Then yield was calculated with reference of air dried basis. Finally the extract were transferred to the air tight amber coloured glass container and stored for further studies.

Chemicals and reagent

DPPH obtained from Sigma Aldrich Ltd. Mumbai. Ascorbic acid, sodium nitroprusside, sulphanilamide, phosphoric acid, α-naphthyl ethylene dihydrochloride, potassium dihydrogen phosphate, potassium ferricyanide, ferric chloride, tricholoroacetic acid, folin-Ciocateu's phenol reagent, ammonium molybdate were obtained from Loba Cheime Ltd. All other chemicals used were analytical grade.

Phytochemical evaluation

Extracts and fractions of *Cassia occidentalis* were studied for its phytoconstituents such as carbohydrates, proteins, steroids, glycosides, saponin alkaloids, flavonoids, tannins and phenolic compounds using different phytochemical tests[7].

DPPH Free radical scavenging activity[8]

Different conc. (20 µl - 100 µl) of test sample and standard sample were prepared. To this add the 3 ml of a 0.004% (w/v) of methanolic solution of DPPH. Shake well and then incubate at room temperature for period of 30 min. A blank was prepared in similar way, without DPPH and absorbance was measured at 517 nm. Scavenging activity was expressed as the percentage inhibition calculated using formula -

Percentage Inhibition= {(Absorbance of control sample – Absorbance of test sample) /Absorbance of control sample} X 100

Ascorbic acid is used as a positive control.

IC_{50} value was calculated from % inhibition[9].

Nitric oxide scavenging activity[10]

The reaction mixture containing 1.5 ml sodium nitroprusside (10 M) in phosphate buffer (7pH) and 1.5 ml of different conc. of test sample (20-100 µg/ml) was incubated at 25^0 C for 150 min. After incubation, 0.5 ml solution of reaction mixture was mixed with 0.5 ml of Griess reagent. The percentage inhibition of nitric oxide generated from sodium nitroprusside was measured using following formula -

Percentage Inhibition= {(Absorbance of control sample – Absorbance of test sample) /Absorbance of control sample} X 100

Absorbance was measured at 546 nm by using UV spectrophotometer. IC_{50} value was calculated from % inhibition. Ascorbic acid was used as positive control.

Hydrogen peroxide scavenging activity[11]

1 ml of test sample / standard solution (20-100 µg/ml) was added to the 0.6 ml of the hydrogen peroxide solution which is prepared in phosphate buffer (pH - 7.4). Incubate for 10 min. at 37^0 C. Hydrogen peroxide in phosphate buffer solution it is used as control. Absorbance was measured at 230 nm.

Scavenging effect (%) was measured using formula –

Scavenging effect (%) = {(Absorbance of control sample – Absorbance of test sample) /Absorbance of control sample} X 100

Ascorbic acid is used as reference compound.

Total antioxidant capacity[12]

Various conc. of test sample (20 - 100µg/ml) and standard Ascorbic acid were prepared. 0.1 ml of test sample was combined with 1 ml of reagent solution (0.6 M Sulphuric acid, 28mM Sodium phosphate and 4 mM Ammonium molybdate). In case of blank, 0.1 ml of methanol was used in place of sample. The tubes were capped properly, and then incubate at 95^0 C for the 90 min. After that cool the sample at room temperature. Measure the absorbance at 695 nm against the blank sample. Antioxidant activity was expressed as equivalent of Ascorbic acid (µg/ml).

Total phenolic content[13]

Various conc. of test sample (20 - 100µg/ml) and standard Gallic acid were prepared. 1.5 ml Folin ciocalteu reagent was added to the volumetric flask containing the test sample and standard solution. After 5 min, 4 ml of sodium carbonate (7%) solution was added. Final volume was made up to 10 ml by using distilled water. Blank determination was done by using the methanol in place of test or standard solution. After 1 hr. measure the absorbance at 760 nm against the

blank solution. Total phenolic content of test sample were expressed in terms of µg Gallic acid equivalent.

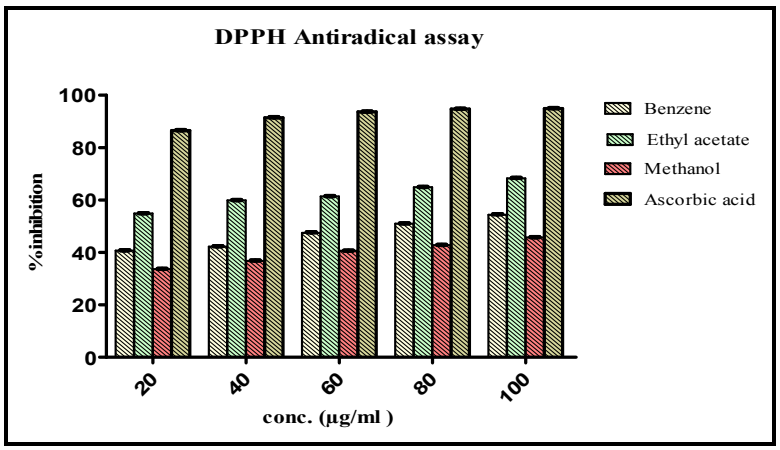
Total reducing power assay[14]

The reducing power of *Cassia occidentalis* was determined on ability of antioxidants to form colored complex with potassium ferricyanide. Various conc. of test sample (20 - 100µg/ml) were mixed with 2.5 ml phosphate buffer (pH 6.6) and 2.5 ml of potassium ferricyanide (1%). The mixture was incubated at 50^0 C for 20 min. 2.5 ml Tricholoroacetic acid (10%) was added to it and then centrifuged at 3000 rpm for the 10 min. The 2.5 ml of supernatant solution was mixed with 2.5 ml of water and 0.5 ml of Ferric chloride (0.1%) was added to it and absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicates increased reducing power.

Statistical analysis

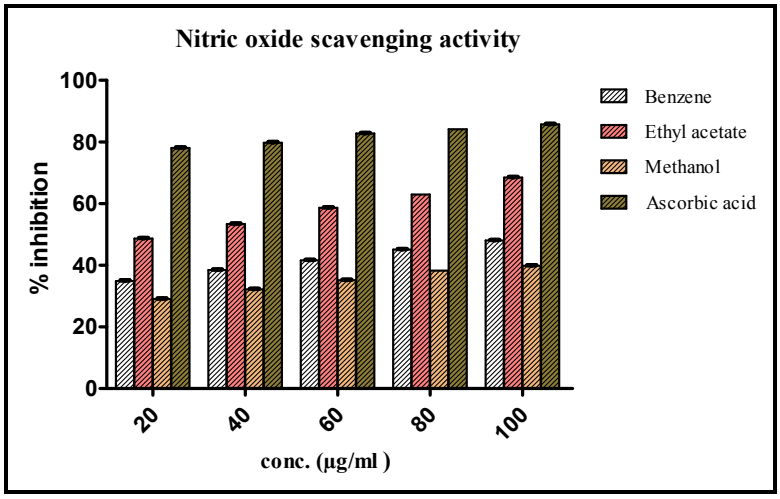
Experimental results were mean ± SEM of three measurements. Analysis of variance was performed by ANOVA followed by Dunnett's test.

Figure 1 Free radical scavenging activity of fractions of ethanol extract of whole plant of *Cassia occidentalis* Linn. on DPPH



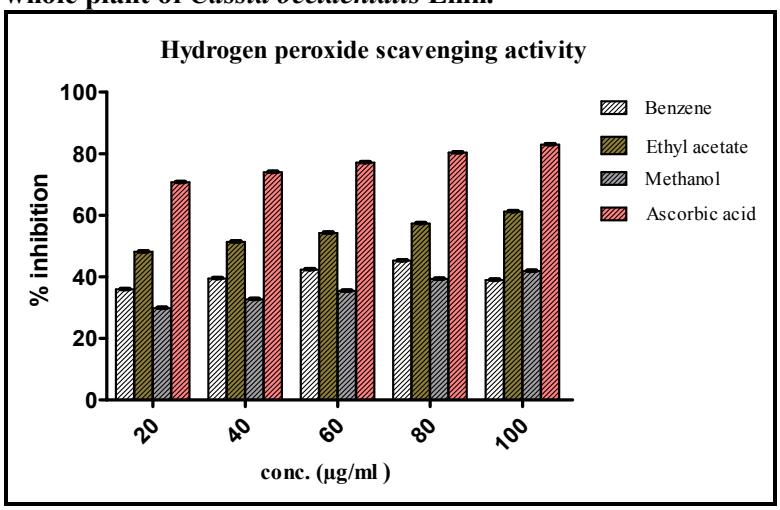
Values are expressed as mean ± SEM, n = 3

Figure 2 Nitric oxide scavenging activity of fractions of ethanol extract of whole plant of *Cassia occidentalis* Linn.



Values are expressed as mean ± SEM, n = 3

Figure 3 Hydrogen peroxide scavenging activity of fractions of ethanol extract of whole plant of *Cassia occidentalis* Linn.



Values are expressed as mean ± SEM, n = 3

Figure 4 Linearity curve for standard Ascorbic acid.

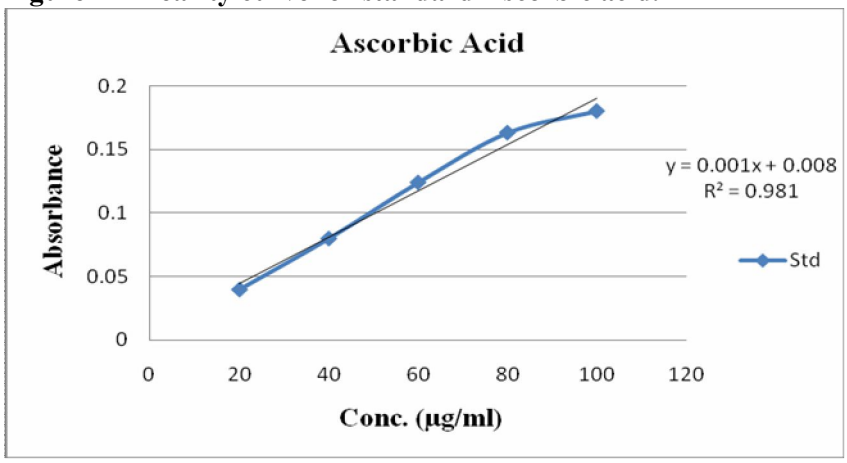
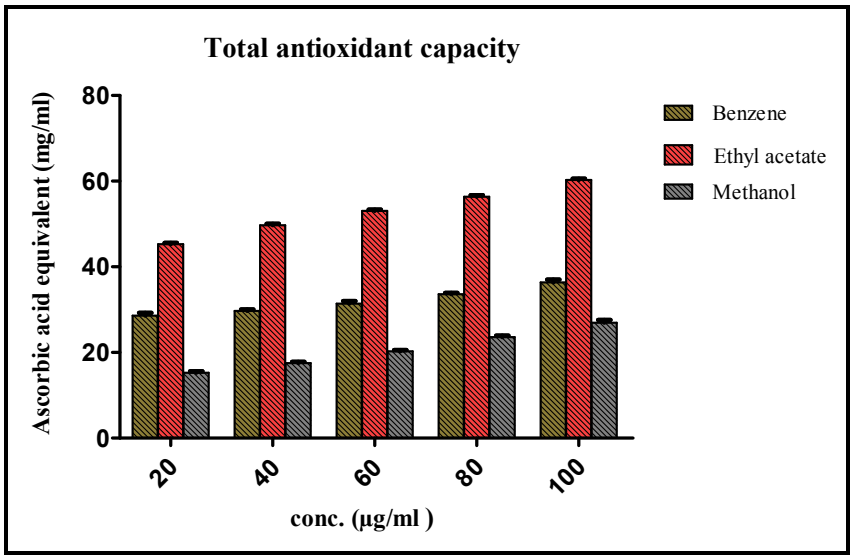


Figure 5 Total antioxidant capacity of fractions of ethanol extract of whole plant of *Cassia occidentalis* Linn.



Values are expressed as mean ± SEM, n = 3

Figure 6 Linearity curve for standard Gallic acid

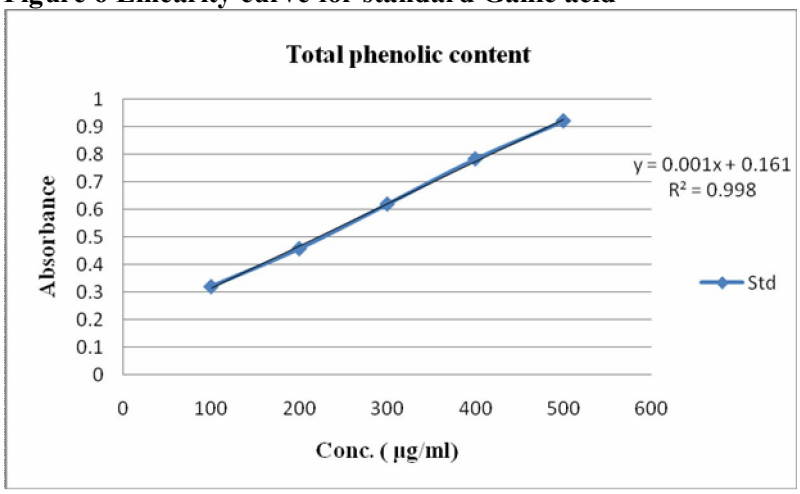
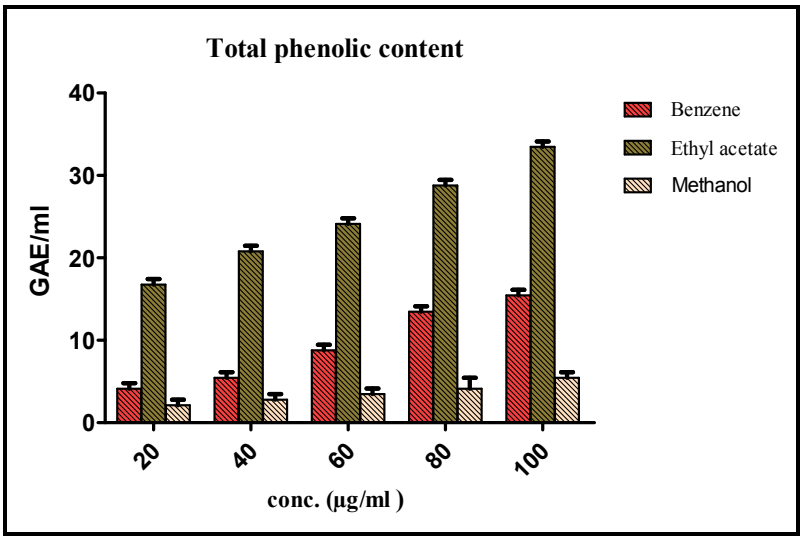
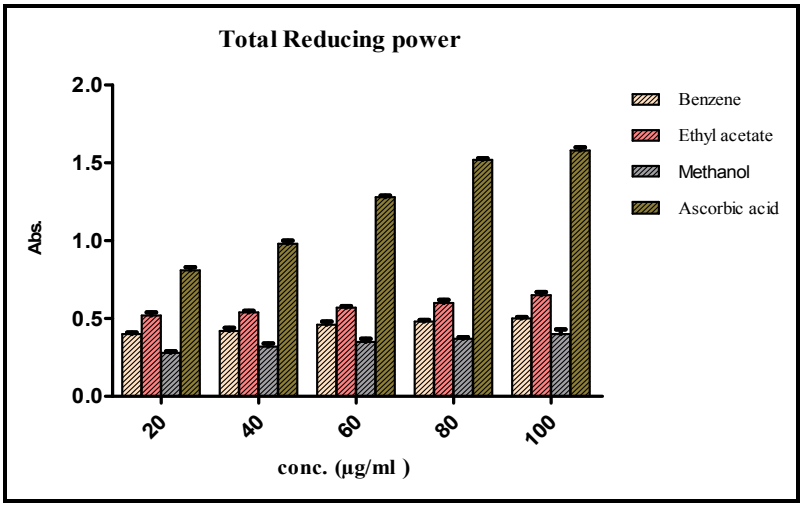


Figure 7 Total phenolic content of fractions of ethanol extract of whole plant of *Cassia occidentalis* Linn.



Values are expressed as mean ± SEM, n = 3

Figure 8 Total reducing power of fractions of ethanol extract of whole plant of *Cassia occidentalis* Linn.



Values are expressed as mean ± SEM, n = 3

RESULTS AND DISCUSSION

Phytochemical evaluation

Phytochemical evaluation of ethanol extract and ethyl acetate revealed presence of carbohydrates, proteins, steroids, glycosides, flavonoids, tannins and phenolic compounds.

In present study the antioxidant activity of benzene, ethyl acetate and methanol fraction were assessed using DPPH radical scavenging, nitric oxide assay and hydrogen peroxide assay. Reducing power, total phenolic contain and total antioxidant capacity was also determined.

DPPH radical scavenging assay

The decrease in absorbance of DPPH radical caused by antioxidants, because of the reaction between antioxidant molecule and radical progresses, results in the scavenging of the radical by hydrogen donation[15].

The results show that whole plant of *Cassia occidentalis* Linn. has concentration dependent scavenging activity against DPPH free radicals. From Figure 1 it is observed that ethyl acetate fraction shows maximum DPPH radical scavenging activity than benzene and methanol fraction. Scavenging ability decreased in the order of Ethyl acetate fraction > Benzene fraction > Methanol fraction which were statistical significant ($p<0.001$) compared to standard.

Nitric oxide scavenging activity

The different fractions of ethanol extract are tested for nitric oxide scavenging activity. It is observed that ethyl acetate fraction shows maximum Nitric oxide radical scavenging activity than benzene and methanol fraction. (Ethyl acetate fraction > Benzene fraction > Methanol fraction) The high Nitric oxide radical scavenging activity was detected in the ethyl acetate fraction with statistical significant ($p<0.001$) compared to standard (Figure 2).

Hydrogen peroxide scavenging activity

H_2O_2 is highly important because of its ability to penetrate biological membranes. H_2O_2 itself is not very reactive, but it can sometimes be toxic to cell because of it may give rise to hydroxyl radical in the cells[15]. The different fractions of ethanol extract are tested for hydrogen peroxide scavenging activity. The results showed that fractions of *Cassia occidentalis* had an effective H_2O_2 scavenging activity. H_2O_2 Scavenging ability decreased in the order of Ethyl acetate fraction > Benzene fraction > Methanol fraction which were statistical significant ($p<0.001$) compared to standard (Figure 3).

Total antioxidant capacity

It is spectroscopic method for the quantitative determination of antioxidant capacity, through formation of phosphomolybdenum complex[16]. Total antioxidant capacity of the extracts is expressed as the number of equivalents of ascorbic acid. Samples show a dose dependent antioxidant capacity, as concentration increases antioxidant capacity increases. Figure 4 shows linearity curve for standard Ascorbic acid. The ethyl acetate fraction was found to be containing higher amount of total antioxidant capacity as compare to benzene fraction and methanol fraction. (Ethyl Acetate fraction > Benzene fraction > Methanol Fraction)

Total phenolic content

Phenolics are very important plant constituents because of their scavenging capability due to their hydroxyl groups. The various phenolic antioxidants such as flavonoids, tannins, coumarins and xanthones scavenge the radicals. The phenolic substance is known to possess ability to reduce oxidative damage and act as antioxidants[17]. Total phenolic content in the extract was determined by Folin-ciocalteu method. The total phenolic content in ethyl acetate fraction, benzene and methanol fraction of whole plant of *Cassia occidentalis* was expressed as gallic acid equivalent per mg of extract.

Figure 6 shows linearity curve for standard gallic acid. From Figure **7** it was found that ethyl acetate fraction contains higher amounts of total phenolics contain as compare to other. Total phenolic content of different fraction of *Cassia occidentalis* Linn are found in order -(Ethyl acetate fraction > Benzene fraction > Methanol fraction)

Total reducing power assay

In this assay the yellow colour of the test solution changes to various shades of green and blue is depending upon the reducing power of each compound[18]. A higher absorbance at 700 nm indicates a higher reducing power. From Figure 8 show reducing capacity of different fraction of *Cassia occidentalis* Linn and Ascorbic acid. It was observed that absorbance of test sample and standard sample was increased with increase in concentration of test and standard. *Cassia occidentalis* Linn shows concentration dependant reducing capacity.

CONCLUSION

The results of the study indicate that ethyl acetate fraction of ethanol extract of whole plant of Cassia occidentalis Linn. possess the significant antioxidant activity. Ethyl acetate fraction of ethanol extract of whole plant of Cassia occidentalis Linn. must contain some principles, which possess antioxidant activity. From phytochemical evaluation, it shows presence of flavonoids, tannins and phenolic compounds, hence proper isolation of the active principles which helps in finding of new lead compounds. Further in vivo studies are required to understand exact mechanism of action.

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