Comparative analysis of antioxidant activity and phytochemical potential of *Cassia absus* Linn., *Cassia auriculata* Linn. and *Cassia fistula* Linn.

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Abstract

Dried seeds of *Cassia auriculata* Linn., *C.absus* Linn. and *C. fistula* Linn. were collected from Javaathu hills, Tiruvannamalai District, Tamil Nadu, India for the comparative analysis of phyto-chemical potential with special reference to antioxidant activity. The active compounds were extracted with ethyl acetate, hexane and methanol. The methanol was found as suitable solvent and hence used for further extraction and analysis. The preliminary phytochemical screening of *C. auriculata*, *C. absus C. fistula* has revealed for the presence of alkaloids, phenolics and flavanoids in all the species. Absence of reducing sugars in *C. absus*, glucosides in *C. auriculata* and saponins in *C. fistula* were also observed. The quantitative determination of phenol and flavanoids was carried out in *C. auriculata*, *C.absus* and *C. fistula* and found that the total phenolic content as 0.18, 0.15, 0.11 and flavonoids 0.08, 0.092, 0.087% respectively. The antioxidant potential of the seed extracts recorded as much as 50% compared to α- tocopherol as standard. The Super oxide assay for the sample shows as 3% comparable to the standard α-tocopherol (5%). The Phosphomolybdenum assay and metal chelating activity indicate that the plant extract is a potential antioxidant. The details are presented in the present study.

Keywords: Phytochemical analysis, antioxidant activity, radical scavenging activity, *Cassia auriculata, Cassia absus* and *Cassia fistula*.

1. Introduction

Medicinal plants constitute an important source of bioactive compounds which are used as both traditional and modern medicine for various human disorders [1]. According to the World Health Organization [2], the current estimate suggests that many developed countries have a great proportion of the population making use of traditional practice of health, especially the use of the medicinal plants. It has been estimated that in developing countries such as China and India, the contribution is as much as 80%, hence the importance of medicinal plants are recognized in our country. A large number of medicinal plants and their purified constituents also have shown beneficial therapeutic potentials and have been reported to exhibit antioxidant activity, which is due to the flavones, isoflavones, flavonoids, anthocyanin, coumarin, lignans, catechins and isocatechins [3, 4, 5]. Primary sources of naturally occurring antioxidants are the whole grains, fruits and vegetables. Some of them are the prime sources of vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens which are known to cure various diseases.

Plants are potential sources of natural antioxidants and were found to correlate with the phenolic compounds. Plants such as turmeric (*Curcuma domestica*), betel leaf (*Piper betel)*, pandan leaf (*Panadanus odorus*), Asam Gelugur (Garcinia *atroviridis*), mengkudu (*Morinda citrifolia*), pegaga (*Centella asiatica*), ginger (*Zingiber officinale*), cassava shoot (*Manihot esculenta*), kesum (*Polygonum minus*), selom (*Oenanthe javanica*) and amla (*Phyllanthus emblica*) show the potential of antioxidant property [6, 7]. Miliauskas *et al.* [8] studied several plant

species for the development of natural antioxidant formulations in the areas of food, medicine and cosmetics. In [9] it is shown that the phenolic compounds exhibit a high antioxidant activity and certain therapeutic uses.

Antioxidants can be of secondary metabolites isolated from plants such as various phenolic compounds [10, 11]. They are the most abundant natural bio-compounds and are synthesized by plants for their protection against biological and environmental stresses. Health promoting effects of antioxidants in plants have been well documented.

Based on the above scientific information, the present study has been designed to carry out for the analysis of antioxidant potential of seeds of *C. auriculata*, *C. absus* and *C. fistula* collected from Javadhu hills of Tamil Nadu. For the determination of antioxidant potential, test for radical scavenging, phosphomolybdenum assay and metal chelating activity were carried out from the above plant seeds. The determination of total phenolic content may be used as an indirect evaluation of the antioxidant potential of the plant extracts. In addition, the overall phyto-chemical profile and the active bio-compounds present in the seed extracts have also been investigated.

2. Materials and methods

2.1. Chemicals and glassware

All the glassware (Borosil, Anumbra or Corning) used were cleaned thoroughly by standard procedures [12]. The chemicals and reagents were of analytical grade.

2.2. Plant seeds collection and sample preparation

Dry seeds of *C. auriculata*, *C. absus* and *C. fistula* were collected from the fields located in Jawadhu hills forest, Thiruvannamalai District, Tamil Nadu, India. The seeds were carefully washed with tap water, rinsed with distilled water, and air-dried in room temperature for 2 to 3 days. Then the seeds were powdered into fine powder and stored for further use.

2.3. Extraction of seeds with solvents

Direct extraction was done with Hexane, Ethyl acetate and Methanol as reported. The seed sample was extracted with Hexane, ethyl acetate and methanol in the ratio of 1:10 in conical flask in shaking condition for overnight. The extract was filtered through the Whatmann No. 1 filter paper in a separate container. The extract was concentrated by evaporation in steam batch and the residues were re-dissolved in solvents to yield 10mg/ml compound for further analysis.

2.4. Antioxidant activity assays DPPH assay

The Radical Scavenging Activity of seed extracts of hexane, chloroform and methanol was determined by DPPH assay according to Chang *et al*. [13].

2.5. Phosphomolybdenum assay

The seed samples were evaluated by the green phosphomolybdenum complex formation according to Prieto e*t al.* [14] and the result was expressed as percent inhibition.

2.6. Super oxide anion radical scavenging activity

The super oxide anion radical scavenging activity of the seed extracts was analysed by the method of Klein *et al.* [15] and expressed in percentage.

2.7. Metal chelating activity

The chelating of ferrous ions in methanol extract of *C. auriculata*, *C. absus* and *C. fistula* was estimated by the method of Dinis *et al.* [16].

2.8. Qualitative Hydroxyl radical scavenging activity

Methanol extract of *C. auriculata*, *C. absus* and *C. fistula* was measured according to the method of Klein e*t al.* [15] and the % hydroxyl radical scavenging activity was calculated.

2.9. Phytochemical analysis Detection of alkaloids:

Solvent free extract (50mg) was stirred with 2 ml of dilute hydrochloric acid (1mL HCl + 1mL H_2O) and filtered. The filtrate was tested carefully with various alkaloidal reagents [17].

Detection of phenolic compound: The extract (50 mg) was dissolved in 5mL of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green color indicated the presence of phenol.

Detection of glycosides: About 50 mg of extract was hydrolysed with 5mL of concentrated HCl for 2h on a water bath, filtered and the hydrolysate was subjected for Borntrager's test. To 2 mL of filtrate hydrolysate, 3mL of chloroform was added and shaken. Chloroform layer was separated and 10% ammonia solution was added to it. Pink colour indicated the presence of glycosides [17].

Detection of flavonoids: About 0.5g of extract was dissolved in 5mL of Distilled water and filtered. Dilute ammonia (5mL) was added to 1mL of the extract filtrate. Concentrated sulphuric acid (1mL) was added. Yellow colorations that disappear on standing indicate the presence of flavonoids.

Detection of reducing sugars [18]: The extract (100mg) was dissolved in 5mL of water and filtered. The filtrate was subjected to the Fehling's test for identification of reducing sugars. For this test, 1 mL of filtrate was boiled on water bath with 1mL each of Fehling's solution I and II and a red precipitate indicated the presence of sugar.

Detection of saponins [19]: About 50mg of extract was diluted with 5mL distilled water. The suspension was shaken in a graduated cylinder for 15 min. A 2cm layer of foam indicated the presence of saponins.

Detection of proteins [20]: About 100mg of extract was dissolved in 10mL distilled water and filtered through Whatmann No.1 filter paper and the filtrate was subjected to tests of proteins.

Millon's test [21]: To 2 mL of filtrate, few drops of Millon's reagent was added. A white precipitate indicated the presence of proteins. To prepare a Millon's reagent , Mercury (0.1g) was dissolved in 0.9mL of fumic nitric acid. When the reaction was completed, equal volume (0.9mL) of distilled water was added.

Estimation of total phenol content: The amount of total phenol content was determined by Folin- Ciocalteu's reagent method [22]. Total phenol values were expressed in terms of gallic acid equivalent (mg/g of extracted compounds).

Total flavonoid content: It was determined using Meda *et al*.[23]method.

2.10. Thin Layer Chromatography [24]

The seed extracts were loaded on pre-coated silica gel plates which were then developed using the solvents methanol, chloroform in the ratio of 0.75:9.25. The compounds were identified both in the UV light, far light and in the iodine chamber and the Rf value were calculated. The extract which showed DPPH inhibition of >90% was examined by thin layer chromatography (TLC). The TLC plates after chromatogram were sprayed with 0.2 % DPPH reagent in methanol or ethanol and left for 30 minutes at room temperature. The plates were observed under white light and antioxidant activity was confirmed by change of purple to yellow colour.

3. Results

The radical scavenging activity of seed extracts from *C. auriculata*, *C. absus* and *C. fistula* show that the methanol extract had higher scavenging activity than the extracts of ethyl acetate and hexane. At a concentration of 200µg/mL, the scavenging activity of methanol extract reached >50% in *C.absus* and *C.fistula* (Table 1) and 40% in *C. auriculata* which is comparable to that of standard chemical.

Table 1: Comparative antioxidant activity (DPPH assay) from different solvent extracts of Cassia spp.

Concentration (µg) of different solvent extract	Percentage of activity in different species		
	C.absus	***C.auriculata***	***C.fistula***
Methanol extract			
20	12.4	6.4	8.5
100	63.6	18.4	28.6
200	89.5	39.6	57.7
Hexane extract			
20	6.56	2.4	2.97
100	18.58	6.4	5.68
200	47.40	18.3	28.42
Ethyl acetate extract			
20	10.80	4.1	4.0
100	22.29	6.1	16.6
200	50.98	41.2	40.3

In vitro superoxide anion radical scavenging activity: The methanol extract of seeds from *C. auriculata*, *C. absus* and *C. fistula* have strong superoxide radical scavenging activity compared to standard and control sample (Table 2). The decrease of absorbance at 560nm with the presence of antioxidants indicates the consumption of superoxide anion in the reaction mixture.

3.1. Phosphomolybdenum assay

The phosphomolybdenum method is quantitative and the antioxidant activity is expressed as the number of equivalents of ascorbic acid. The methanolic extract of *C. auriculata*, *C. absus* and *C. fistula* were observed to have formation of green phosphompolybdenum complex and the OD values were recorded for interpretation (Table 2).

3.2. Metal chelating activity

The presence of chelating agents in the ethyle acetate extract of *C. auriculata*, *C. absus* and *C. fistula* disrupts the ferrozine - Fe 2+ complex formation, thus decreasing the red colour. Among the three plants used, *C.auriculata* exhibited higher activity (Table 2).

Table 2: Comparative analysis showing Phosphomolybdenum assay, Superoxide radical scavenging assay and Metal chelating activity of Cassia spp.

Name of the test	Activity (OD values) in different species		
	C. absus	*C. auriculata*	*C. fistula*
Phosphomolybdenum assay in methanol extract			
Control	0.695	0.695	0.695
Test	0.272	0.220	0.112
Standard	0.161	0.161	0.161
Superoxide radical scavenging assay in methanol extract			
Control	1.457	1.457	1.457
Test	0.856	0.975	1.212
Standard	0.632	0.632	0.632
Metal chelating activity in ethyl acetate extract			
Control	0.074	0.074	0.074
Test	0.032	0.011	0.043
Standard	0.024	0.024	0.024

Hydroxyl radical scavenging activity: Hydroxyl radical scavenging activity of methanol extract and standard is presented in the Table 3. The radical scavenging percentage activity observed from 15% from 73% at the concentration of 50 to 200 ug on different species. The higher percentage activity was observed in *C. absus* compared to other species like *C. auriculata* and *C. fistula*. The value of *C.absus* at the concentration of 200ug is equal to the standard tocopherol.

Table 3: Comparative study of Hydroxyl radical scavenging assay of Cassia spp. extracted in methanol

Concentration (µg)	**Activity (%)**			
	C. absus	*C. auriculata*	*C. fistula*	α- tocopherol
50	30.30	27.27	15.15	34
100	34.85	34.85	25.76	37
150	63.64	50.00	36.36	67
200	72.73	57.58	48.48	75

The qualitative phytochemical screening of *C. auriculata*, *C. absus* and *C. fistula* has revealed that the presence of alkaloids, phenolics and flavanoids present in all the three plants. Whereas, the absence of reducing sugars in *C. absus*, absence of glycosides and non reducing sugars in *C.auriculata* and absence of glycosides and saponins in *C.fistula*. However, higher amount of alkaloids, flavanoides and saponins in *C.absus*. terpinoids and tannins were present in moderate amount. Alkaloids, glycosides and saponins were also present in trace amount. A protein was completely absent (Table 4) in all the 3 samples. Based upon the preliminary phytochemical test, quantitative determination of phytoconstituents was carried out for the extracts of *C. auriculata*, *C. absus* and *C. fistula*. The total phenol 0.182%, 0.151% & 0.106% and flavonoids 0.080%, 0.092% & 0.087% was present in methanolic extract (Table 4).

Table 4: Comparative analysis of Phyto-chemicals in Cassia spp.

	Test	***Test Results in different species***		
		C. absus	*C. auriculata*	*C. fistula*
Detection of alkaloids	Mayer's test	+++	++	+
Detection of phenolic compound	Ferric chloride test	+ 0.182%	+++ 0.151%	+ 0.106%
Detection of glycosides	Borntrager's test	+++	-	-
Detection of flavonoids	Shinoda's test	++	++	+++
Detection of flavonoids	Alkaline reagent test	++ 0.080%	+++ 0.092%	+++ 0.087%
Detection of reducing sugars	Fehling's test	-	++	++
Detection of non reducing sugars	Benedict's test	++	-	++
Detection of saponins	Saponification test	+++	+++	-
+++ : Present in good amount; ++: in moderate; +: in trace amount; - : Completely absent				

TLC developed with 10% methanol in chloroform (10:90) revealed the presence of major compounds with their corresponding Rf value for *C. absus*, *C. auriculata* and *C. fistula* visualized under iodine vapour and ordinary light illumination (Table 5). The specific compound (band) which has anti oxidative properties shows in the Rf value of 0.44, which was chosen as effective compound and collected which yields partially purified compound. The purity of the compound was checked by TLC with 20% methanol in chloroform (20 : 80). The antioxidant activity of these compound was confirmed when the DPPH purple color changed to yellow.

Table 5: Rf values of compounds in the seeds of Cassia spp. as separated by TLC

Detecting agent			
Short light (UV)		**Iodine**	**DPPH**
C. absus	0.12	0.12	0.44
	0.24	0.24	
	0.34	0.34	
	0.44	0.44	
		0.48, 0.62, 0.7	
C. auriculata	0.22	0.22	0.62
	0.35	0.35	
		0.5, 0.62, 0.7	
C. fistula	0.17	0.17	0.32
	0.32	0.32	
		0.5, 0.75	

4. Discussion and conclusion

The methanolic extract of cassia seeds exhibited a significant level of activity of antioxidant compound which is in accordance with the earlier report [25]. The DPPH radical scavenging ability of *C. auriculata*, *C. absus* and *C. fistula* methanolic seed extract was significant when α-tocopherol was used as standard; it reveals the proton donating capacity of the extract. The methonolic extracts of cassia seeds were separated on TLC and a compound isolated at Rf value 0.12 exhibited free radical scavenging potential as analysed by DPPH assay. Velioglu *et al*.[26] studied total phenolics and the antioxidant activity in selected fruits, vegetables and grain products and found significant level. The reducing capacity of the plant extract components may serve as a significant indicator of its potential antioxidant activity [27]. Phenolic compounds are dietary constituents widely existing in plants and have been considered to have high antioxidant capacity and free radical scavenging capacity [28, 29]. Phenolic compounds have attracted more attention as potential agents for preventing and treating many oxidative stress-related diseases [30]. Studies showed that phenolic compounds were the main antioxidant ingredients in various medicinal plants [31]. Flavonoids are well-known antioxidant constituents of plants and possess a broad spectrum of chemical and biological activity, including radical scavenging properties [32]. Phytochemical screening of the crude extracts revealed the presence of secondary compounds such as alkaloids, flavonoids, steroids, tannins, and Phenols. Presence of these phytochemicals in methanol extract of *C. auriculata*, *C. absus* and *C. fistula* attributed to their excellent antioxidant activity. The results of the present study indicate that methanol extract of *C. auriculata*, *C. absus* and *C. fistula* seeds are high in phenolic contents and these extract exhibit strong antioxidant activities. The scavenging activities observed against DPPH and hydroxyl radicals, lead us to propose the above plant seeds as promising natural sources of antioxidants suitable for application in nutritional/pharmaceutical fields. Further studies are needed to explore *in vivo* response for better understanding.

5. References

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