**Anti-oxidative activity of Cassia L. species of Southern India**

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**ABSTRACT**

To establish a comparative account within the taxa by assessing its anti-oxidative property and mapping it with the morphometric characters. The methanolic leaf extracts of 12 Cassia L. species were screened for their antioxidant activity using 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging assay and reducing power capability with reference to standard Ascorbic acid. Chamaecrista kleinii exhibited strong antioxidant activity with IC₅₀ 2.17 µg mL⁻¹, followed with Senna auriculata (IC₅₀ 11.51 µg mL⁻¹) and Senna polyphylla (15.17 µg mL⁻¹). Highest reducing ability was observed in Senna auriculata extract with 0.676 nm absorbance. The correlation observed between the reducing power and DPPH radical scavenging assay supports the contribution of the phytoconstituents like phenolics and flavonoids towards managing oxidative stress. The present study reveals the beneficiary effects of the selected plants by virtue of their antioxidant activity that can be harnessed in drug formulations.

**Keywords:** Antioxidant, 1, 1-diphenyl-2-picryl hydrazyl, reducing power, Cassia.

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Received 16 June 2019, Accepted 24 June 2019
INTRODUCTION

Antioxidants are compounds that protect cells against damaging effects of reactive oxygen species (ROS) such as singlet oxygen ($^1$O$_2$), superoxide anion (O$_2^-$), per-oxo radicals (H$_2$O$_2$), hydroxyl radicals (OH) and per-oxo nitrite (NO$_3^-$). An imbalance between antioxidants and ROS results in oxidative stress, leading to cellular damage. These highly reactive transient chemical species formed in all tissues during normal aerobic cellular metabolism, have the potential to initiate damage to various intracellular components (nucleic acids, lipids, proteins) on which normal cell functioning depends. Oxidative process is one of the most important routes for producing free radicals in food, drugs and even in living systems (Haliwell 1994). Oxidative stress is among the major causative factors which induce many chronic and degenerative diseases like cancer, aging, atherosclerosis, ischemic injury, inflammation and neurodegenerative diseases (Elmastas 2006).

The most effective path to eliminate and diminish the action of free radicals which cause the oxidative stress is antioxidative defense mechanism which includes superoxide dismutase, catalase, glutathione-s-transferase, glutathione peroxidase and reductase and also molecules such as Vitamin C, Vitamin E, glutathione etc. (Ames 1983; Fattman et al. 2003).

A great number of aromatic medicinal plants contain chemical compounds exhibiting antioxidant properties. Natural antioxidants have been studied extensively for decades in order to find compounds protecting against a number of diseases related to oxidative stress and free radical induced damage. The potential of higher plants as a source for new drugs is still largely unexplained. Among the estimated 250,000-500,000 plants, only a small percent has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller (Mahesh and Satish 2008). In this scenario, the screening of plant extracts has been of great interest to discover new drugs effective in the treatment of several diseases. The phytochemical research based on ethnopharmacological information is considered an effective approach in the discovery of new bioactive compounds from higher plants (Chen et al. 2008; Duraipandiyan 2006).

The genus *Cassia* L. ranks among the twenty five largest genera of the dicotyledonous plants in the world (Irwin and Turner 1960) that consists of 500 to 600 species (Airy Shaw 1973). Irwin and Barneby (1981, 1982) proposed a revised classification with the genus *Cassia* L. of Leguminosae-Caesalpinioideae being split into three smaller genera viz., *Cassia* sensu stricto, *Senna* Mill. and *Chamaecrista* Moench.
Ethnobotanical survey of India mentions Cassia plants as one of the widely used species in therapeutic medicine for treating skin disorders. Some species of Cassia have been reported to be antidiabetic, antipyretic, anti-inflammatory, antimicrobial, anthelmintic, antiemetic, antioxidant etc (Dave and Ledwani 2012). Cassia, Senna and Chamaecrista are well known for its laxative properties. These medicinal plants are extensively used within indigenous health care system in India and several other countries (Seethapathy et al. 2014). Phytochemical screening of the bioactive extracts performed for the 12 Cassia species (Usha and Bopaiah 2011, 2012), indicates the presence of secondary metabolites like phenolics, flavonoids, tannins, steroids, alkaloids and carbohydrates. These bioactive compounds are associated with various biological activities. The current study aims at relating the antioxidant activity with the various phytoconstituents present in twelve species of Cassia. The crude methanolic extracts of different species of Cassia, were subjected to in vitro DPPH radical scavenging assay and reducing activity.

MATERIALS AND METHOD

Sample collection

Twelve species from the genus Cassia, one from genera Cassia L., ten from Senna Mill., and one from Chamaecrista Moench., were chosen for the analysis (antioxidant activity), viz., Senna alata, Senna auriculata, Cassia fistula, Senna hirsuta, Chamaecrista kleinii, Senna occidentalis, Senna polyphylla, Senna uniflora, Senna siamea, Senna surattensis, Senna spectabilis and Senna tora.

These plants collected from different regions of Bangalore were authenticated using morphological characters by the Centre for Ecological studies, Indian Institute of Science, Bangalore. Voucher specimens were prepared for each of these species and deposited at the Herbaria, National Ayurveda Dietetics Research Institute, Bangalore.

Morphological studies

At the preliminary step, the morphological characters were observed and a comparative study was done with 23 external characters. These qualitative and quantitative, morphological traits were subjected to cluster analysis and cladogram constructed for the same to analyse the relationship amongst the 18 (1 from Chamaecrista, 6 from Cassia and 11 from Senna) species with respect to their similarities and dissimilarities.

Morphological studies supplemented with bioactivity guided assay possess definite promises for future in various fields hence the 12 species of Cassia L. were subjected to antioxidant activity studies (Note: Out of the 18 species taken for morphological studies, only 12 were considered for the antioxidant activity studies) (Figure 1, 3).
Figure 1: UPGMA (Unweighted pair group method with arithmetic mean) tree of genus *Cassia* using 23 morphological traits.
(Note: 18 species (1 from *Chamaecrista*, 6 from *Cassia* and 11 from *Senna*) were subjected to Cluster analysis based on their similarities and dissimilarities).

![Figure 1: UPGMA tree of genus *Cassia* using 23 morphological traits.](image)

**IC$_{50}$ values of DPPH radical inhibitory activity**

Figure 2: IC$_{50}$ values of 12 *Cassia* species on DPPH radical scavenging activity (Values are expressed for n=3).
Figure 3: UPGMA tree (Unweighted pair group method with arithmetic mean) of genus Cassia using 23 morphological traits with IC₅₀ values mapped on it.
(Note: Out of the 18 species taken for morphological studies, only 12 were considered for the antioxidant activity analysis)

Figure 4: Reducing abilities of Cassia species in terms of absorbance (Values are expressed for n=3)

In vitro Antioxidant activity
Antioxidant activity assay (DPPH free radical scavenging activity)
Aliquots of 50 µL extract (20-100 µg) were added to 3 mL 0.1 mM ethanolic DPPH solution. The change in absorbance was measured using Shimadzu UV-Visible spectrophotometer after 30 minutes at 517 nm (Leong and Shui 2002). The inhibitory percentage of the plant extracts were calculated using the formula (Chang et al. 2002).

\[
\text{Inhibition (\%)} = \left(\frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}}\right) \times 100
\]

**Reducing antioxidant power**

The reducing antioxidant power of plant methanolic extracts was determined by the method of Oyaizu (1986). Different concentrations of plant extracts in 1mL distilled water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and 1% potassium ferricyanide (2.5 mL). The mixture was incubated at 50 °C for 20 min. 2.5 mL of trichloroacetic acid (10%) was added to the mixture, centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and FeCl₃ (0.5 mL, 0.1%). The absorbance was measured at 700 nm against a blank using visible spectrophotometer.

**Statistical analyses:**

The experimental results were expressed as mean±SD of three parallel measurements. IC₅₀ values were calculated by regression analysis quoting regression coefficient. The relativeness between two parameters was expressed in terms of correlation coefficient. Data was evaluated by one-way ANOVA, using Dunnett’s method of multiple comparisons with Graphpad Instat 3.0 software.

**RESULTS AND DISCUSSION**

**Morphometric analysis**

The totality of morphological characters is of great importance in traditional taxonomy and also a preliminary step in chemosystematic investigations. Hence these characters have to be carefully checked when decisions regarding delimitations and classifications are to be made. A dendrogram which depicts the degree of relationships among the taxa was produced on the basis of a few qualitative and quantitative morphological traits of *Cassia* L. observed (Figure 1.)

**Qualitative and quantitative morphological traits (Binary state) used for cluster analysis.**

**Qualitative characters**

1. Habit: Herb(0)/Shrub(1)/Tree(2)
2. Stem: Erect(0) /Creeping(1)
3. Stem angle: Absent(0) /Present(1)
4. Stem surface: Glabrous(0) /Pubescent(1)
5. Stipules: Absent(0) /Present(1)
6. Stipule nature: Absent(0) /Auricled(1)/Foliaceous(2)
7. Leaflet shape: Oblong(0) /Elliptic(1)/Ovate(2)
8. Leaflet apex: Obtuse /Retuse(0); Acute/Acuminate(1);Mucronate(2)
9. Leaflet glands: Absent(0) /Present(1)
10. Leaf texture: Membranous(0) /Chartaceous(1)/Hirsutus(2)
11. Dorsal surface of Leaf : Glaucous(0) /Aglacous(1)
12. Inflorescence: Cymose(0) /Racemose(1)
13. Position of inflorescence: Terminal(0) /Axillary(1)
14. Extra Floral Nectaries(Nectarines): Absent(0) /Present(1)
15. Bracts: Absent(0) /Present(1)
16. Bracteoles: Absent(0) /Present(1)
17. Floral fragrance: Absent(0) /Present(1)
18. Fruit structure: Flat(0) /Terete(1)/Angular(2)
19. Fruit colour (mature): Copper brown(0) /Blackish(1)/Greenish(2)
20. Flower colour: Yellow(0) /Pink/Red(1)
21. Life span: Annual (0) /Perennial(1)

Quantitative characters
22. Number of leaflets: (3-5) (0), (6-10) (1), (more than 10) (2)
23. Number of seeds: (1-10) (0), (10-30) (1), (more than 30) (2)

The cladogram (Figure 1) constructed based on UPGMA (Unweighted pair group method with arithmetic mean) method reveals the 3 distinct intra-generic clustering in the genus Cassia i.e. Cassia, Chamaecrista & Senna. Clade 3 consists of branch II (Senna auriculata, Senna surattensis, Senna polyphylla) with perennial shrubs, branch III (Senna tora), branch IV (Senna alata), branch V (Senna uniflora, Senna occidentalis, Senna hirsuta), and branch VI (Senna sophora), which are all annual shrubs except Senna alata and Senna sophora. Members of branch II and VI possess 6-10 pairs of leaflets, branch III, IV, V have 3-5 pairs of leaflets. Except Senna sophora, the others are all stipulate. All the taxa of Clade 3, have yellow coloured flowers and possess extra floral nectaries but for Senna alata which has no EFN’s. Only species of branch II exhibit floral fragrance.

Clade 2 branches out into two subclades, subclade 2 with branch VII (Senna siamea, Senna spectabilis, Cassia fistula), and subclade 1 with branch VIII (Cassia roxburghii), branch IX (Cassia nodosa, Cassia bakeriana, Cassia grandiflora), branch X (Cassia javanica), all of which are trees with floral fragrance and without extra floral nectarines. Branch VII are extipulate
members with more than 10 pairs of leaflets except Cassia fistula with 3-5 pairs of leaflets but all the three possess yellow coloured flowers. Branches, VIII, IX and X are stipulate possessing more than 10 pairs of leaflets with pink or red flowers.

Chamaecrista kleinii of Clade I, an annual prostrate herb, is stipulate, with 6-10 pairs of leaflets and is yellow flowered which sits as a sister to the two clades, Clade 3 and Clade 2. In the analysis of the full taxon set taken for the cluster analysis, the cladogram reveals the three subgenera trifurcating into three clades with all Senna species, Cassia species and Chamaecrista species getting resolved separately.

Evaluation of morphological characters and their interpretations have often led to disagreement regarding classification of plants due to which taxonomists as a rule look for anatomical, embryological, palynological and cytological characters which may be convincing at times (Giulietti 2012). But, thorough phytochemical investigations and chemical characters so obtained may become useful guides to taxonomists in such instances (Ankanna et al. 2012). Hence qualitative and quantitative phytochemical analysis yields good chemical data that adds on to the classical taxonomical schemes which provides confirmatory evidences to plant classification and also comparative evaluation.

In support to the morphometric data an attempt was made to check the bioactivity and whether the bioconstituents present in them exhibit correlation with respect to a group of Cassia L. species.

**In vitro Antioxidant activity**

**Antioxidant activity assay (DPPH free radical scavenging activity):**

The efficacies of the antioxidants are associated with their ability to scavenge free radicals. DPPH assay is a sensitive and rapid method that provides accurate information regarding the reactivity of the plant compounds towards radical scavenging activity. DPPH has absorption maxima (Perl’s Prussian Blue colour) at 517 nm which becomes colourless on reduction by plant constituents. Reduced absorbance reflects the antiradical activity of the extracts which is due to electron transfer process (Sowndhararajan 2013).

Chamaecrista kleinii, Senna auriculata and Senna polyphylla presented excellent DPPH scavenging activity of 93.46%, 93.44% and 93.16% showing IC$_{50}$ values 2.17, 11.51 and 15.17 µg mL$^{-1}$ compared to Ascorbic acid which showed 50% inhibition at 3.623 µg mL$^{-1}$. Senna tora, Senna surattensis, Senna alata and Senna uniflora presented good radical inhibitory activity and were able to scavenge DPPH radicals to an extent of 86.68%, 74.31%, 62.11% and 52.70% respectively with IC$_{50}$ value 53.11, 62.93, 65.03 and 96.37µg mL$^{-1}$. The inhibitory activity of all
the species was found to be concentration dependent (p<0.01). The IC\textsubscript{50} values of the plant extracts for DPPH radical scavenging activity is represented in Figure 2.

The potential antioxidant activity of the plant extracts primarily rely on the reducing property as a major defensive action against various free radicals. Various types of secondary metabolites present in the plants are known to have reducing capabilities and hence act as antioxidants. Phenolics and flavonoids are known for their antioxidant property which accounts for the potential scavenging capabilities (Stefek 2011). Previous data reported regarding the total phenolics and flavonoid contents of the Cassia species (Usha and Bopaiah 2013) supports the DPPH radical scavenging activity and reducing power.

**Reducing antioxidant power:**

The impact of reducing power on the DPPH radical scavenging activity of the leaf extracts from Cassia fistula and Senna tora were observed to be high when expressed in terms of correlation co-efficient (Table 1). The radical scavenging activity of Senna alata, Chamaecrista kleinii, Senna polyphylla and Senna occidentalis also showed potential relatedness to reducing capabilities followed by a comparable correlation in Senna spectabilis, Senna hirsuta and Senna siamea while Senna auriculata indicated a lesser impact. Strong correlation existing between DPPH radical scavenging activity and the reducing power assay of all the species gives additive effect with respect to their phytoconstituents.

**Table 1 Correlation co-efficient of DPPH radical scavenging and reducing power assay of Cassia species.**

<table>
<thead>
<tr>
<th>Cassia species</th>
<th>Correlation co-efficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chamaecrista kleinii</td>
<td>0.9806</td>
</tr>
<tr>
<td>Senna auriculata</td>
<td>0.7872</td>
</tr>
<tr>
<td>Senna polyphylla</td>
<td>0.9787</td>
</tr>
<tr>
<td>Senna tora</td>
<td>0.9900</td>
</tr>
<tr>
<td>Senna surattensis</td>
<td>0.8454</td>
</tr>
<tr>
<td>Senna alata</td>
<td>0.9832</td>
</tr>
<tr>
<td>Senna uniflora</td>
<td>0.8466</td>
</tr>
<tr>
<td>Senna siamea</td>
<td>0.9337</td>
</tr>
<tr>
<td>Senna hirsuta</td>
<td>0.9437</td>
</tr>
<tr>
<td>Senna spectabilis</td>
<td>0.9533</td>
</tr>
<tr>
<td>Cassia fistula</td>
<td>0.9908</td>
</tr>
<tr>
<td>Senna occidentalis</td>
<td>0.9705</td>
</tr>
</tbody>
</table>

All the species of Cassia exhibited radical scavenging activity and has found to be related with the reducing power. The antiradical activity of the species may be due to the presence of various phytoconstituents such as alkaloids, tannins, saponins, anthraquinones, anthocyanosides,
flavonoids, carbohydrates, proteins, steroids, terpenoids and cardiac glycosides. The result substantiates the effective usage of these medicinal plants in ethnomedicine as laxative, diuretic, antimicrobial, against constipation, stomach pain, snake bite, skin diseases, ringworm, liver problems and asthma (Chatterjee al. 2012). Previous reports on the study of antioxidant activity of Cassia species from Egypt by Sayed (2010) indicate that the fruit pulp, leaf extract of Cassia fistula and leaf extract of Senna occidentalis are good antioxidants. Senna tora leaf extracts are also reported to be potent antioxidants (Sirappuselvi 2012). The ethanolic leaf extract of Senna hirsuta has showed significant antioxidant activity according to the investigations of Dey et al. (2013). Antioxidant studies are reported from the flower extracts of Senna alata, Senna auriculata, Cassia fistula and Senna siamea by Priyadarshini 2013. However, there are no reports on the activity studies reported from the plant extracts of Chamaecrista kleinii, Senna spectabilis, Senna polyphylla, Senna uniflora and Senna surattensis till date.

CONCLUSION

The bioactivity exhibited by the Cassia species was mapped with the qualitative and quantitative characters obtained from the morphological traits in the dendrogram (Figure 3) constructed by the UPGMA method. The numbers above the lines in the dendrogram indicate the linkage distance of the species. The herbaceous Chamaecrista kleinii, is a sole representative of this genus taken for the study exhibits maximum activity. The shrubs (Clade 3) and trees/small trees (Clade 2) which are segregated into two clades according to the morphometric characters more or less resolves in the same pattern when mapped with the IC_{50} values derived from the antioxidant activity assay with slight variation. The dendrogram (Figure 3) indicates that the activity exhibited by the Cassia L. species decreases down from Clade 3 (Shrub Clade) to Clade 2 (Tree Clade) except that observed in Senna surattensis and Senna occidentalis. Cassia and Senna contain active ingredients which are anthraquinines derivatives and glucosides called sennosoides (Srivastava et al. 2010). These genera along with Chamaecrista also contain water soluble polysaccharides, alkaloids, triterpene derivatives and polyphenolics comprising of flavonoids, catechins and proanthocyanidines (Chaubey and Kapoor 2001; Luximon et al. 2002; Kashiwada et al. 1990; Gupta et al. 1993). Many commercial herbal products and pharmaceuticals are derived from these species which are used in Indian and Western medicines (Anonymous, 1999; 2008). Hence the present work not only gives clarity regarding the activity exhibited by each of the 12 Cassia species but also depicts their reductive capabilities that may be attributed to
the phenolics present in the leaf extracts. This is also indicative of the active ingredients, which are responsible for these species to be used in medicinal products.

ACKNOWLEDGEMENT:

The authors thank Prof. Shankar Rao, Prof. Seetharam Y N and Late Dr. Harish Bhat, Centre for Ecological Sciences, IISc, Bangalore, for their help towards authentication of plants. We are grateful to Prof. UmaShaanker R, Dept of Crop Physiology, School of Ecology and Conservation, UAS, GKV, Bangalore, Dr. Thamizh Seran, Professor, Department of Botany, St.Joseph’s PG and Research Centre, Bengaluru, Sahana C. S. Research Scholar, for their kind help and valuable suggestions.

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