African Journal of Biotechnology Vol. 11(10), pp. 2490-2503, 2 February, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.855
ISSN 1684–5315 ©2012 Academic Journals

Full Length Research Paper

Antioxidant and antimicrobial properties of various polar solvent extracts of stem and leaves of four *Cassia* species

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Accepted 8 October, 2010

Antioxidant and antimicrobial activities of leaves and stem of four *Cassia* species are reported in solvents like methanol, acetone and water after defatting with petroleum ether. *In vitro* antioxidant activities like 2,2-diphenyl-1-picryl-hydrazyl (DPPH), hydroxyl and superoxide anion radical scavenging activities and reducing capacity assessment was done. Total phenol and flavonoid content were measured. The antimicrobial activity was tested against eight bacterial and four fungal strains. There was a direct correlation between phenol content and antioxidant activity. The acetone extract of stem of *Cassia auriculata* showed significantly higher antioxidant activity while acetone and methanol extracts of stem and leaves showed a broad range of antimicrobial property. Therefore, it is concluded that *C. auriculata* possess phenolic properties that has considerable antioxidant and antimicrobial properties.

Key words: Antimicrobial activity, antioxidant activity, total phenol content, *Cassia* species.

INTRODUCTION

Medicinal plants represent a rich source of antimicrobial and antioxidant agents. In different countries, plants are a main source of many potent and powerful drugs (Srivastava et al., 1996). Screening of plants with validated methods is a primary source of discovery, in terms of identifying potentially useful molecules against infectious disease. For the past two decades, there has been an increasing interest in the investigation of different extracts, obtained from traditional medicinal plants, as potential sources of new antimicrobial (Bonjar and Farrokhi, 2004) and antioxidative agents (Amakura et al., 2002; Orhan et al., 2003). In recent years, multiple drug/chemical resistance in both human and plant pathogenic organisms has developed due to the indiscriminate use of commercial antimicrobial drugs/chemicals in the treatment of infectious diseases. Therefore, researchers have been trying to develop new broad spectrum antibiotics to treat infectious disease caused by bacteria, fungi and parasites. Prolonged usage of these broad spectrum antibiotics has led to the emergence of drug resistance. There is a tremendous need for novel antimicrobial agents from different sources. Medicinal plants are used traditionally to prevent or cure diseases all over the world (Nair et al., 2005). The medicinal values of plants lie in bioactive phytochemicals flavonoids, phenolic compounds, tannins, anthracene derivatives and essential oils which produce definite physiological actions in the human body (Akinmoladun et al., 2007).

Oxidative stress plays a major role in the development of chronic and degenerative ailments such as cancer, autoimmune disorders, rheumatoid arthritis, cataract, aging, cardiovascular and neurodegenerative diseases (Willcox et al., 2004; Pham Huy et al. 2008). Several natural compounds extracted from medicinal plants have been shown to exhibit antioxidant and/or radical scavenging properties, which protect the human body from chronic diseases.

The genus *Cassia* belongs to the family Caesal-

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Abbreviations: DPPH, 2,2-Diphenyl-1-picryl-hydrazyl; **EDTA,** ethylenediaminetetraacetic acid; **NBT,** nitroblue tetrazolium; **PMS,** phenazine methosulfate; **DMSO,** dimethylsulphoxide; **MIC,** minimum inhibition concentration.

Table 1. The ethno botanical information of the screened *Cassia* species of family Caesalpiniaceae.

No.	Botanical name (voucher specimen number)	Vernacular name	Therapeutic use	Reference
1	*Cassia auriculata* L. (PSN249)	Awal	Anthemintic, Laxative, Refrigerant, Fever, Conjunctivitis, Rheumatism, Skin diseases, Asthma, Renal disorders, Diabetes mellitus, Constipation, Diseases of the Urinary tract.	Jayaweera, 1982; Joshi, 2000; Anjaria et al., 2002
2	*Cassia fistula* L. (PSN250)	Garmalo	Skin diseases, Rheumatism, Anorexia, Jaundice, Ringworm and other Fungal skin infections, Nasal infection, Intestinal disorders, Hypoglycemic, Hepatoprotective, wound healing, Abdominal pain, Leprosy, Demulcent, Refrigerant, Diabetes, Analgesic, Diarrhea, Fever.	Patel et al.,1965; Kirtikar and Basu, 1975; Perry, 1980; Bhakta et al.,1997; Samy et al., 1998; Bhakta et al.,1999; Rajan et al., 2001
3	*Cassia siamea* L. (PSN256)	Kashod	Analgesic, Anti-inflammatory, Antipyretic, Constipation, Malaria, Fever, Jaundice, Ringworm, Hypertension, Insomnia, Asthma, Diabetes, Purgative, Anti-tumor.	Ahn et al., 1978; Anjaria et al., 2002; Mbatchi et al., 2006; Kaur et al., 2006
4	*Cassia tora* L. (PSN258)	Kuvadio	Ringworm and Skin diseases, Laxative, Maturant, Anodyne action, Mucilaginous, Leprosy, Psoriasis, Gout, Sciatica, Snake bites.	Anjaria et al., 2002

piniaceae and it is well known that members of this family are traditionally used for curing many diseases (Anjaria et al., 2002). There are many therapeutic uses and medicinal properties are reported in different parts (leaves, stem, seeds, flowers, fruits, stem bark, etc) of the plants. They are known for antihyperglycemic activity (Gupta et al., 2009), hepatoprotective activity (Das et al., 2008), antihelmintic activity (Deore et al., 2009), anti-microbial activity (Duraipandiyan and Ignacimuthu, 2007) and antioxidant activity (Kaur et al., 2006). Therefore it was thought to be of interest to screen few *Cassia* species for antimicrobial and antioxidant properties.

The objectives of the present investigation are to determine the antimicrobial and antioxidant potential of stem and leave of four *Cassia* species viz. *Cassia auriculata, Cassia fistula, Cassia siamea* and *Cassia tora*.

MATERIALS AND METHODS

Plant material

The leaves and stem of four *Cassia* species, were collected in the month of August - September 2009 from Rajkot, Gujarat, India. The ethnobotanical information and voucher specimen number of the screened *Cassia* species is given in Table I. The taxonomic identification was confirmed by the voucher specimen deposited at the Department of Biosciences, Saurashtra University, Rajkot, Gujarat, India. The stem and leaves were thoroughly washed, shade dried, homogenized to fine powder and stored in air tight bottles.

Extraction

The dried powder of leaf and stem of different *Cassia* species was individually extracted by cold percolation method (Parekh and Chanda, 2007) using different organic solvents like petroleum ether, acetone, methanol and water. 10 g of dried powder was taken in 100 ml of petroleum ether in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 120 rpm for 24 h. After 24 h, the extract was filtered with eight layers of muslin cloth and then centrifuged at 5000 rpm for 10 min. The supernatant was collected and the solvent was evaporated. The residue was then taken in 100 ml of solvent (acetone, methanol and water) in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 120 rpm for 24 h. The procedure followed was same as above, and the dry extract was stored at 4°C in air tight bottles. The extract was weighed to obtain the extractive yield.

Determination of total phenol content

The amount of total phenol content was determined by Folin-Ciocalteu's reagent method (Mc Donald et al., 2001). 0.5 ml of extract (1 mg/ml) and 0.1 ml Folin-Ciocalteu's reagent (0.5 N) was mixed and the mixture was incubated at room temperature for 15 min. Then 2.5 ml saturated sodium carbonate solution was added and further incubated for 30 min at room temperature and the absorbance was measured at 760 nm. Gallic acid was used as a positive control. Total phenol values are expressed in terms of gallic acid equivalent (mg/g of extracted compound).

Determination of flavonoid content

The amount of flavonoid content was determined by Aluminium chloride colorimetric method (Chang et al., 2002). The reaction mixture of 3 ml consisted of 1.0 ml of sample (1 mg/ml), 1.0 ml of methanol, 0.5 ml of aluminium chloride (1.2 %) and 0.5 ml potassium acetate (120 mM); this was incubated at room temperature for 30 min. The absorbance of all the samples was measured at 415 nm. Quercetin was used as a positive control. Flavonoid content is expressed in terms of quercetin equivalent (mg/g of extracted compound).

2,2-Diphenyl-1-picryl-hydrazyl (DPPH) free radical scavenging activity

The free radical scavenging activity was measured using DPPH by the modified method of McCune and Johns (2002). The reaction mixture of 3.0 ml consisted of 1.0 ml of DPPH in methanol (0.3 mM), 1.0 ml of extract (10 to 1000 µg/ml) and 1.0 ml of methanol; it was incubated in dark for 10 min after which the absorbance was measured at 517 nm. Ascorbic acid was used as a positive control. The percentage inhibition was calculated.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and plant extract for hydroxyl radicals generated by Fe^{3+}-ascorbic acid- ethylenediaminetetraacetic acid (EDTA)-H_2O_2 system (Fenton reaction) according to the method of Kunchandy and Rao (1990). The reaction mixture of 1.0 ml consisted of 100 µl of 2-deoxy-D-ribose (28 mM in 20 mM KH_2PO_4 -KOH buffer, pH 7.4), 500 µl of the various solvent extracts (500 to 1000 µg/ml), 200 µl EDTA (1.04 mM) and 200 µM $FeCl_3$ (1:1 v/v), 100 µl H_2O_2 (1.0 mM) and 100 µl ascorbic acid (1.0 mM); it was incubated at 37°C for 1 h. One milliliter (1.0 ml) of thiobarbituric acid (1%) and 1.0 ml of trichloroacetic acid (2.8%) were added and incubated at 100°C for 20 min. After cooling, absorbance of pink color was measured at 532 nm. Gallic acid was used as a positive control.

Superoxide anion radical scavenging activity

The superoxide anion radical scavenging activity of various extracts was measured as described by Robak and Gryglewski (1988). In the PMS/NADH-NBT system, the superoxide anion derived from dissolved oxygen from PMS/NADH coupling reaction reduces nitroblue tetrazolium (NBT). The superoxide radicals were generated in 3.0 ml of Tris–HCl buffer (16 mM, pH 8.0), containing 0.5 ml of NBT (0.3 mM), 0.5 ml of NADH (0.936 mM) solution, 1.0 ml of extracts (100 to 1000 µg/ml) and 0.5 ml of Tris-HCl buffer (16 mM, pH 8.0). The reaction was initiated by adding 0.5 ml of phenazine methosulfate (PMS) solution (0.12 mM) to the mixture, and incubated at 25°C for 5 min. The absorbance was measured at 560 nm. Gallic acid was used as a positive control.

Reducing capacity assessment

The reducing capacity assessment was determined using the modified method of Athukorala et al. (2006). The reaction mixture of 6.0 ml consisted of 1 ml of extract (20 to 200 µg/ml) mixed with 2.5 ml of phosphate buffer (200 mM, and pH 6.6) and 2.5 ml of potassium ferricyanide (30 mM). The mixture was incubated at 50°C for 20 min. Then 2.5 ml of trichloroacetic acid (600 mM) was added and centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 6 mM) and the absorbance was measured at 700 nm. Ascorbic acid was used as a positive control.

Bacterial and fungal species

The microbial strains used were obtained from the National Chemical Laboratory (NCL), Pune. The microorganisms investigated are four Gram positive bacteria *Staphylococcus aureus* ATCC29737, *Staphylococcus subflava* NCIM2178, *Bacillus megaterium* ATCC9885, *Bacillus subtilis* ATCC6633; four Gram negative bacteria *Klebsiella pneumoniae* NCIM2719, *Proteus mirabilis* NCIM2241, *Proteus morganii* NCIM2040, *Pseudomonas testosteroni* NCIM5098 and four Fungi *Candida albicans* ATCC2091, *Candida tropicalis* ATCC4563, *Candida glabrata* NCIM3448, *Candida neoformans* NCIM3542.

Antibacterial and antifungal assay

In vitro antimicrobial activity of the crude extracts was studied against eight bacterial strains and four fungal strains by the agar well diffusion method (Perez et al., 1990; Kaneria et al., 2009). Mueller Hinton agar No. 2/Sabouraud dextrose agar (Hi Media, India) was used as the bacteriological/mycological medium. The extracts were diluted in 100% dimethylsulphoxide (DMSO) at the concentration of 25 mg/ml. The antimicrobial activity was evaluated at the concentration of 2.5 mg/well. The Mueller Hinton agar/ Sabouraud dextrose agar was melted and cooled to between 48 to 50°C and a standardized inoculum (1.5×10^8 CFU/ml, 0.5 McFarland) was then added aseptically to the molten agar and poured into sterile Petri dishes to give a solid plate. Wells were prepared in the seeded agar plates. The plant extract (100 µl) was introduced into the well (8.5 mm). The plates were incubated over night at 37 and 28°C for 24 and 48 h for bacteria and fungi, respectively. The antimicrobial spectrum of the extract was determined for the bacterial and fungal species in terms of zone sizes around each well. The diameter of zone of inhibition produced by the extracts was compared with those produced by the commercial control antibiotics, ciprofloxacin (5 µg), amikacin (30 µg), gentamicin (10 µg), fluconazole (10 µg) and ketoconazole (10 µg). These are commonly used antibiotics to treat infections caused by several bacteria and fungi. So they were selected as control antibiotics. DMSO was used as negative control. The control zones were subtracted from the test zones and the resulting zone diameter is shown in Tables 4 to 6. The experiment was performed three times to minimize the error and the mean values ± SEM are presented.

Determination of minimum inhibition concentration (MIC)

Agar well diffusion method was used in the present study to determine MIC of methanol and acetone extracts of leaves of *C. auriculata* by two- fold serial dilution prepared in the range of 25000 to 97 µg/ml as reported by Habeeb et al. (2007).

Statistical analysis

All the experiments were performed in triplicate and results are presented as mean ± SEM (Standard Error of Mean).

RESULTS AND DISCUSSION

Extractive yield

The extractive yield was different in stem and leaves of *Cassia* species. It also varied among the solvents used. The yield was more in leaves than in stem. In *C. siamea* and *C. tora,* the yield was more in water than in organic solvents and in *C. auriculata* and *C. fistula* the yield was more in methanol. Amongst the 16 extracts of leaves, maximum yield was in methanolic extract of *C. auriculata* (1.48%) while minimum was in acetone extract of *C. tora*

Table 2. % yield, total phenol and flavonoid content of methanol, acetone and aqueous extract of *Cassia* species.

Plant name	Parts used	Extract	Yield (%, w/w)	Total Phenol content (mg/g)	Flavonoid content (mg/g)
CA	Leaves	PE	0.21	ND	ND
		ME	1.48	80.02 ± 1.42	34.76 ± 0.02
		AC	0.64	86.81 ± 2.50	21.71± 0.44
		AQ	1.09	38.78 ± 0.39	0.93 ± 0.03
	Stem	PE	0.05	ND	ND
		ME	0.96	96.62 ± 3.60	3.21 ± 0.18
		AC	0.49	96.55 ± 6.29	4.06 ± 0.17
		AQ	0.63	78.01 ± 0.94	3.36 ± 0.07
CF	Leaves	PE	0.38	ND	ND
		ME	1.15	74.61 ± 0.46	45.08 ± 1.37
		AC	0.40	94.10 ± 1.11	25.34 ± 1.27
		AQ	1.06	64.98 ± 0.29	13.98 ± 0.64
	Stem	PE	0.06	ND	ND
		ME	0.63	106.90 ± 0.64	4.17 ± 0.20
		AC	0.27	113.19 ± 1.52	4.43 ± 0.05
		AQ	0.36	78.57 ± 0.46	1.97 ±0.12
CS	Leaves	PE	0.19	ND	ND
		ME	0.84	75.81 ± 0.34	16.57 ± 0.36
		AC	0.18	53.81 ± 0.45	46.25 ± 0.33
		AQ	1.44	72.12 ± 4.56	1.40 ± 0.12
	Stem	PE	0.06	ND	ND
		ME	0.38	45.79 ± 0.67	7.23 ± 0.37
		AC	0.07	45.95 ± 0.49	17.54 ± 0.52
		AQ	0.62	58.83 ± 1.68	0.98 ± 0.12
CT	Leaves	PE	0.30	ND	ND
		ME	0.78	53.12 ± 0.92	35.48 ± 1.03
		AC	0.15	51.03 ± 0.18	65.46 ± 0.23
		AQ	1.25	35.67 ± 0.11	1.61 ± 0.07
	Stem	PE	0.08	ND	ND
		ME	0.53	57.19 ± 1.04	20.53 ± 0.23
		AC	0.09	79.23 ± 0.94	46.67 ± 1.04
		AQ	0.87	20.77 ± 0.20	2.12 ± 0.18

CA, *Cassia auriculata*; CF, *Cassia fistula*; CS, *Cassia siamea*; CT, *Cassia tora*; PE, petroleum ether; ME, methanol; AC, acetone; AQ, aqueous; ND, not done. Values are expressed as mean ± SEM (n = 3).

(0.15%) (Table 2). The stem of four *Cassia* species showed a considerably low yield in water as well as in organic solvents. Maximum yield was in methanolic extract of *C. auriculata.* There are many reports in the literature where extractive yield varied with different solvents (Vaghasiya and Chanda, 2007; Yang et al., 2007).

Total phenol and flavonoid contents

Phenolics are secondary metabolites that are commonly found in both edible and non edible plants and are reported to have multiple biological effects, including antioxidant activity. The action of phenolics is believed to be mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching single and triplet oxygen, or decomposing peroxidase (Itagaki et al., 2009).

In the present study, leaves and stem of four *Cassia* species show the presence of more quantity of total phenolic content than the flavonoid, except in the acetone extract of leaves of *C. tora*. Among all the extracts, highest total phenolic content was shown by acetone extract of stem of *C. fistula* and flavonoid content was maximum in acetone extract of leaves of *C. tora* (Table 2).

Antioxidant activity

DPPH free radical scavenging activity

The DPPH radical scavenging activity was known to

Table 3. IC_{50} Values of DPPH free radical scavenging activity (DPPH), hydroxyl radical scavenging activity (OH) and superoxide anion radical scavenging activity (SO) of different polar solvent extracts of *Cassia* species.

No.	Plant name	Parts used	IC_{50} Values (µg/ml)								
			DPPH			OH			SO		
			ME	AC	AQ	ME	AC	AQ	ME	AC	AQ
1	*C. auriculata*	Leaves	76.5	45	-	-	-	-	210	167	660
		Stem	30.7	29	83	-	-	-	190	115	285
2	*C. fistula*	Leaves	170	93	465	-	-	720	-	525	540
		Stem	38.5	21.8	500	-	-	-	290	145	545
3	*C. siamea*	Leaves	305	487	-	-	-	910	-	-	437
		Stem	345	175	-	-	-	-	-	-	738
4	*C. tora*	Leaves	310	215	-	-	-	620	-	-	-
		Stem	177	970	-	-	-	-	970	370	-
Std	Ascorbic acid		11.4			ND			ND		
	Gallic acid		ND			140			185		

ME, Methanol extract; AC, acetone extract; AQ, aqueous; Std, standard; -: >1000 µg/ml; ND, not done.

correlate well with the inhibitory capacity of lipid peroxidation of plant extracts (Rekka and Kourounakis, 1991). DPPH is a stable and nitrogen centered violet colored free radical that upon reduction is converted to yellow by electron or hydrogen donating ability of the antioxidant compound found in the extract (Oliveira et al., 2009). The degree of discoloration indicates the scavenging potential of antioxidant compounds of extracts in terms of H_2 donating ability.

In the present study, leaves and stem of four *Cassia* species, in various polar solvents were evaluated for their DPPH free radical scavenging activity. Out of the 24 extracts investigated, 5 extracts showed IC_{50} value more than 1000 µg/ml (Table 3), the remaining 19 extracts showed a varied level of DPPH free radical scavenging activity. Methanol and acetone extracts of leaves and stem of all the four *Cassia* species showed remarkable DPPH free radical scavenging activity. IC_{50} values ranged from 21.8 to 970 µg/ml (Table 3). The IC_{50} value of acetone extract of *C. fistula* was 21.8 µg/ml and IC_{50} values of methanol and acetone extracts of stem of *C. auriculata* were 30.7 and 29 µg/ml, respectively which were almost near to that of standard ascorbic acid 11.4 µg/ml (Table 3). Low IC_{50} value indicates high antioxidant activity (Tenpe et al., 2008). Our above observations clearly reveal the high antioxidant activity of the methanol and acetone extracts of stem and leaves of *C. auriculata* and *C. fistula*.

Hydroxyl radical scavenging activity

The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cell (Lee et al., 2002). This radical has the capacity to cause strand breakage in DNA, which contributes to carcinogenesis, mutagenesis and cytotoxicity (Spencer et al., 1994). Methanol and acetone extracts of leaves and stem of all the four *Cassia* species showed poor hydroxyl radical scavenging activity (>1000 µg/ml, Table 3). Best hydroxyl radical scavenging activity was shown by aqueous extract of leaves of *C. tora*; its IC_{50} value was 620 µg/ml.

Superoxide anion radical scavenging activity

Scavenging of superoxide anion radicals is of importance for protection against early event in oxidative damages (Hu and Skibsted, 2002). Superoxide anion is a free radical created from the normal process of energy generation in the human body and first reduction product of molecular oxygen, a highly toxic radical, the most abundantly produced in all aerobic cells by several enzymatic and non-enzymatic pathways, attacks a number of biological molecules and leads to unfavorable alterations of bimolecules including DNA (Waris and Alam, 2004). It also forms an important source of other deleterious radicals such as hydroxyl and hydro-peroxides, which initiate free radical chain reactions (Andlauer and Frust, 1998).

The acetone extract of leaves and stem of *C. auriculata* and stem of *C. fistula* showed very good superoxide anion radical scavenging activity which was comparable or even better (some extracts) to that of the standard (Table 3). The ability of *C. auriculata* extracts to quench superoxide anion radicals seems to be directly related to

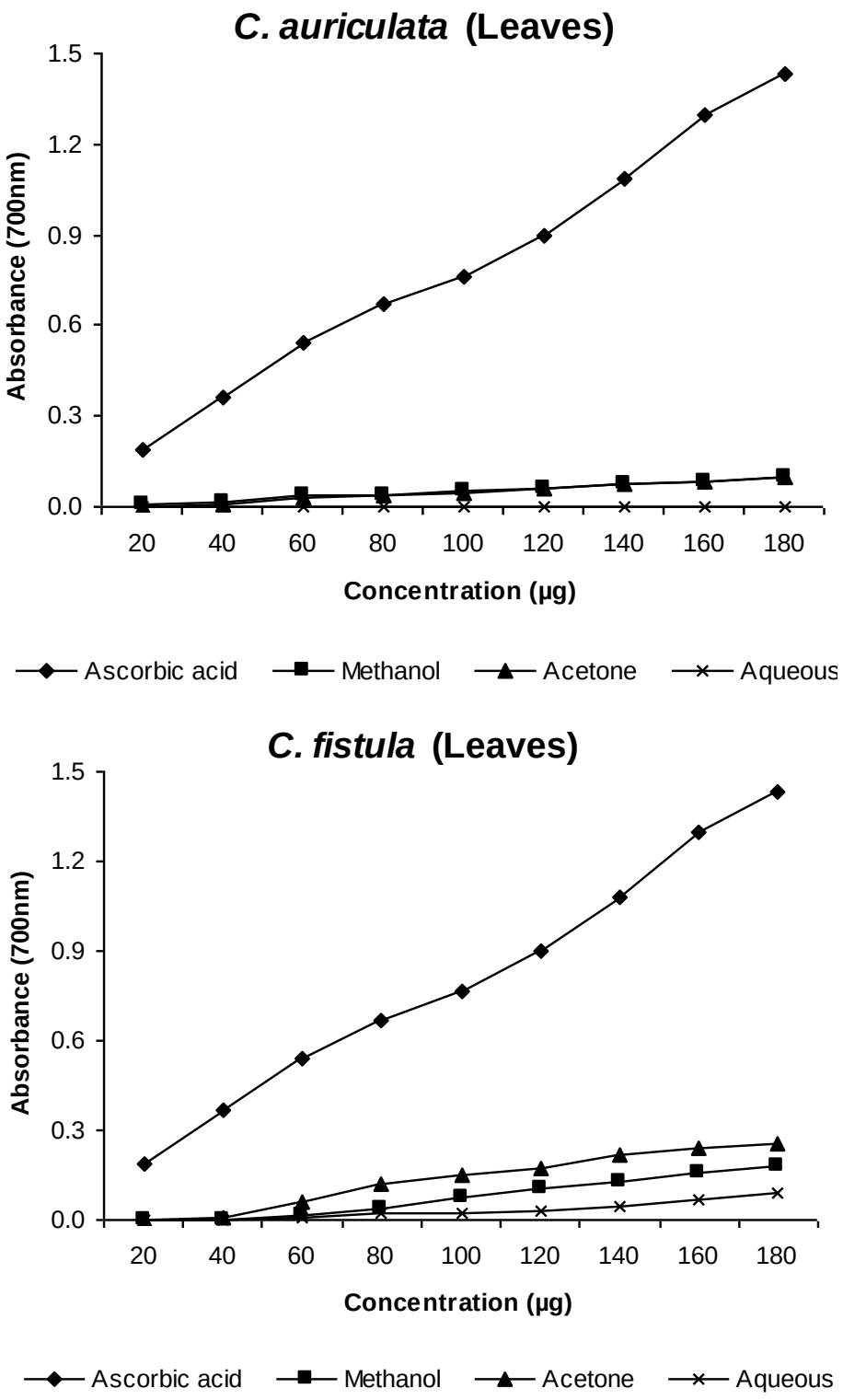

Figure 1. Reducing capacity assessment of different polar solvents extracts of leaves of *Cassia* species.

the prevention of lipid peroxidation and seems to be a good scavenger of active oxygen species thus reducing the rate of chain reaction.

Reducing capacity assessment

Reducing power is associated with antioxidant activity

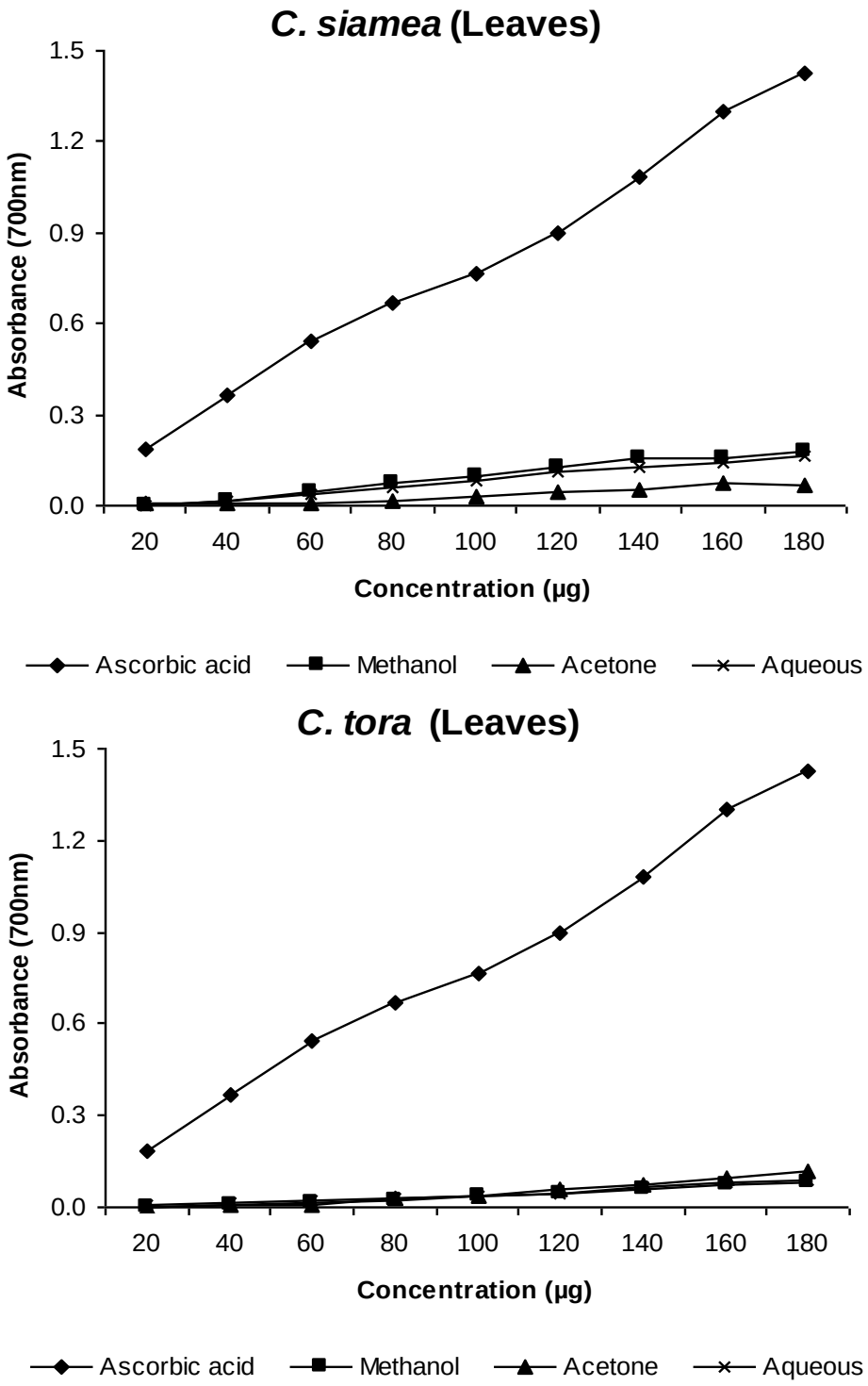

Figure 1. Contd.

and may serve as a significant reflection of the antioxidant activity (Oktay et al., 2003). Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants (Yen and Chen, 1995).

Amongst the 24 extracts, only the stem of *C. auriculata* (Figure 2a) and *C. fistula* (Figure 2b) showed reducing capacity assessment. The acetone extract of stem of *C. auriculata* showed maximum reducing capacity assessment. The leaves showed poor reducing capacity assessment (Figure 1).

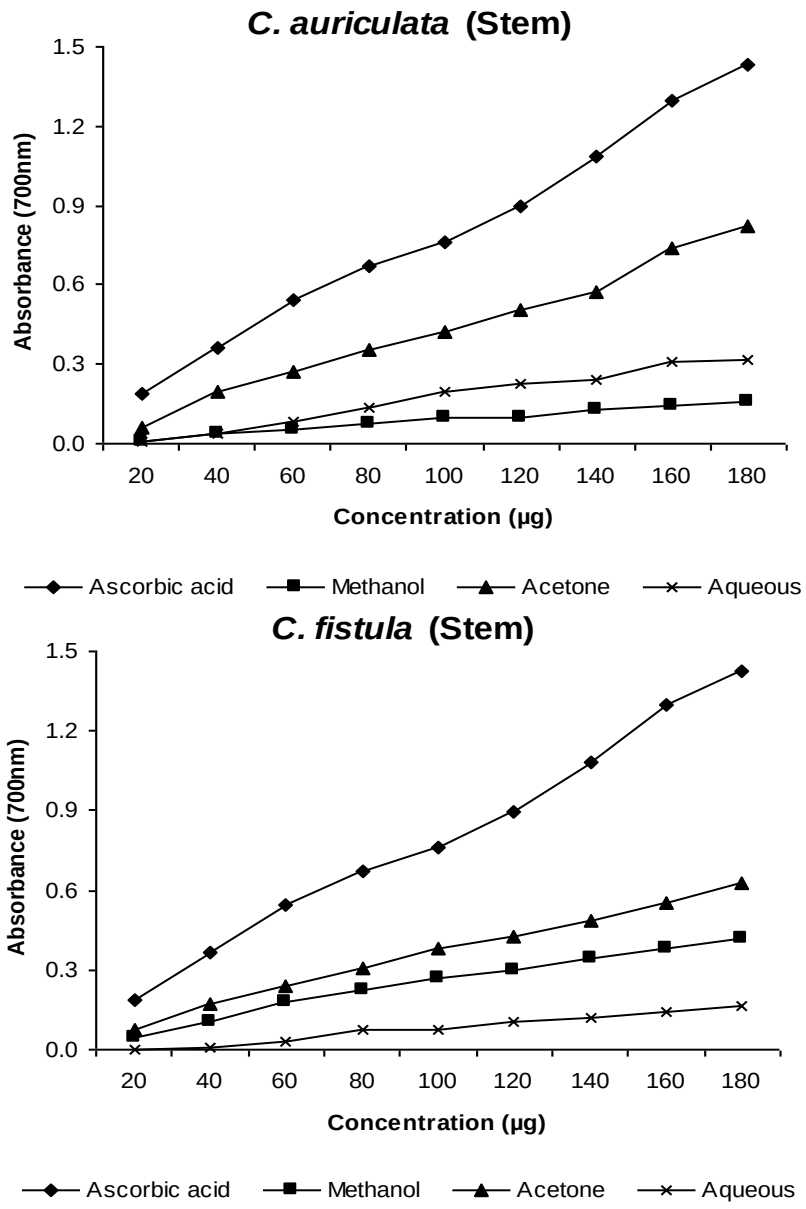

Figure 2. Reducing capacity assessment of different polar solvents extracts of stem of *Cassia* species.

The results of the present study revealed a direct correlation between phenolic content and antioxidant activity. The acetone extract of stem of *C. auriculata* had maximum phenolic content and antioxidant activity. This is in agreement with many reports in the literature (Brighente et al., 2007; Biglari et al., 2008; Baravalia et al., 2009). The phenolic content of a plant can be a good indicator of its antioxidant capacity.

Antimicrobial activity

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases. Plant based antimicrobial compounds have enormous therapeutical potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials (Murray, 1995). Many phytomedicines exert their beneficial effects through the additive or synergistic action of several chemical compounds acting at single or multiple target sites (Briskin, 2000).

The results of antimicrobial activity are shown in Tables 4 to 6. The antimicrobial activity of some of the solvent

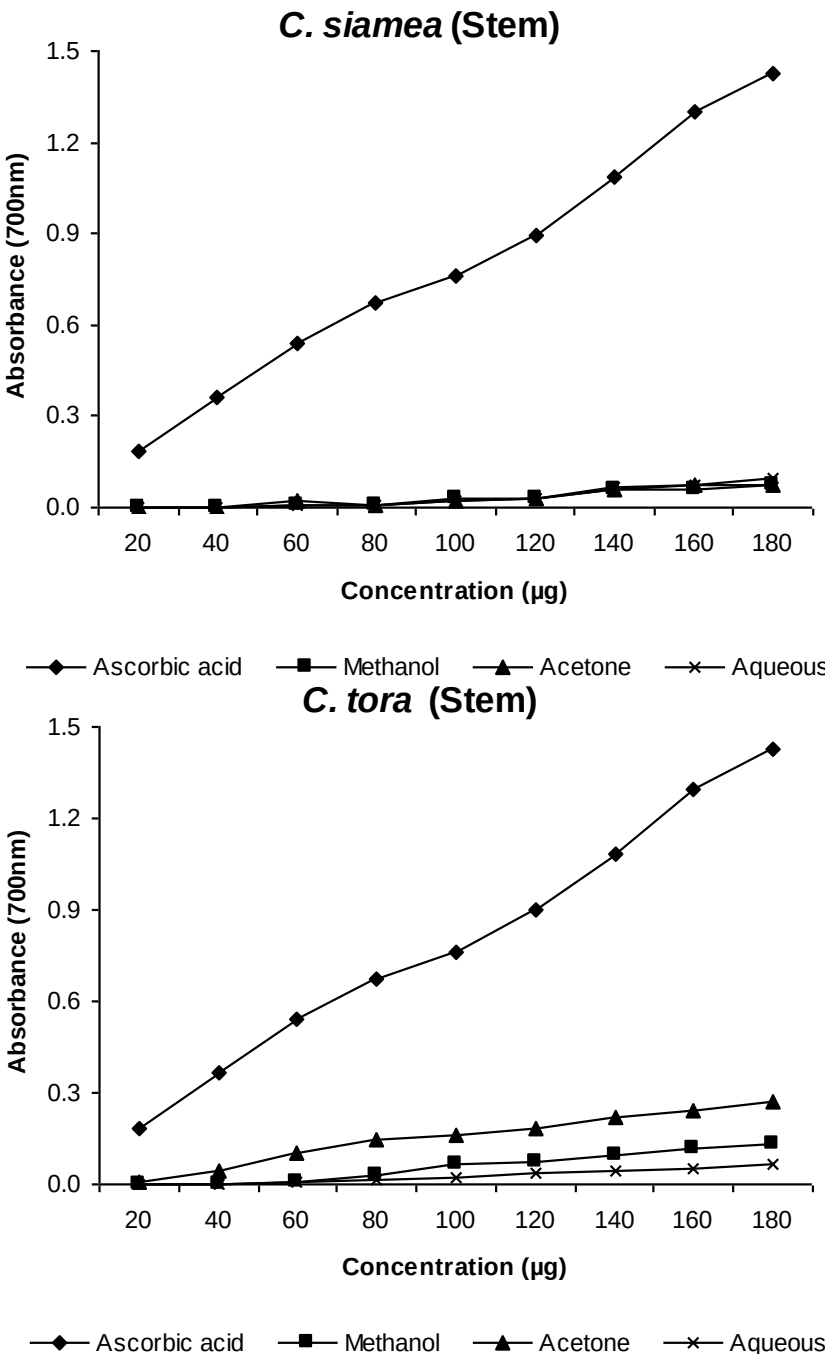

Figure 2. Contd.

extracts is comparable with that of standard antibiotics. The methanol and acetone extracts of leaves of *C. auriculata* showed maximum antibacterial activity against *S. aureus* which was better than standard antibiotics (Table 4). Amongst the Gram negative bacteria, *K. pneumoniae* was susceptible to all the 24 extracts of *Cassia* species; though maximum activity was shown by methanol and acetone extracts of leaves of *C. auriculata* which was comparable with standard antibiotic gentamicin (Table 5). The antifungal activity was more than Gram positive bacteria. The most susceptible fungal strain was *C. auriculata* (Table 6).

From the results of antimicrobial activity, the most active extracts that is, acetone and methanol extract of *C. auriculata*, were subjected to the evaluation of MIC (Table 7). The acetone extract showed least MIC value against *B. megaterium* and *K. pneumoniae* (Table 7). From the above results, it can be concluded that *S.*

Table 4. Antibacterial activity of different polar solvent extracts of *Cassia* species against Gram positive bacteria.

Plant name	Parts used	Extract	Zone of Inhibition (mm)			
			SA	BS	SS	BM
CA	Leaves	PE	-	-	-	-
		ME	17.50 ± 0.29	13.50 ± 0.29	11.50 ± 0.29	14.50 ± 0.29
		AC	17.50 ± 0.29	16.00 ± 0.00	12.50 ± 0.29	15.00 ± 0.00
		AQ	-	-	-	-
	Stem	PE	9.00 ± 0.00	-	-	-
		ME	13.50 ± 0.29	12.00 ± 0.00	10.50 ± 0.29	13.00 ± 0.00
		AC	14.00 ± 0.58	14.00 ± 0.00	11.50 ± 0.29	14.50 ± 0.29
		AQ	11.00 ± 0.00	-	-	-
CF	Leaves	PE	-	-	-	-
		ME	-	-	-	-
		AC	13.50 ± 0.29	-	-	12.50 ± 0.29
		AQ	-	-	-	-
	Stem	PE	-	-	9.00 ± 0.00	-
		ME	11.50 ± 0.29	10.00 ± 0.00	10.00 ± 0.00	13.50 ± 0.29
		AC	13.50 ± 0.87	13.00 ± 0.00	11.00 ± 0.00	14.50 ± 0.29
		AQ	9.50 ± 0.29	-	-	-
CS	Leaves	PE	-	-	-	-
		ME	-	-	-	-
		AC	9.50 ± 0.29	-	9.50 ± 0.29	-
		AQ	10.50 ± 0.29	10.00 ± 0.00	9.00 ± 0.00	-
	Stem	PE	-	-	-	-
		ME	-	-	-	-
		AC	-	-	-	-
		AQ	-	-	-	-
CT	Leaves	PE	-	-	-	-
		ME	-	-	-	-
		AC	-	-	-	-
		AQ	-	-	-	-
	Stem	PE	-	11.00 ± 0.00	10.00 ± 0.00	-
		ME	-	9.00 ± 0.00	10.00 ± 0.00	-
		AC	-	11.00 ± 0.58	9.00 ± 0.00	11.50 ± 0.29
		AQ	-	-	-	-
Ciprofloxacin			14	30	19	20
Amikacin			13.5	15	ND	18
Gentamicin			18	15	18	20

CA, *Cassia auriculata*; CF, *Cassia fistula*; CS, *Cassia siamea*; CT, *Cassia tora*; SA, *Staphylococcus aureus*; BS, *Bacillus subtilis*; SS, *Staphylococcus subflava*; BM, *Bacillus megaterium*; PE, petroleum ether; ME, methanol; AC, acetone; AQ, aqueous; -: no inhibition; ND, note done. Values are expressed in mean ± SEM (n = 3).

aureus and *K. pneumoniae* were the most susceptible bacterial strains and *C. albicans was* the most susceptible fungal strain. The leaves of *C. auriculata* showed a broad spectrum of antimicrobial activity suggesting that the leaves possess certain constituents with antibacterial properties that can be used as antimicrobial agents in new drug therapy of infectious diseases caused by pathogens. The present study of *in vitro* antioxidant and antibacterial evaluation of *Cassia* species forms a primary platform for further isolation to discover new bioactive molecules and carry out further pharmacological evaluation.

Table 5. Antibacterial activity of different polar solvent extracts of *Cassia* species against Gram negative bacteria.

Plant name	Part used	Extract	Zone of Inhibition (mm)			
			KP	PMo	PT	PMi
CA	Leaves	PE	13.00 ± 0.00	-	-	-
		ME	17.50 ± 0.29	14.50 ± 0.29	12.00 ± 0.00	15.00 ± 0.00
		AC	17.50 ± 0.29	16.00 ± 0.00	14.50 ± 0.29	15.50 ± 0.29
		AQ	11.50 ± 0.29	-	-	-
	Stem	PE	12.00 ±0.00	12.00 ±0.58	13.50 ± 0.29	-
		ME	15.50 ± 0.29	14.50 ± 0.29	12.50 ± 0.29	13.00 ± 0.00
		AC	15.00 ± 0.58	15.00 ± 0.00	12.50 ± 0.29	12.50 ± 0.29
		AQ	12.00 ± 0.00	10.50 ± 0.29	9.00 ±0.00	-
CF	Leaves	PE	15.00 ± 0.00	-	-	-
		ME	15.50 ± 0.29	-	-	-
		AC	15.50 ± 0.29	11.00 ±	9.00 ± 0.00	11.50 ± 0.29
		AQ	13.50 ± 0.29	12.00 ± 0.00	9.00 ± 0.00	-
	Stem	PE	14.50 ± 0.29	11.00 ± 0.00	11.00 ± 0.58	9.00 ± 0.00
		ME	14.50 ± 0.29	11.50 ± 0.29	10.50 ± 0.29	10.50 ± 0.29
		AC	17.00 ± 0.00	13.50 ± 0.29	11.50 ± 0.29	13.00 ± 0.58
		AQ	13.00 ± 0.58	-	10.00 ± 0.58	-
CS	Leaves	PE	12.00 ± 0.00	-	11.00 ± 0.00	-
		ME	11.50 ± 0.29	-	-	-
		AC	13.50 ± 0.29	10.00 ± 0.00	-	9.50 ± 0.29
		AQ	16.00 ± 0.00	10.50 ± 0.29	11.00 ± 0.00	10.00 ± 0.00
	Stem	PE	15.00 ± 0.00	-	-	-
		ME	14.00 ± 0.00	-	-	-
		AC	15.50 ± 0.29	-	-	9.00 ± 0.00
		AQ	13.50 ± 0.29	-	-	9.00 ± 0.00
CT	Leaves	PE	13.00 ± 0.00	-	-	-
		ME	14.50 ± 0.29	-	-	-
		AC	15.00 ± 0.58	-	-	9.00 ± 0.00
		AQ	15.00 ± 0.00	11.00 ± 0.00	-	9.00 ± 0.00
	Stem	PE	15.00 ± 0.00	10.50 ± 0.29	11.00 ± 0.00	9.00 ± 0.00
		ME	14.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00
		AC	13.00 ± 0.00	12.00 ± 0.00	10.50 ± 0.29	-
		AQ	12.00 ± 0.00	-	-	9.00 ± 0.00
Ciprofloxacin			25	ND	ND	16
Amikacin			21.5	ND	ND	12
Gentamicin			20	ND	ND	14

CA, *Cassia auriculata*; CF, *Cassia fistula*; CS, *Cassia siamea*; CT, *Cassia tora*; KP, *Klebsiella pneumoniae*; PMo, *Proteus morganii*; PT, *Pseudomonas testosteroni*; PMi, *Proteus mirabilis*; PE, petroleum ether; ME, methanol; AC, acetone; AQ, aqueous; -: no inhibition; ND, note done. Values are expressed as mean ± SEM (n = 3).

Conclusion

The acetone extract of stem of *C. auriculata* had maximum phenolic content and showed good antioxidant activity; while acetone and methanol extracts of stem and leaves of *C. auriculata* showed a broad range of antimicrobial property. This information can serve as an important platform for the development of inexpensive, safe and effective natural antioxidants and antimicrobics. Further studies are necessary for isolation and

Table 6. Antifungal activity of different polar solvent extracts of *Cassia* species against fungi.

Plant name	Part used	Extract	Zone of Inhibition (mm)			
			CAl	CN	CTr	CG
CA	Leaves	PE	-	-	9.00 ± 0.00	-
		ME	9.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.00
		AC	10.00 ± 0.00	10.50 ± 0.29	10.00 ± 0.00	10.00 ± 0.00
		AQ	9.00 ± 0.00	-	9.00 ± 0.00	-
	Stem	PE	10.00 ± 0.00	-	9.00 ± 0.00	-
		ME	10.00 ± 0.00	10.50 ± 0.29	-	10.00 ± 0.00
		AC	9.50 ± 0.29	10.50 ± 0.29	-	-
		AQ	10.00 ± 0.00	10.00 ± 0.00	-	-
CF	Leaves	PE	11.00 ± 0.00	10.00 ± 0.00	-	-
		ME	11.00 ± 0.00	10.50 ± 0.29	9.00 ± 0.00	-
		AC	10.00 ± 0.00	11.00 ± 0.58	-	-
		AQ	10.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	-
	Stem	PE	11.00 ± 0.00	-	10.00 ± 0.00	-
		ME	11.00 ± 0.00	10.50 ± 0.29	9.50 ± 0.29	9.50 ± 0.29
		AC	10.00 ± 0.00	10.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00
		AQ	10.00 ± 0.00	11.00 ± 0.00	10.50 ± 0.29	9.00 ± 0.00
CS	Leaves	PE	-	-	-	-
		ME	-	-	-	-
		AC	9.00 ± 0.00	-	9.50 ± 0.29	-
		AQ	9.00 ± 0.00	10.00 ± 0.00	9.50 ± 0.29	-
	Stem	PE	-	-	9.00 ± 0.00	-
		ME	10.00 ± 0.00	-	10.00 ± 0.00	-
		AC	10.00 ± 0.00	-	9.00 ± 0.00	-
		AQ	-	-	-	-
CT	Leaves	PE	9.00 ± 0.00	-	-	-
		ME	9.00 ± 0.00	-	10.00 ± 0.00	-
		AC	9.50 ± 0.29	-	-	-
		AQ	9.00 ± 0.00	-	-	-
	Stem	PE	10.00 ± 0.00	-	9.00 ± 0.00	-
		ME	-	-	10.00 ± 0.00	-
		AC	-	-	10.00 ± 0.00	-
		AQ	9.00 ± 0.00	-	-	-
Fluconazole			0	27	0	22
Ketoconazole			20	23.5	0	ND

CA, *Cassia auriculata*; CF, *Cassia fistula*; CS, *Cassia siamea*; CT, *Cassia tora*; Cal, *Candida albicans*; CN, *Candida neoformans*; CTr, *Candida tropicalis*; CG, *Candida glabrata*; PE, petroleum ether; ME, methanol; AC, acetone; AQ, aqueous; -: no inhibition; ND, not done. Values are expressed as mean ± SEM (n = 3).

Table 7. Minimum inhibitory concentration of methanol and acetone extracts of leaves of *C. auriculata.*

Microorganism	Botanical name	MIC (µg/ml)	
		Methanol	Acetone
Gram positive bacteria	*S. aureus*	25000	25000
	B. subtilis	25000	25000
	S. subflava	25000	25000
	B. megaterium	6250	<97
Gram negative bacteria	*K. pneumoniae*	390	97
	P. morganii	25000	12500
	P. testosteroni	25000	25000
	P. mirabilis	25000	25000

Table 7. Contd.

Fungi	*C. albicans*	12500	12500
	C. neoformans	25000	25000
	C. tropicalis	12500	6250
	C. glabrata	25000	25000

characterization of the active compounds of the plant.

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