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# Determination of four Cassia species for their antioxidant and antimicrobial properties

KEYWORDS: Cassia, antioxidant, β-carotene bleaching, erythrocytes hemolysis

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**ABSTRACT** *Flowers of Cassia alata, Cassia auriculata, Cassia fistula and Cassia siamea belonging to family Fabaceae were extracted using solvents like hexane, chloroform, ethyl acetate, methanol, hydroalcohol, for whom the immense presence of phytoconstituents has been revealed by preliminary screening tests. In the present scenario, with a necessity to explore in vitro enzymatic antioxidant activity, assays like superoxide, hydrogen peroxide, inhibition of β-carotene bleaching, inhibition of erythrocytes hemolysis accompanied with carbohydrate, protein content, antibacterial and antifungal activities were performed. All among 20 extracts, for a comparative analysis, the methanol extracts of Cassia fistula and Cassia auriculata has demonstrated their excellence in all the above said assays, in particular. Cassia alata and Cassia siamea has presented satisfactory values for the above assays, in contrast to other species. The extensive research work on the Cassia species in a systematic way has presented a wide dimension to be easily accessible antioxidants.*

## INTRODUCTION

Working on plants was a good start to a research career, since plants are of enormous importance in the free radical-antioxidant field. Natural antioxidant substances are presumed to be safe since they occur in plant foods and are seen as more desirable than their synthetic counterparts. Owing to this concept, in tune with an effort to trigger the positive scale of the flowers of four species viz. Cassia alata, Cassia auriculata, Cassia fistula, Cassia siamea is of interest. The comprehensive phytochemical and biological evaluations of the different morphological parts of the numerous local Cassia species are scanty (Abo et al., 1999). Cassia species (Leguminoseae- Caesalpiniaceae), are well-known for their laxative and purgative properties and are also used in the treatment of skin diseases (Dalziel, 1956), healing ulcers (Biswas & Ghosh, 1973) exerts an antipyretic and analgesic effect (Patel et al., 1965), an antiperiodic agent (Dutta & De, 1998). In this paper, we describe the various enzymatic and non-enzymatic assays to contend their nutritive value and in turn the antioxidant capacity.

## MATERIALS AND METHODS

### Plant selection and authentification:

Fresh and healthy flowers were collected [July- December] in Shevaroys hills and open grounds and authenticated [Cassia alata & Cassia siamea- BSI/SRC/5/23/11-12/TECH-781, Cassia auriculata & Cassia fistula- BSI/SRC/5/23/10-11/TECH-780] by a Botanist (Dr.G.V.S.Murthy, Scientist-F, Head of Office), BSI, TNAU, Coimbatore.

### Extraction procedure:

The old, insect-damaged, fungus-infected flowers were removed and only the fresh, healthy flowers were selected and washed well with dechlorinated water prior to distilled water, deprived of dusts. Soxhlet extraction of the powdered flower samples was carried out using each of the following solvents in increasing polarity: n-hexane (defatting), ethyl acetate, chloroform, methanol and finally with water. All the crude solvent extracts were stored at 4–5˚C until further use.

### Estimation of carbohydrate content:

Total carbohydrate contents were estimated by anthrone method (Hedge & Hofreiter, 1962). Glucose was used to calculate the standard curve (20-120 µg/mL, Y= 0.0263x+0.0532, R2= 0.9992) and the results were expressed has µg of glucose equivalents per mg of extract.

### Estimation of protein content:

Total proteins were estimated by Lowry's method (Lowry et al., 1951). Bovine serum albumin was used to calculate the standard curve (20-160 µg/mL, Y = 0.0159x + 0.0319, R2 = 0.9569) and the results were expressed as µg of bovine serum albumin equivalents per mg of extract.

### Superoxide anion radical scavenging assay:

The assay for superoxide anion radical scavenging activity was based in on a riboflavin – light –NBT system (Beauchamp & Fridobich, 1971). Ascorbic acid was used as standard. The percent inhibition of superoxide anion generation was calculated using the following formula:

Scavenging activity (%) =

1- absorbance of sample x 100
absorbance of control

### Hydrogen peroxide scavenging assay:

The ability of the extracts to scavenge hydrogen peroxide was determined according to the method of Ruch et al., (1989). Ascorbic acid was used as the standard. The abilities to scavenge the hydrogen peroxide were calculated using the following equation:

Hydrogen peroxide scavenging activity =

1- absorbance of sample x 100
absorbance of control

### Inhibition of b-carotene bleaching:

The antioxidant activity of Cassia extracts was evaluated by the b-carotene linoleate model system (Barros et al., 2007). Lipid peroxidation (LPO) inhibition was calculated using the following equation: LPO inhibition= (b-carotene content after 2 h of assay- initial b-carotene content) x 100. The extract concentration providing 50% antioxidant activity (EC50) was calculated from the graph of antioxidant activity percentage against extract concentration. TBHQ was used as a standard.

### Inhibition of erythrocyte hemolysis mediated by peroxyl free radicals:

The antioxidant activity of Cassia extracts was measured as the inhibition of erythrocyte hemolysis (Barros et al., 2007). The percentage hemolysis inhibition was calculated by the equation % hemolysis inhibition= [(AAAPH-AS)/AAAPH] x 100, where AS is the absorbance of the sample containing the Cassia extracts and AAAPH is the absorbance of the control sample containing Cassia extracts. The extract concentration providing 50% inhibition (EC50) was calculated from the graph of hemolysis inhibition percentage against extract concentration. Ascorbic acid was used as a standard.

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**Antibacterial assay:**
The antibacterial activity assay was performed by agar disc diffusion method (Bauer et al., 1966). Standard antibiotic discs (Kanamycin 30 µg/ disc, Neomycin 10 µg/disc) and blank discs (impregnated with solvent and water) were used as positive and negative control. The zone of inhibition were expressed in millimeter for the organisms- Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Salmonella typhil, and repeated thrice to calculate the mean.

**Antifungal assay:**
The antifungal assay (Lam & Ng, 2009b) was performed by the agar dilution method using 100 mm × 15 mm petri dishes (Falcon), in triplicates. The zone of inhibition in millimeter (Aspergillus niger and,Aspergillus fumigatus) was defined as the lowest concentration of extract that inhibited visible growth on agar.

## RESULTS AND DISCUSSION

**Carbohydrate content:**
Significant and satisfactory contents were seen in EA, HX and CH extracts of all the species (Table.1). Among the various fractions the CH extract of Cassia auriculata showed the highest carbohydrate content (0.78 mg). The next highest was presented by Cassia fistula CH extract (0.63 mg). On the other hand, the next highest content was seen in CH extract of Cassia siamea and Cassia alata, being equal amount of about 0.60 mg. These differences of the richer contents of those secondary metabolites among the conducted extracts were due to their polarity range of extracting constituents.

**Table 1. Dietary assessment**

| Species | Carbohydrate Content (mg/mL) | | | | | Protein Content (mg/mL) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | a | b | c | d | e | a | b | c | d | e |
| Cassia alata | 0.53 | 0.60 | 0.42 | 0.29 | 0.35 | 0.28 | 0.37 | 0.20 | 0.09 | 0.13 |
| Cassia auriculata | 0.61 | 0.78 | 0.58 | 0.33 | 0.44 | 0.50 | 0.56 | 0.46 | 0.33 | 0.38 |
| Cassia fistula | 0.41 | 0.63 | 0.57 | 0.36 | 0.38 | 0.27 | 0.34 | 0.44 | 0.16 | 0.22 |
| Cassia siamea | 0.38 | 0.60 | 0.42 | 0.24 | 0.35 | 0.29 | 0.36 | 0.31 | 0.07 | 0.15 |

a- Hexane; b- Chloroform; c- Ethyl acetate; d- Methanol; e- Hydroalcohol

**Protein content:**
The overall increasing order of protein content among all the extracts (Table.1) of all species obtained was: Cassia auriculata [CH] > Cassia auriculata [HX] > Cassia auriculata [EA] > Cassia fistula [EA] > Cassia auriculata [WA] > Cassia alata [CH] > Cassia siamea [CH] > Cassia fistula [CH] > Cassia auriculata [ME] > Cassia siamea [EA] > Cassia siamea [HX] > Cassia alata [HX] > Cassia fistula [HX] > Cassia fistula [WA] > Cassia alata [EA] > Cassia fistula [ME] > Cassia siamea [WA] > Cassia alata [WA] > Cassia alata [ME] > Cassia siamea [ME]. It is evident from the results that more or less about 50% of the plant extracts contained protein and carbohydrate in 2 mg/mL.

Furthermore, Cassia alata and Cassia siamea represented the least contents of about 0.09 and 0.07 mg whom gave excellent phenolic and flavonoid contents in our previous studies (Deepika priyadharshini & Sujatha, 2011).

**Superoxide anion radical scavenging assay:**
Radical scavenging assay is a quick, sensitive, and widely used method to measure antioxidant activity of several compounds. In contrast to the above contents, Cassia fistula [ME] fraction has exhibited its highest scavenging activity of about 78.2±0.23% in 2 mg/mL amongst 20 extracts, against the standard ascorbic acid which produced 84.25±0.08% scavenging effect. The very next significant activity was presented by Cassia auriculata [ME] and Cassia fistula [EA] crude of about 73±0.09% and 71±0.16% respectively. For a keen observation (Fig.1), all the species enclosing Cassia siamea has revealed their excellence for their ME fraction except Cassia alata whose EA extract has given its highest scavenging activity. It is noteworthy to display that the least activities exhibited by the HX and HA extracts of all the species has presented their activities in and around 50±0.02% scavenging effects which is really appreciable.

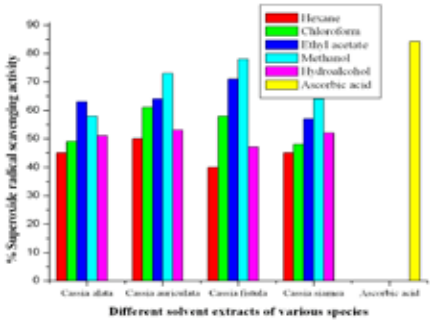


Figure 1. In vitro superoxide radical scavenging activity

**Hydrogen peroxide scavenging assay:**
Top activities were produced by Cassia fistula ME, EA, CH, Cassia auriculata ME and Cassia alata EA extracts above 70±0.11% percentage of scavenging effects compared to the standard ascorbic acid which had 93±0.08% scavenging activity. On the other hand, the least activities were produced by the extracts of Cassia siamea, HX extract in particular. To deal with the HA extract, it had revealed satisfactory scavenging effects too, greater than the HX extracts, uniformly for all the species and moreover the % peroxide scavenging activities were above 50±0.20. The percentage scavenging effects of all the extracts are depicted in Fig. 2. To discuss with, inconsistent to other species, Cassia alata has showed its excellence in EA, abiding with enzymatic and non-enzymatic assays in our former reports (Deepika priyadharshini & Sujatha, 2011).

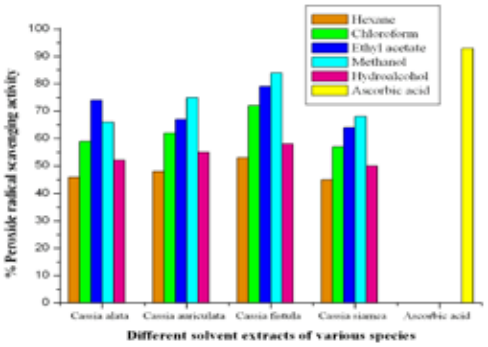


Figure 2. In vitro peroxide radical scavenging activity

**Inhibition of b-carotene bleaching:**
All the species have produced good bleaching inhibition which has resulted in the peroxidation of β-carotene being decreased. The absorption values were seen to decrease as the color of the reaction mixture diminished, and after the introduction of the antioxidants from the extracts the samples retained its color. Almost all the extracts possess such inhibitions, for instance, the ME extract (90±0.09%) of Cassia fistula produced the highest bleaching effects in contrast to other species. The very next intense activity was generated by Cassia auriculata ME (82±0.06%) and Cassia fistula EA (76±0.08%) extract. An interesting part of this assay is that the HX extract of Cassia fistula which produced richest protein content, has recorded the least % inhibition of about 42±0.11%. Antioxidant capacity was classified as high (70%), intermediate (40- 70%) or low (< 40%) levels of oxidation inhibition (OI) for the sample's ability to inhibit β-carotene bleaching caused by free radicals generated during linoleic acid peroxidation.

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**Inhibition of erythrocytes hemolysis:**
The oxidative hemolysis in erythrocytes induced by AAPH has been extensively studied as a model for peroxidative damage to the biomembrane. The protective effect of the Cassia species on hemolysis has been measured to be best, in which almost all the solvent fractions have exhibited above 50% scavenging activity except few HX and HA extracts. As has already been observed in previous antioxidant assays, Cassia fistula showed a high protective effect against erythrocytes hemolysis, when compared to other studied species. It is probable that the antioxidant components in Cassia fisluta extracts can reduce the extent of β-carotene destruction by neutralizing the linoleate free radical and other free radicals formed in the system by showing 78±0.03% of inhibition. The highest inhibition in Cassia alata was presented by its EA extract (65±0.01%), in Cassia siamea and Cassia auriculata, their ME extracts (76%). However, the inhibition percentage of the standard L-ascorbic acid on hemolysis of red blood cells was much higher (84±0.22%) than those of Cassia extracts. For a deeper evaluation, it is interesting that the lowest activity/ inhibition were extended by the HX extract of Cassia fistula which on the other hand projected its intense inhibition of about 78±0.15%.

**Antibacterial activity:**
The ME extract of Cassia fistula, Cassia siamea, Cassia auriculata and the EA extract of Cassia alata were found to be effective against all the pathogenic bacteria when tested by disc diffusion assay. Other extracts with respect to the species failed to exhibit their zone of inhibition in comparison with the above said extracts and the results were not shown for better understanding. The zone of inhibition as shown in Table.2 has revealed their potency against pathogens, among which Bacillus subtilis has been inhibited effectively in contrast to others. A maximum inhibition was 6 mm by Cassia fistula ME fraction.

**Table 2. Antimicrobial activity**

| Organisms | Zone of inhibition (mm) | | | | |
|---|---|---|---|---|---|
| | Cassia alata | Cassia auriculata | Cassia fistula | Cassia siamea | Standard |
| Staphylococcus aureus | 1 | NA | 2 | NA | 7 |
| Bacillus subtilis | 5.5 | 5.5 | 6 | 4 | 6.5 |
| Escherichia coli | NA | 3.5 | 6 | NA | 8 |
| Salmonella typhi | NA | NA | NA | NA | 3 |
| Aspergillus niger | 1 | 2 | 9 | NA | 4 |
| Aspergillus fumigatus | NA | 2 | 8 | NA | 2 |

**Antifungal activity:**
Antifungal activity exhibited by the Cassia species has showed their significance of inhibiting the growth of fungi viz. Aspergillus niger, Aspergillus fumigatus. Antifungal activity was not much effective like antibacterial activity, as there was no activity in most of the test samples. Amidst, Cassia fistula was the only extract that exhibited excellent inhibition against the pathogens. The ME extract of all the species and EA extract of Cassia alata has exhibited potent antibacterial and antifungal activity in inhibiting the subjected pathogens at 40 µL concentration. Prabha et al., (1997) reported that the fouling microorganisms were particularly sensitive to the action of the crude ME extracts.

**CONCLUSION**
The data of this study so far has generated a description to set the basis for a clear understanding on the phytochemistry of Cassia species, and opens the possibility of the potential utilization of the flower extracts in food system or as prophylactics in nutritional/food supplement programs. Cassia fistula and Cassia auriculata flowers comparatively can be appraised to be involved in clinical trials against pathogens. There is no doubt that most flower exhibit their effects owing to a variety of constituents and the idea of synergy within and between them is also gaining acceptance.

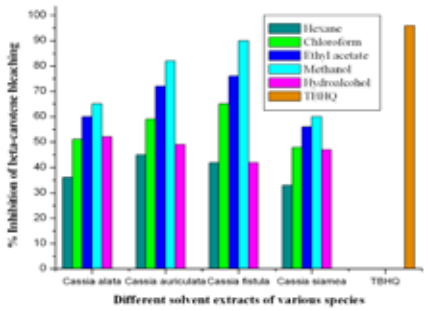


Figure 3. Inhibition of β-carotene bleaching activity

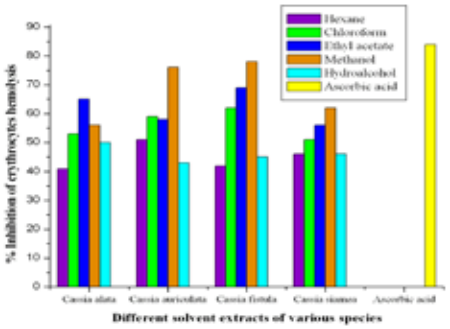


Figure 4. Inhibition of erythrocytes hemolysis

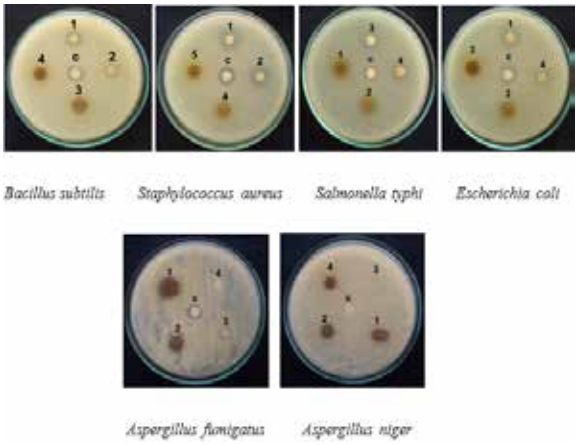


Figure 5. Antimicrobial activity

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