



Evaluation of free radical scavenging activity of selected parts of *Cassia senna*

Amutha Priya R *, Suganya V¹, Suganthi B², Aishwarya K³

Avinashilingam Institute for Home Science and Higher Education for women, Coimbatore, Tamilnadu, India.

*Corresponding author's E-mail: amuthabiochem@gmail.com

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ABSTRACT

Medicinal plants containing high antioxidant properties play an important role in the prevention of various degenerative diseases in the society. *Cassia* species have been of keen interest in phytochemical and pharmacological research due to their excellent medicinal values. Hence the present study is aimed at investigating the free radical scavenging activity of selected parts of *Cassia senna*. The different solvent extracts of leaf and pod parts of *Cassia senna* were analysed for the various free radical scavenging activity. All solvent extracts of the leaves and pods exhibited varying degree of free radical scavenging effect on DPPH radical, inhibition of super oxide radical generation, inhibition of nitric oxide radical generation, hydroxyl radical, hydrogen peroxide and ABTS radical. The petroleum ether extract of both leaf and pod parts showed the maximum level of radical scavenging activity, while a moderate activity was observed in all the other solvent extracts of leaf and pod.

Keywords: *Cassia senna*, free radical scavenging activity, petroleum ether extract.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Medicinal plants have been used for centuries as remedies for human and animal diseases as they contain phytochemicals of therapeutic values¹. The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities, no side effects and economic viability².

Living cells may generate free radicals and other reactive oxygen species by-products as a result of physiological and biochemical processes. Free radicals can cause oxidative damage to lipids, proteins and DNA, eventually leading to many chronic diseases such as cancer, diabetes, aging and other degenerative diseases in humans³. Recently interest in naturally occurring antioxidants has considerably increased for use in food, cosmetic and pharmaceutical products, because they possess multifacetedness in their multitude and magnitude of activity and provide enormous scope in correcting imbalance⁴.

In search of novel sources of antioxidants in the last years, medicinal plants have been extensively studied for their antioxidant activity⁵. The therapeutic basis of herbal medication are by the presence of diverse bioactive compounds like alkaloids, flavonoids, terpenoids, phenolic compounds, glycosides etc., in plant and also for the treatment of diseases which are still incurable, medicinal plants can serve as a source of novel therapeutic agent⁶.

Cassia species have been of keen interest in phytochemical and pharmacological research due to their excellent medicinal values⁷. All *Cassia* species are rich source of secondary metabolites, notably anthraquinone derivatives and has been used in Chinese and Ayurvedic preparations. A review of phytochemistry of some medicinally important *Cassia* species has been presented, considering the fact that there are about 580 species of this genus scattered all around the world⁸.

Cassia senna (Common Names-Senna, Indian senna, English – sanay, Hindi- sana ka pat) belonging to the family Fabaceae. *Cassia senna* leaves have been investigated for the presence of its secondary metabolites and evaluation of biological activities of the crude extracts with special emphasis to the antimicrobial activity, cytotoxic activity and thrombolytic activity⁹. The ethanolic extract of *Cassia senna* has good suppression of Prostate tumor growth in *in vivo* model using Testosterone and N-methyl N-nitrosourea for induction of prostate carcinogenesis¹⁰

MATERIALS AND METHODS

Collection of the plant material

The fresh plant of *Cassia senna* was collected from the Madurai district, Tamilnadu. Then the plant was identified and was authenticated in Tamilnadu Agricultural University, Coimbatore. The plant material was cleaned, separated into leaves and pods and they were shade dried. When the plant materials were thoroughly dried, they were coarsely powdered using a mechanical grinder. The powder was stored in an air tight, light resistant container for further analysis.



Preparation of the extracts from leaf and pods

Extraction procedure using organic solvents by Successive soxhlet apparatus

The powdered plant material of leaf and pod was subjected separately to successive solvent extraction taking from non-polar to polar solvents like petroleum ether, benzene, chloroform, ethyl acetate and ethanol. 15gms of powdered plant material was subjected to soxhlet extraction for 8 hrs with 250ml of the above solvents. The extracts obtained were later kept for evaporation to remove the excessive solvents. These extracts were stored in a cool dry place for the analysis of free radical scavenging activity¹¹.

Extraction procedure using water

For aqueous extraction, 10 g of air-dried powder of leaf and pod was added to distilled water and boiled on slow heat for 2 hours. It was then filtered through 8 layers of muslin cloth and centrifuged at 5000g for 10 min. The supernatant was collected. This procedure was repeated twice. After 6 hours, the supernatant collected at an interval of every 2 hours was pooled together and concentrated to make the final volume one-fourth of the original volume¹².

Evaluation of free radical scavenging activity

DPPH radical scavenging activity

DPPH(2,2'-diphenyl-1-picrylhydrazyl) radical scavenging activity was carried out by the method of Mensor *et al.*, (2001)¹³. The different solvent extracts of leaf and pod parts of *Cassia senna* (25µl) and 0.48 ml of methanol were added to 0.5 ml of methanolic solution of DPPH. The mixture was allowed to react at room temperature for 30 minutes. Methanol alone served as blank and DPPH in methanol, without the plant extracts, served as positive control. After 30 minutes of incubation, the discolourisation of the purple colour was measured at 518nm. The radical scavenging activity was calculated as follows.

$$\% \text{ of scavenging activity} = \frac{A_{518} \text{Control} - A_{518} \text{Sample}}{A_{518} \text{Control}} \times 100$$

Inhibition of superoxide radical generation

Determination of inhibition of superoxide radical generation was carried out by the method of Winterbourn *et al.*, (1975)¹⁴. The reaction mixture consisted of 2.63ml phosphate buffer, 20µl of plant extract, 200µl of EDTA, 100µl of NBT and 50µl of riboflavin. The control tubes were also set up where DMSO was added instead of sample. All the tubes were vortexed and measured the initial optical density at 560nm. After that, these tubes were placed in an area where they received uniform illumination for 30 minutes. Again the optical density was measured at 560nm. The difference in optical density before and after illumination is the generation of superoxide by the test sample and

calculated by comparing with the optical density of the control.

$$(\% \text{ inhibition}) = \frac{\text{O.D after illumination} - \text{O.D (Initial)}}{\text{Control O.D}}$$

Inhibition of nitric oxide radical generation

Determination of Inhibition of nitric oxide radical generation was carried out by the method of Green and Hill, (1984)¹⁵. The reaction mixture (3ml) containing 2ml of sodium nitroprusside, 0.5ml of phosphate buffered saline and 0.5ml of extracted sample was incubated at 25°C for 150 minutes. Control without test compound was kept in an identical manner. After incubation, 0.5ml of griess reagent was added. Incubate for 30 minutes. The absorbance of the chromophore formed was read at 546nm and the percentage inhibition was calculated by the following formula.

$$\% \text{ inhibition} = \frac{A_e}{A_c} \times 100 \text{ Where,}$$

$$A_c = \text{Absorbance of control}$$

$$A_e = \text{Absorbance in the presence of plant extract}$$

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was carried out by Apak *et al.*, (2007)¹⁶. To a test tube add 1.3ml of phosphate buffer (pH-7.0), 0.5 ml of 10mM 2-deoxy-D-ribose, 0.25ml of 20mM Na₂-EDTA, 0.25ml of 20mM FeCl₂ solution, 0.1ml of sample solution, 1.9ml distilled water and 0.5ml of 10mM H₂O₂ rapidly in this order. The mixture in a total volume of 10ml is incubated for 4hours at 37°C in a waterbath. At the end of the period, the reaction is arrested by adding 2.5ml of 2.8% TCA. To this add 2.5ml of 1%TBA and keep the reaction mixture at 100°C in a boiling waterbath for 10minutes. Cool the mixture under running tap water and record the absorbance at 520nm. Express the hydroxyl radical scavenging ability in percentage

$$[1 - \frac{\text{test sample absorbance}}{\text{blank sample absorbance}}] \times 100\%$$

Hydrogen peroxide scavenging activity

The ability of the plant extracts to scavenge hydrogen peroxide was determined according to the method of Ruch *et al.*, (1989)¹⁷. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Plant extracts (100 µg/ml) were added to a hydrogen peroxide solution (0.6 mL, 40mM). Absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of extracts were calculated.

$$\% \text{ Scavenged [H}_2\text{O}_2] = \frac{[(AC - AS)/AC] \times 100}{100}$$

Where AC is the absorbance of the control and AS is the absorbance in the presence of the sample.



ABTS radical scavenging activity

ABTS [2, 2'-azinobis (3-ethylbenzothiazoline-6- sulfonic acid)] radical scavenging activity was carried out by the method of Shirwaikar *et al.*, (2006)¹⁸. The different extracts (100µl each) were added to ABTS solution (300µl) and the final volume of each was made up to 1ml with ethanol. The absorbance was read at 740nm and the percentage inhibition was calculated using the formula,

$$\% \text{ of scavenging activity} = \frac{A_{740} \text{Control} - A_{740} \text{Sample}}{A_{740} \text{Control}} \times 100$$

RESULTS AND DISCUSSION

DPPH radical scavenging activity

Figure 1 shows the DPPH radical scavenging activity of the different solvent extracts of leaf and pod of *Cassia senna*. From the present results, it is evident that benzene extract of leaf and petroleum ether extract of pod exhibited maximum percentage of scavenging activity of 94% and 97% respectively.

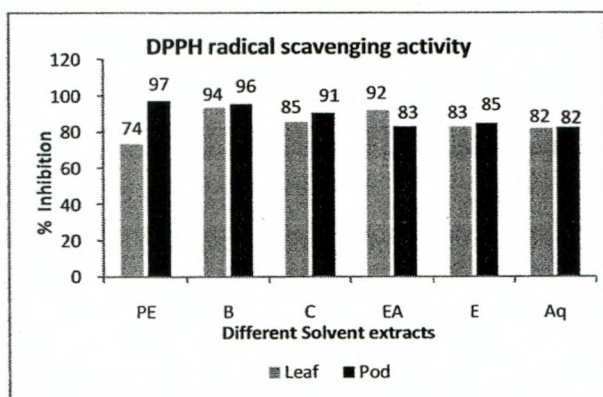


Figure 1: DPPH radical scavenging activity

The antioxidant activity of *Cassia spectabilis* was evaluated by Sangetha *et al.*, (2008)¹⁹ using the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay. It was reported that the flower, stem, leaf and pod extracts exhibited 54.29%, 53.28%, 45.17% and 6.18% of radical-scavenging activities, respectively, at 1.0 mg/ml of extract tested.

The antioxidant potential of flowers and seeds of *Cassia occidentalis* in different solvent extracts were evaluated by various biochemical assays namely, DPPH radical scavenging activity, reducing power activity, in which seeds were found to have higher antioxidant activity²⁰.

In a similar manner leaf extracts of *Cassia occidentalis* and *Cassia hirsuta* were also shown to possess the highest scavenging activity of DPPH radical^{21,22}.

Inhibition of superoxide radical generation

From the Figure 2 it is evident that among the various solvent extracts, ethyl acetate followed by ethanolic extract of the pod showed maximum percentage inhibition of superoxide radical generation, 90% and 89% respectively.

In the various leaf extracts studied the ethyl acetate extract exhibited maximum percentage of inhibition of 32%.

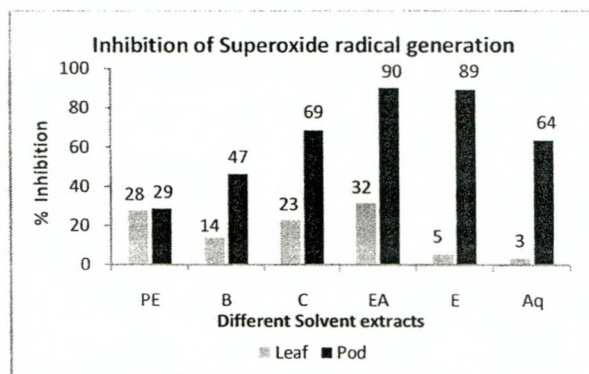


Figure 2: Inhibition of superoxide radical generation

Inhibition of Nitric oxide radical generation

It is evident from Figure 3 that percentage inhibition of nitric oxide radical generation by petroleum ether extract of both leaf and pod showed the highest value, while that in other solvent extracts of leaf and pod did not differ significantly.

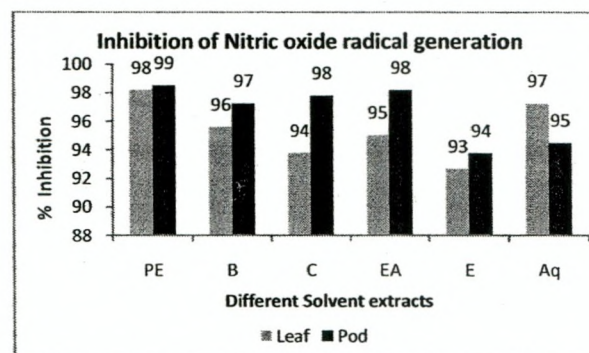


Figure 3: Inhibition of Nitric oxide radical generation

Hydroxyl radical scavenging activity

From the results shown in Figure 4, the petroleum ether extract of both leaf and pod parts showed the highest percentage of hydroxyl radical scavenging activity, while a moderate activity was observed in all the other solvent extracts of leaf and pod.

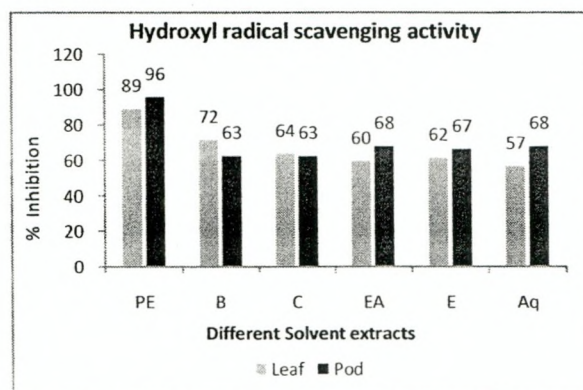


Figure 4: Hydroxyl radical scavenging activity



Hydrogen peroxide scavenging activity

It is evident from Figure 5 that the hydrogen peroxide scavenging activity of aqueous extract of leaf and pod were found to be 87% and 89% respectively followed by the ethanolic extract of both leaf and pod.

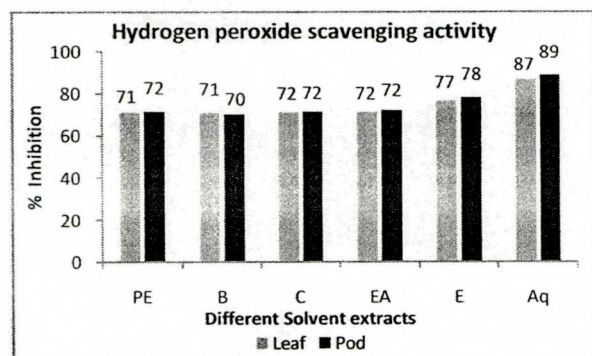


Figure 5: Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging activities of ethanolic leaf extracts of *Cassia occidentalis* (COLEX), BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) were respectively 53.0%, 85.3% and 97.8% for COLEX, BHA and BHT at 50 $\mu\text{g}\cdot\text{ml}^{-1}$ concentration²¹.

ABTS radical scavenging activity

From the results shown in Figure 6, it is clear that the ABTS radical scavenging activity of the various leaf extracts was found to be greater than that of pod. The chloroform extract of leaf showed maximum scavenging effect when compared to the other solvent extracts. In pod petroleum ether extract exhibited maximum scavenging of ABTS radical. The ability of *Cassia senna* to scavenge ABTS radical might be due to the presence of antioxidants particularly polyphenols and flavonoids which are highly responsible for the ABTS radical scavenging activity.

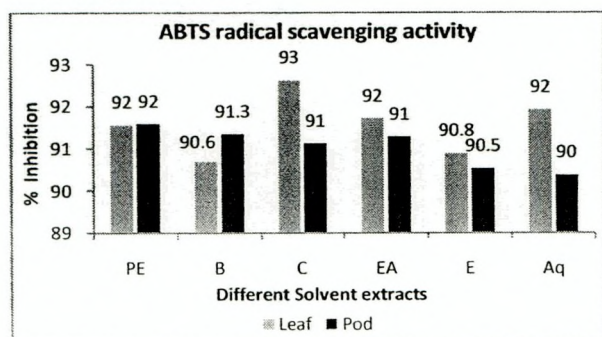


Figure 6: ABTS radical scavenging activity

CONCLUSION

In conclusion, it could be stated that the ability of free radical scavenging activity of leaf and pod parts of *Cassia senna* might be due to the presence of antioxidants and secondary metabolites both of which could serve as free radical inhibitors or scavenger. The present study revealed that the leaves and pods of *Cassia senna* appear

to be good sources of essential antioxidants by their radical scavenging activity which can have novel therapeutic value against various degenerative diseases.

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