Antioxidant Behavior of Two Bangladeshi Medicinal Plants: *Kigelia pinnata* and *Mesua nagassarium*

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Abstract

Different extractives of leaves of *Kigelia pinnata* and *Mesua nagassarium* were screened for total phenolic constituents and free radical scavenging activity by using butylated hydroxytoluene (BHT) and ascorbic acid (ASA) as standard antioxidants. The total phenolic content was determined and expressed in gallic acid equivalent/ gm of extractives. Among all extractives of leaves of *K. pinnata*, the highest phenolic content was found in pet-ether soluble fraction (147.55 mg of GAE / gm of extractives), while the carbon tetrachloride soluble fraction (75.25 mg of GAE / gm of extractives) of leaves of *M. nagassarium* were found to contain the highest phenolic constituents. In the present studies, the methanol extracts and their pet-ether soluble partitionates of both plants demonstrated significant antioxidant potentials.

Keywords: *Kigelia pinnata*, *Mesua nagassarium*, Antioxidant, Free radical scavenging activity.

Introduction

Oxygen free radicals can cause damage to cells and tissues during infections and various degenerative disorders such as cardiovascular diseases, aging and neurodegenerative diseases, like Alzheimer's disease, mutations and cancer (Lee *et al.,* 2003; Tadhani *et al.,* 2007). The most widely used synthetic antioxidants, butylated hydroxyanisole (BHA) and butylated hydroxytoulene (BHT) have been restricted because of serious concerns about their carcinogenic potential (Tadhani *et al.,* 2007; Baydar *et al.,* 2007). Natural antioxidants, especially phenolics and flavonoids are safe; they protect the human body from free radicals and retard the progress of many chronic diseases as well as lipid oxidative rancidity in food (Kumaran and Karunakaran, 2006). Numerous studies have been carried out on plants with antioxidant properties (Baydar *et al.,* 2007; Prakash *et al.,* 2007). However, there is still great interest in finding new antioxidants from natural sources. Our present study was aimed to find out potential sources of antioxidants, especially from plant origin.

Kigelia pinnata (sausage tree), belonging to the family Bignoniaceae, has a wide geographical distribution in west and central Africa. The tree grows on riverbanks, wet areas along streams and on floodplains of Nigeria, Cameroon, Kenya, Guinea and Senegal (Owolabi and Omogbai, 2007) and also in the subcontinent. *K. pinnata* is widely used for antidiarrhoeal (Akah, 1996), antileprotic (Lal, 1983), antimalarial (Weenen *et al.,* 1990; Carvalho *et al.,* 1988) anti-inflammatory (Owolabi and Omogbai, 2007), anticancer (Msouthi and Mangombo, 1983), gynecological disorders (Grace *et al.,* 2002), anti-microbial activities (Kela *et al.,* 1989) and rheumatism (Gill, 1992).

On the other hand, *Mesua nagassarium* (Bengali - nagesar, nageswar, Family- Clusiaceae) is a medium-sized or fairly large evergreen flowering tree up to 36 m tall. It is the most widely studied plant under this genus and is a well-known tropical tree. It is used in folk medicine for the treatment of fever, dyspepsis and renal diseases (Kumar *et al.,* 2006). Different aerial parts of *M. nagassarium* are traditionally used in the preparation of

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cosmetics and unguents. The pounded kernels of the seeds are applied externally as a poultice on wounds and all forms of skin eruptions. *M. nagassarium* is also reported to have antiasthmatic activity (Singh *et al.*, 1993).

Materials and Methods

Plant materials: K. pinnata and *M. nagassarium* were collected from Rangpur and Dhaka, respectively in March 2010. Herbarium sheet for *K. pinnata* (DACB 34998) and *M. nagassarium* (DACB 35158) have been deposited in Bangladesh National Herbarium for future reference. Leaves of *K. pinnata* and *M. nagassarium* were sun dried for several days. The plant materials were then oven dried for 24 hours at considerably low temperature (40 °C) and then ground into coarse powder. The powdered materials (300 gm each) were then soaked in 2.0 and 2.5 liters of methanol separately and kept for 10 days with occasional shaking. The crude extracts were filtered through cotton plug followed by Whatman No. 1 filter paper separately and the extracts were concentrated by using a rotary evaporator. The concentrated methanolic extract was partitioned by modified Kupchan method (Van Wagenen *et al.*, 1993) and the resultant partitionates i.e., pet-ether (PESF), carbon tetrachloride (CTCSF), chloroform (CSF), and aqueous (AQSF) soluble fractions were used for the experimental processes.

Total phenolics analysis: Total phenolic content of *K. pinnata* and *M. nagassarium* extractives were measured by employing the method described by Skerget *et al.*, 2005 involving Folin-Ciocalteu reagent as an oxidizing agent and gallic acid as a standard. To 0.5 ml of extract solution (2 mg/ml) in water, 2.5 ml of Folin-Ciocalteu reagent (diluted 10 times with water) and 2.0 ml of sodium carbonate (7.5 % w/v) solution were added. After 20 minutes of incubation at room temperature the absorbance was measured at 760 nm using a UV-visible spectrophotometer. Total phenolics were quantified by calibration curve obtained from measuring the known concentrations of gallic acid (0-100 µg/ml) and were expressed as mg of GAE (gallic acid equivalent) / gm of the dried extract.

Free radical scavenging activity: The free radical scavenging activity (antioxidant capacity) of the plant extractives on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was estimated by the method established by Brand-Williams *et al.*, 1995. Here, 2.0 ml of a methanol solution of the sample (extractive/standard) at different concentration (500 µg/ml to 0.977 µg/ml) were mixed with 3.0 ml of a DPPH methanol solution (20 µg/ml). After 30 minutes of reaction at room temperature in dark place, the absorbance was measured at 517 nm against methanol as blank by UV visible spectrophotometer. Inhibition of free radical DPPH in percent (I %) was calculated as follows:

$$(I\ \%) = (1 - A_{sample}/A_{blank}) \times 100$$

where, A_{blank} is the absorbance of the control reaction (containing all reagents except the test material) and Asample is the absorbance of extractive. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted with percentage inhibition against extractive/standard concentration.

Statistical analysis: Three replicates of each sample were used for statistical analysis and the values are reported as mean ± SD.

Results and Discussion

The methanol extracts of leaves of *K. pinnata* and *M. nagassarium* as well as their pet-ether (PESF), carbon tetrachloride (CTCSF), chloroform (CSF) and aqueous (AQSF) soluble partitionates were subjected to total phenolic content determination. Among all extractives of leaves of *K. pinnata*, the highest phenolic content was found in PESF (147.55 mg of GAE/gm of extractives) followed by ME (142.66 mg of GAE/gm of extractives). Significant amount of phenolic compounds were also present in CTCSF (134.38 mg of GAE/gm of extractives), CSF (108.57 mg of GAE/gm of extractives) and AQSF (57.69 mg of GAE/gm of extractives) of leaf extracts. On the other hand, among all extractives of *M. nagassarium*, the highest phenolic content was found in CTCSF (75.25 mg of GAE/gm of extractives) followed by PESF (55.25 mg of GAE/gm of extractives), CSF (26.56 mg of GAE/gm of extractives), ME (20.43 mg of GAE/gm of extractives) and AQSF (5.44 mg of GAE / gm of extractives) (Table 1).

In free radical scavenging (DPPH) assay, the methanol extract (ME) of leaves of *K. pinnata* and its pet-ether soluble partitionate (PESF) revealed significant antioxidant property with the IC_{50} value of 21.68 µg/ml (Figure 1) and 10.21µg/ml (Figure 2), respectively, whereas the pet-ether soluble fraction (PESF) and

methanolic crude extract of *M. nagassarium* exhibited IC_{50} value 12.5 µg/ml (Figure 3), 10.5 µg/ml (Figure 4) respectively in comparison to the standard *tert*-butyl-1-hydroxytoluene (BHT, IC_{50} 27.5) and ascorbic acid (ASA, IC_{50} 5.8) (Table 2).

Table 1. Total phenolic content of leaves of *K. pinnata* and *M. nagassarium*

Test material	Total phenolic content (mg of GAE / gm ofextractive)	
	K. pinnata	*M. nagassarium*
ME	142.66±2.03	20.43±1.04
PESF	147.55±1.58	55.25±0.89
CTCSF	134.38±2.57	75.25±0.95
CSF	108.57±1.05	26.56±0.47
AQSF	57.69±1.32	5.44±0.41

ME = Methanol extract; PESF = pet-ether soluble fraction of crude methanol extract, CTCSF = carbon tetrachloride soluble fraction of crude methanol extract; CSF = chloroform soluble fraction of crude methanol extract and AQSF = aqueous soluble fraction of crude methanol extract

Table 2. Free radical scavenging activity of leaves of *K. pinnata* and *M. nagassarium*

Test material	Free radical scavenging activity (IC_{50} µg/ml)		
	K. pinnata	Standard	*M. nagassarium*
ME	10.21±0.56		10.5±0.42
PESF	21.68±0.51		12.5±0.29
BHT		27.5±0.54	
ASA		5.8±0.21	

From the above investigations it was evident that different extractives of both *K. pinnata* and *M. nagassarium* revealed significant antioxidant potentials. These bioactivities substantiate the folk use of these plants in inflammation, joint pain, cancer, rheumatism etc. Further studies are underway to isolate the bioactive compounds from these extractives.

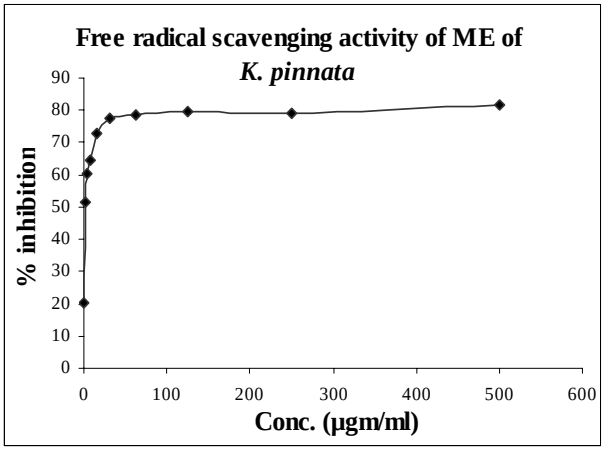

Figure 1: Free radical scavenging activity of ME of *K. pinnata*

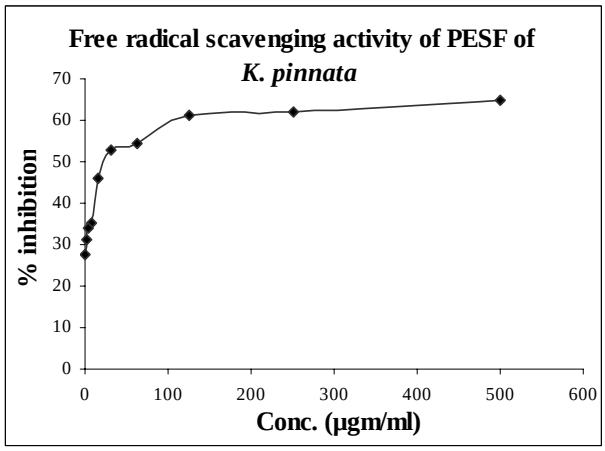

Figure 2: Free radical scavenging activity of PESF of *K. pinnata*

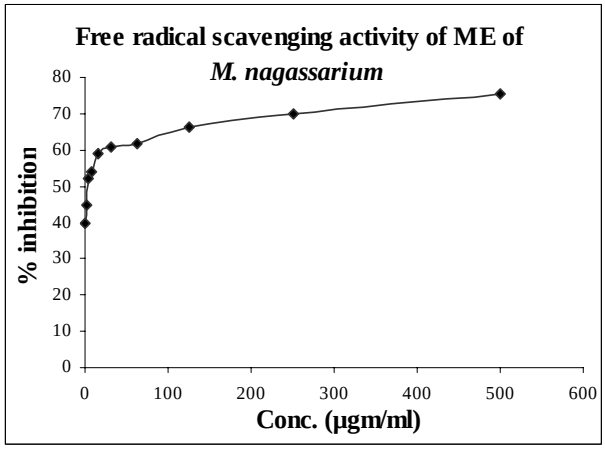

Figure 3: Free radical scavenging activity of ME of *M. nagassarium*

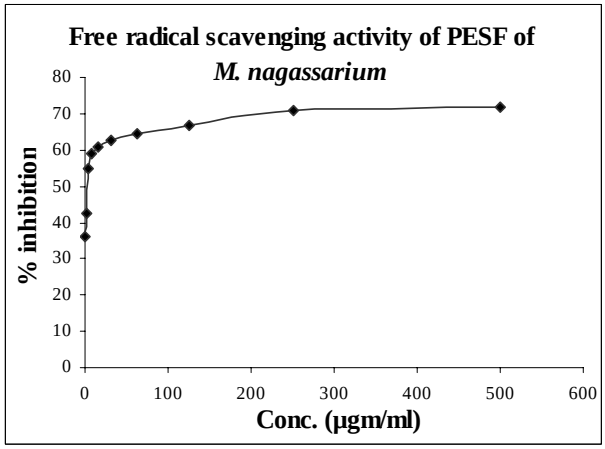

Figure 4: Free radical scavenging activity of PESF of *M. nagassarium*

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