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Free radical scavenging activity of some Bangladeshi plant extracts

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SUMMARY

A number of plants from different geographical origins have been shown to possess antioxidant activity. Some of them have been developed as natural antioxidant formulations for food, cosmetic and other applications. Bangladeshi flora is a rich source of a range of plant species, many of which are medicinal plants, and have been used in the preparations of the Unani and Ayurvedic traditional medicines. There are no, or just a few, reports on any systematic screening of the extracts of Bangladeshi plants for free radical scavenging activity using DPPH assay available to date. As part of our on-going search for biological activity in Bangladeshi plants, Kadam (*Anthocephalus chinensis*), Goran (*Ceriops decandra*), Swarnalata (*Cuscuta reflexa*), Gab (*Diospyros peregrina*), Sundari (*Heritiera fomes*), Dhundul (*Xylocarpus granatum*) and Possur (*Xylocarpus mekongensis*) have been selected for the assessment of their free radical scavenging activity, and studies on the contents of alkaloids, anthraqunones, flavonoids and tannins in these extracts. Most of these species have been used in traditional medicine in Bangladesh and other countries for the treatment of various illnesses ranging from common cold to cancer. All extracts, except the methanol extract of *Cuscuta reflexa*, displayed significant free radical scavenging activity in the DPPH assay (RC_{50} values within the range of 2.75×10^{-2} to 4.7×10^{-3} mg/mL). Among these extracts, the methanol extract of *Xylocarpus granatum* exhibited the most potent activity (4.7×10^{-3} mg/mL) and that of *Cuscuta reflexa* had the least activity (1.64×10^{-1} mg/mL). While none of these plants showed positive tests with Dragendorff's reagent, presence of low to moderate amounts of phenolic compounds, *e.g.* anthraquinones, flavonoids and tannins was evident in all of these plants, except for the methanolic extracts of *C. reflexa* and the barks of *D. peregrina*, which did not display any evidence for the presence of flavonoids and anthraquinones, respectively.

Key words: Convolvulaceae; Ebenaceae; Meliaceae; Rhizophoraceae; Rubiaceae; Sterculiaceae; DPPH assay; Natural antioxidant

INTRODUCTION

Anthocephalus chinensis (Lamk.) Rich. Ex Walp. (Rubiaceae), commonly known as 'kadam', is a medium-sized deciduous tree that grows in the sub-Himalayan tract at latitudes from 9°S to 27°N, from Nepal eastward to Bangladesh, India, Myanmar, Sri Lanka, Indonesia, the Phillippines and Papua New Guinea (Whitmore, 1984; Kirtikar and Basu, 1999; ARCBC database, 2004; GRIN database, 2004). *Ceriops decandra* (Griff.)

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Ding Hou (Rhizophoraceae), common name 'goran', is a glabrous shrub or small tree, found abundantly in the mangrove vegetation of the Sundarbans in Bangladesh and India, and also grows in many other countries of south-east Asia (Tomlinson, 1986; Kirtikar and Basu, 1999; ARCBC database, 2004). The Sundarbans, the largest single tract of mangrove forests in the world, covers an area of over 10,000 km^2, and is also the habitat for the tree species, *Heritiera fomes* Buch.-Ham. (Sterculiaceae), common name 'sundari' (Rahman, 2000), *Xylocarpus granatum* J. Knig (Meliaceae), known as 'dhundul' and *Xylocarpus mekongensis* (Prain) Pierre. (Meliaceae), synonym *Carapa molucensis* Lam., common name 'possur' (GRIN database, 2004). Both *X. granatum* and *X. mekongensis* are also well distributed in a number of other countries of south-east Asia, Australia and east Africa (Tomlinson, 1986). *Cuscuta refelxa* Roxb. (Convolvulaceae), commonly known as 'swarnalata' or 'Indian dodder', is a leafless and rootless parasitic Bangladeshi annual climber herb, and also distributed in the countries of temperate, tropical and sub-tropical Asia (Ghani, 1998; GRIN database, 2004). *Diospyros peregrina* Gurke (Ebenaceae), Bengali name 'gab', is a medium-sized evergreen tree indigenous to Bangladesh and India, and also found in many other countries of Asia and America (Ghani, 1998; GRIN database, 2004).

The ethnobotanical and traditional medicinal uses of *A. chinensis* include its use as a remedy for fever, chest congestion and stomatitis, and as an astringent and tonic (Yusuf *et al.*, 1994; ARCBC database, 2004; Phytochemical and Ethnobotanical Databases, 2004). It is also used to treat snake-bites. However, its major economical importance lies in its application as a source of wood for matchstick boxes, tea boxes, bobbins, veneer, plywood, crates, furniture, light construction, root structure, etc. (Grijpma, 1967). While there is no proven record for any medicinal value of *C. decandra*, the decoction of the barks of this plant is used traditionally in the treatment haemorrhages (ARCBC database, 2004). In a recent study, it was observed that the water and alkaline extracts from the leaves of *C. decandra* had radical modulation activity in scavenging superoxide anions produced by hypoxanthine-xanthine oxidase (Sakagami *et al.*, 1998). It is used extensively as cottage poles, pillars, piles and fuel woods. Owing to its high tannin content (19.0 % in the stem barks), it is largely used by the fishermen for tanning their fishing nets. The non-medicinal uses of *H. fomes* are similar to those of *C. decandra*. However, to our knowledge, there are no traditional medicinal uses of this plant available to date. *Xylocarpus granatum* has been used traditionally to treat diarrhoea, cholera and fever, and as an astringent and emollient (ARCBC databases, 2004; Phytochemical and Ethnobotanical databases, 2004). The barks of this plant are used for tanning and for the preparation of dyes of umber colour. The aqueous extract of different parts of this plant was also reported to have significant antifilarial activity (Wan Omar *et al.*, 1997; Zaridah *et al.*, 2001). The traditional medicinal and non-medicinal uses of *X. mekongensis* are similar to those of *X. granatum*, *e.g.*, as an astringent and febrifuge, for the treatment of dysentery and diarrhoea, and in boat-building and furniture (Ghani, 1998). *Cuscuta reflexa* plays an important role in traditional medicine in Bangladesh, China, Thailand and other Asian countries. The ethnobotanical uses of this plant comprise its use for the treatment of stomach ache, cancer, bone fracture, conjunctivitis, eczema, night blindness, rickets and skin diseases, and as an anthelmintic, depurative, diaphoretic, purgative and tonic (Yusuf *et al.*, 1994; Phytochemical and Ethnobotanical databases, 2004). In Ayurvedic medicine, *C. reflexa* has been described to be useful in eye and heart diseases (Chopra *et al.*,

1958; Sing and Garg, 1973). An alcoholic extract of this plant demonstrated inotropic and cardiotonic properties on perfused frog heart, and smooth muscle relaxant effect on rabbit duodenum (Sing and Garg, 1973). Most recently, antifertility activity of a methanolic extract of the stems of *C. reflexa* was demonstrated in male mice (Pal *et al.*, 2003). The onset of puberty in mice was also observed with the administration of this extract (Gupta *et al.*, 2003). A crude water extract of this plant showed anti-HIV activity, and led to the isolation of a number of active phenolic compounds (Mahmood *et al.*, 1997). *Diospyros pregerina* has traditionally been used as an aphrodisiac, astringent, bactericide and tonic, and for the treatment of many ailments, *e.g.* diarrhoea, cholera, dysentery, fever, malaria, menorrhagia and sore throat (Singh *et al.*, 1988; Kirtikar and Basu, 1999; Phytochemical and Ethnobotanical databases, 2004). It has also been used to treat snake-bites (Kirtikar and Basu, 1933). The water extract of the 'gab' fruits is used as a dye for fishing nets and boats. Singh *et al.* (1988) reported the anti-stress activity of an EtOAc extract of the whole plant parts of *D. peregrina* which was similar to *Panax ginseng*. The alcoholic extract of stem barks of this plant has been reported to have hypoglycemic, diuretic and anti-cancer properties (Ghani, 1998).

Most of the previous phytochemical or pharmacological studies on these plants were carried out on non-polar or medium polarity extracts, only a few on polar extracts. A number of limonoids (Connolly *et al.*, 1976; Taylor, 1983; Khisal *et al.*, 1991; Kokpol *et al.*, 1996; Wu *et al.*, 2003, 2004) and sterols (Hogg and Gillian, 1984) were reported from *X. granatum* and *X. mekongensis*. The methanol (MeOH) extract of *C. reflexa* was reported to have phenolic compounds, mainly caffeic acid derivatives and other phenyl propanoids, and flavonol glycosides (Dandapani and Nagarajan, 1989; Lffler *et al.*, 1995; Ghani, 1998; Phytochemical and Ethnobotanical databases, 2004). An aliphatic ketol, onadecan-7-ol-2-one and triterpenes were isolated from the stem, fruits and seeds of *D. peregrina* (Misra *et al.*, 1971; Chauhan and Kumari, 1980; Jain and Yadav, 1994). The fruits and roots of this plant were also found to produce flavonoids (Chauhan *et al.*, 1982; Jain and Yadava, 1997). Phytochemical investigations of a dichloromethane (DCM)-MeOH extract of the roots of *C. decandra* revealed the presence of a number of diterpenes, *e.g.* ceriopsins A-G (Anjaneyulu and Rao, 2002, 2003; Anjaneyulu *et al.*, 2002). Pentacyclic triterpenoids and sterols were also found in the leaves of this plant (Ghosh *et al.*, 1985). Secoiridoid glucosides and phenolic glycosides (Kitagawa *et al.*, 1996) and quinoline alkaloids (Handa *et al.*, 1983; 1984) were isolated from the bark of *A. chinensis*.

As part of our continuing evaluation of plants from Bangladeshi flora for their phytochemistry and biological activities (Datta *et al.*, 2000a-c; 2001a, b; 2002a, b; 2004), we now report on the free radical scavenging activity of *Anthocephalus chinensis, Ceriops decandra, Cuscuta reflexa, Diospyros peregrina, Heritiera fomes, Xylocarpus granatum* and *Xylocarpus mekongensis* and comaprative studies on the contents of alkaloids, anthraqunones, flavonoids and tannins in these extracts.

MATERIALS AND METHODS

Plant materials

Plant parts of *Anthocephalus chinensis, Ceriops decandra, Cuscuta reflexa, Diospyros peregrina, Heritiera fomes, Xylocarpus granatum* and *Xylocarpus mekongensis* were collected from the tidal forest in the coastal Sundarbans (a swamp region in the Ganges delta) or other places in the district of Khulna (Table 1), and identified by Sarder Nasir Uddin, Scientific Officer, The Bangladesh

National Herbarium, Dhaka, Bangladesh. Voucher specimens (Table 1) representing these collections have been deposited in the Bangladesh National Herbarium, Dhaka, Bangladesh.

Extraction

Shade-dried and ground plant parts (100-250 g) were extracted by maceration over 24-72 h using MeOH, ethanol (EtOH) or water (Table 1) at room temperature. The extracts were filtered and dried using a rotary evaporator at a temperature not exceeding 55 ℃.

Preparation of the extract solutions for DPPH assay

The extracts (0.05 g) were dissolved in 5 mL MeOH to obtain stock solutions of 10 mg/mL concentration.

Antioxidant assay (DPPH assay)

2,2-Diphenyl-1-picrylhydrazyl (DPPH), molecular formula $C_{18}H_{12}N_5O_6$, was obtained from Fluka Chemie AG, Bucks. Quercetin was obtained from Avocado Research Chemicals Ltd, Shore road, Heysham, Lancs. The method used by Takao *et al.* (1994) was adopted with suitable modifications (Kumarasamy *et al.*, 2002; Sarker *et al.*, 2003). DPPH (4 mg) was dissolved in MeOH (50 mL) to obtain a concentration of 80 g/mL.

Qualitative

Test samples were applied on a TLC plate and sprayed with DPPH solution using an atomiser. It was allowed to develop for 30 min. The colour

Table 1. Collection details and antioxidant (free radical scavenging) activity of *Anthocephalus chinensis, Ceriops decandra, Cuscuta reflexa, Diospyros peregrina, Heritiera fomes, Xylocarpus granatum* and *Xylocarpus mekongensis*

Botanical names (Family)	Common Bengali names	Collection details				Extract used	RC_{50} value mg/mL**
		Plant parts (code)	Place	Date	Voucher number*		
Anthocephalus chinensis (Rubiaceae)	Kadam	Barks (MKL)	Shaikpara, Khulna, Bangladesh	October 2003	DACB30321	MeOH	1.23×10^{-2}
Ceriops decandra (Rhizophoraceae)	Goran	Pneumatophore (EGR)	The Sundarbans, Khulna, Bangladesh	August 2003	DACB30322	EtOH	9.5×10^{-3}
Cuscuta reflexa (Convolvulaceae)	Swarnalata	Aerial parts (MSB)	Khulna university Campus, Bamgladesh	November 2003	DACB12790	MeOH	1.64×10^{-1}
Diospyros peregrina (Ebenaceae)	Gab	Barks (MGB)	South Khalishpur, Khulna, Bangladesh	July 2003	DACB30323	MeOH	2.75×10^{-2}
Heritiera fomes (Sterculiaceae)	Sundori	Leaves (ESL)	The Sundarbans, Khulna, Bangladesh	August 2003	DACB30324	EtOH	2.5×10^{-2}
		Barks (ESB)				EtOH	8.1×10^{-3}
Xylocarpus granatum (Meliaceae)	Dhundul	Barks (MDB)	The Sundarbans, Khulna, Bangladesh	August 2003	DACB12789	MeOH	4.7×10^{-3}
Xylocarpus mekongensis (Meliaceae)	Possur	Barks (MPB)	The Sundarbans, Khulna, Bangladesh	August 2003	DACB30320	MeOH	6.4×10^{-3}
		Barks (HPB)				Water	8.8×10^{-3}
		Pneumatophore (MPR)				MeOH	8.4×10^{-3}

*Voucher specimens have been retained in the Bangladesh National Herbarium, Dhaka, Bangladesh

**Free radical scavenging activity obtained from DPPH assay (the RC_{50} value of the positive control quercetin was 2.88×10^{-5} mg/mL)

changes (purple on white) were noted.

Quantitative

Stock solutions (10 mg/mL) of the plant extracts were prepared in MeOH. Serial dilutions were carried out to obtain concentrations of 5×10^{-1}, 5×10^{-2}, 5×10^{-3}, 5×10^{-4}, 5×10^{-5}, 5×10^{-6} 5×10^{-7}, 5×10^{-8}, 5×10^{-9}, 5×10^{-10} mg/mL. Diluted solutions (1 mL each) were mixed with DPPH (1 mL) and allowed to stand for 30 min for any reaction to occur. The UV absorbance was recorded at 517 nm. The experiment was performed in duplicate and the average absorption was noted for each concentration. The same procedure was followed for the positive standard (quercetin).

Phytochemical tests

The following tests were carried out according to the methods described by Harborne (1998).

Test for alkaloids

Dragendorff's reagent was used to assess the presence of alkaloids in these extracts.

Test for anthraquinones

Anthraquinones were detected by 5 % methanolic KOH. A colour change from the original yellow and yellowish brown to red, violet, green or purple was the indicator of the presence of anthraquinones.

Test for tannins

Liquid extract (1 mg/mL) was mixed with the methylene blue solution (7.0×10^{-5} M) followed by the determination of residual methylene blue by its absorbance at 668 nm.

Test for flavonoids

Cyanidin test was used to determine the presence of flavonoids. Methanolic solutions of the plant extracts were used. In the presence HCl and metallic magnesium, flavonoids present in these extracts were reduced to anthocyanins which were determined by their absorbance at 510 nm.

RESULTS AND DISCUSSION

The DPPH antioxidant assay is based on the ability of 2,2-diphenyl-1-picryl-hydrazyl (DPPH), a stable free radical, to decolourise in the presence of antioxidants. The DPPH radical contains an odd electron which is responsible for the absorbance at 517 nm, and also for visible deep purple colour. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolourised which can be quantitatively measured from the changes in absorbance. In the TLC-based qualitative antioxidant assay using DPPH spray, all extracts, except the methanol extract of *Cuscuta reflexa,* showed free radical scavenging properties indicated by the presence of a yellow/white spot on a purple background on the TLC plates. In the quantitative assay, apart from the MeOH extract of *C. reflexa,* all other extracts displayed significant free radical scavenging activity in the DPPH assay (RC_{50} values within the range 2.75×10^{-2} to 4.7×10^{-3} mg/mL). Among these extracts, the MeOH extract of *Xylocarpus granatum* exhibited the most potent activity (4.7×10^{-3} mg/mL) and the methanol extract of *Cuscuta reflexa* had a low level of activity (1.64×10^{-1} mg/mL). The RC_{50} value for quercetin, a well known plant-derived antioxidant, was found to be 2.88×10^{-5} mg/mL (Fig. 1).

While none of these plant extracts exhibited positive results for alkaloids with Dragendorff's reagent (Table 2), presence of low to moderate amounts of phenolic compounds, e.g. anthraquinones, flavonoids and tannins was evident in all of these plants, except for the MeOH extracts of *C. reflexa* and barks of *D. peregrina,* which did not display any evidence for the presence of

Table 2. Contents of alkaloids, flavonoids, anthraquinones and tannins in the plant extracts, *Anthocephalus chinensis, Ceriops decandra, Cuscuta reflexa, Diospyros peregrina, Heritiera fomes, Xylocarpus granatum* and *Xylocarpus mekongensis*

Type of compounds	Plant extract codes									
	ESL	MDB	MKL	HPB	MGB	EGR	MSB	MPR	ESB	MPB
Alkaloids	-	-	-	-	-	-	-	-	-	-
Anthraquinones	++++	++++	+++	++	-	++	+	+++	+++	+++++
Flavonoids	+	+++	+	+++++	+++++	++	-	+++	++++	+++++
Tannins	++++	+++++	++++	+++++	+++	++++	+	++++	+++	++

- = Not detected; + = Very low level; ++ = Low level; +++ = Moderate level; ++++ = High level; +++++ = Very high level

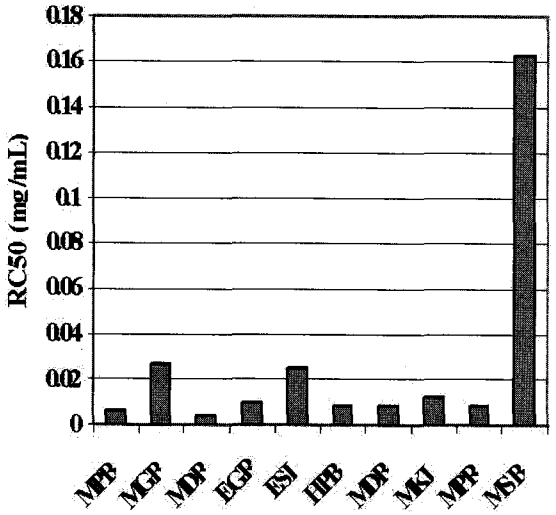

Fig. 1. Comparative antioxidant (free radical scavenging) activity of the extracts in DPPH assay
* Lower RC_{50} value = higher free radical scavenging activity
The RC_{50} value of the positive control quercetin was 2.88×10^{-5} mg/mL (not shown in the graph)

flavonoids and anthraquinones, respectively. The high content of tannins in *C. decandra, H. fomes, X. granatum* and X. *mekongensis* found in this study was in good agreement with a number of previous reports on the tannin contents in these species (Basak *et al.*, 1997; ISI database, 2004).

It has previously been outlined in a number of scientific publications that plant phenolic compounds constitute one of the major groups of compounds that can act as primary antioxidants or free radical terminator (ISI database, 2004; Miliauskas *et al.*, 2004). In this study the presence and levels of phenolic compounds in the extracts corresponded well with their potency of free radical scavenging activity. For example, the levels of phenolic compounds in the MeOH extracts of the barks of *X. granatum*, the pneumatophore and barks of *X. mekongensis*, the water extract of the barks of *X. mekongensis* and the EtOH extracts of the barks of *H. fomes* and *C. decandra* were extremely high (Table 2), and all these extracts showed high degree of free radical scavenging activities (RC_{50} 4.7×10^{-3} to 9.5×10^{-3} mg/mL).

CONCLUSION

From the results obtained in the present study, it can be concluded that the high amounts of phenolic compounds in most of the test extracts contributed to their potent free radical scavenging activity.

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