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Phytochemical Assessment and Antioxidant Activities of Alcoholic and Aqueous Extracts of *Acacia mearnsii* De Wild

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Abstract: Phenolic compounds are well known for their antioxidant activities. The objectives of this study were to determine the phenolic content of the crude extracts of *Acacia mearnsii* De Wild and to evaluate the antioxidant properties of these extracts. The Folin-ciocalteu procedure was used to assess the total phenolic compositions of the extracts as gallic acid equivalents. Antioxidant activity was evaluated using 2,2-Azinobis-3-ethyl benzothiazoline-6-sulfonic acid (ABTS) diammonium salt and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging methods. All the extracts showed antioxidant potential. Ethanolic extract had the highest total flavonoids. Acetone extract had the highest total phenolic contents. The total proanthocyanidins was highest in the methanol extract while aqueous extracts had the least of these phytochemicals. The reducing power of the extracts of *A. mearnsii* was dose dependent. Aqueous extract showed the least reducing power, methanol extract indicated the highest reducing power. The reducing power of the extracts is lower than those obtained from the reference standard such as Butylated Hydroxytoluene (BHT), Rutin and ascorbic acid. 2,2-Azinobis-3-ethyl Benzothiazoline-6-Sulfonic acid (ABTS) diammonium salt showed that ethanol extract exhibited the highest activity at the highest concentration tested. 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay indicated that ethanol extract had the highest activity at the lowest concentration and the activities of all the extracts decreased with increase in their concentrations. This study revealed a positive linear correlation between the total phenolic content and antioxidant activity of the extracts of *A. mearnsii*.

Key words: Antioxidant activity, *Acacia mearnsii* extracts 2, 2-azinobis-3-ethyl benzothiazoline-6-sulfonic acid, radical scavenging, reducing power

INTRODUCTION

Acacia is one of the important genera of the family Fabaceae. It is a cosmopolitan genus containing more than 1350 species (Seigler, 2003). In Australia, there are approximately 960 species, which makes *Acacia* the largest genus of vascular plants in that region. The *Acacia species* are of immense value for reforestation and reclamation of wastelands, for fuel wood, timber and shelter (Palmberg and Pasca, 1981). They are also used for conservation and improvement of soil fertility through nitrogen fixation. These species can provide the nutrients and therapeutic ingredients to prevent, alleviate or treat many diseases in humans. They contain a variety of bioactive components such as flavonoids, alkaloids, tannins and phenolic acids. The most prominent substances in many *Acacia* species, however, are complex phenolic compounds (condensed tannins) and polysaccharides or gums (Maslin and Stirton, 1997). These compounds are responsible for numerous

biological and pharmacological properties of acacia due to their strong antioxidant and free radical scavenging activities (Chopra *et al.*, 1999).

Antioxidants are vital substances with the ability to protect the body from damages caused by free radical-induced oxidative stress. While free radicals have been reported to cause cellular damages that result in chronic diseases, many studies have indicated that phenolic compounds play a crucial role in oxidative scavenging (Mateos *et al.*, 2005). The antioxidative effect is mainly due to phenolic components such as phenolic acids, phenolic diterpenes, anthocyanins, caumarins and flavonoids (Cai *et al.*, 2004; Chye and Sim, 2009). These phenolic compounds with antioxidant activity are believed to account mainly for the antioxidant capacity of many plants (Wu *et al.*, 2004).

Acacia mearnsii de Wild (Fabaceae) is a fast-growing leguminous tree. It was introduced to South Africa about 150 years ago primarily for the tanning industry. The bark of *A. mearnsii* is known to contain about 20-40% tannins

and 70% proanthocyanidins (Young *et al.*, 1986). Although, the *A.* species is widespread, relatively little is known about its chemistry and antioxidant potentials. This may be due to the difficulty associated with the identification of *Acacia* species and the insufficient clarity about their taxonomic relationships (Seigler, 2003).

Prior to this study, there was a dearth of information on the phytochemical and antioxidant activity of *A. mearnsii* in the literatures. Hence, this study was designed to investigate the phytochemical composition and antioxidant potential of this plant.

MATERIALS AND METHODS

Collection of plant material: The bark materials of *A. mearnsii* were collected from the plant growing within the University of Fort Hare campus in Alice, South Africa in September, 2010 while the study was carried out immediately after the plant material was dried. The plant was authenticated in the Department of Botany and a voucher specimen was prepared and deposited in the Griffen Herbarium of the University.

The bark samples were air-dried at room temperature and pulverized using a milling machine. Portions of about 100 g each of the pulverized samples were extracted separately with acetone, methanol, ethanol and water for 48 h. The extracts were filtered through Whatman No. 1 filter paper and evaporated to dryness under reduced pressure at 40°C using a rotary evaporator. The extracts were redissolved in their respective solvents to the required concentrations for the bioassay analysis.

Chemicals and reagents used: 2,2-azinobis-3-ethyl benzothiazoline-6-sulfonic acid (ABTS) diammonium salt, 1,1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), gallic acid, rutin, ascorbic acid (VC), quercetin and FeCl₃, were purchased from Sigma Chemical Co. (St. Louis, MO, USA) vanillin was from BDH Chemicals Ltd. (Poole, England) and Folin-Ciocalteu phenol reagent and sodium carbonate were from Merck Chemical Supplies (Darmstadt, Germany). All other chemicals used, including the solvents, were of analytical grade.

Determination of total flavonoids: Total flavonoids were estimated using the method of Ordonez *et al.* (2006).

Determination of ferric reducing power: The ferric reducing potential of the extract was determined according to the method of Kumar *et al.* (2005).

Determination of total phenol: The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method (Wolfe *et al.*, 2003).

Determination of total proanthocyanidins: The total proanthocyanidins were determined by using the procedure reported by Sun *et al.* (1998).

DPPH radical scavenging assay: For DPPH assay, the method of Liyana-Pathirana and Shahidi (2005) was adopted.

ABTS radical scavenging assay: The total antioxidant activity of the samples was measured by ABTS radical cation decolorization assay according to the method of Siddhuraju and Manian (2007).

Statistical analysis: The experimental results were expressed as the Mean±Standard Deviation. All assays were performed in triplicates. The data were subjected to one way analysis of variance using SPSS version 14.0. Differences between means at the 5% level were considered significant.

RESULTS AND DISCUSSION

Phytochemical compositions: The result showed that *A. mearnsii* has a considerable amount of total phenolic and flavonoids contents while the proanthocyanidins were very low in all the extracts. The quantities of the phenolic contents, total flavonoids and total proanthocyanidins were in descending order depending on the extract as shown in Fig. 1. The results indicated that acetone extract had the highest total phenolic content (47.88 mg g⁻¹) while ethanol extracts had the highest total flavonoid contents (7.98 mg g⁻¹). While total

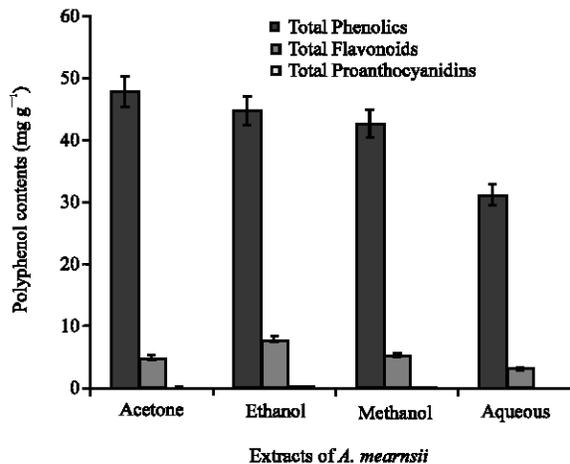


Fig. 1: Polyphenolic contents (mg g⁻¹) of *A. mearnsii*. Data are presented as Means±Standard Deviation of three replicate with significant increases from all samples tested

proanthocyanidin content (0.51 mg g^{-1}) was the highest in methanol extract, the polyphenols contents of this plant were least obtained in the aqueous extract. This result is similar to earlier report of Zongo *et al.* (2010) indicating that alcoholic extract exhibited higher level of total polyphenol contents than water extracts. Quantitatively, in *A. mearnsii*, total phenolic contents were more than the flavonoids while the proanthocyanidins were the least. Many related polyphenols, commonly found in plants, have been reported to have several different biological activities, including antioxidant property (Luo *et al.*, 2002; Afolayan *et al.*, 2008; Krishna *et al.*, 2010). According to Jayaprakasha and Patil (2007) and Hussein *et al.* (2010), there is a relationship between total phenolic content and antioxidant activity of plants. This is believed to be mainly due to redox properties of the phenolic compounds (Zheng and Wang, 2001) adsorbing and neutralizing free radicals, quenching active oxygen species as well as decomposing superoxide hydroxyl radicals. These phenolic compounds act as free radical terminators (Galvez *et al.*, 2003) while flavonoids show antioxidant activity through scavenging or chelating process (Torane *et al.*, 2011). El-Hela and Abdullah (2010) and Saikia and Adhyaya (2011) also noted a significant relationship between the free radical scavenging potency, the total phenolic and flavonoids contents of plant extracts. In this study, the extract having highest amount of flavonoids and phenolic compounds exhibited the highest antioxidant activity.

Total antioxidant power of extracts from *A. mearnsii* by the FRAP assay:

The ferric reducing power of the different extracts of *A. mearnsii* was presented in Fig. 2. In both extracts and the standards studied, the reducing power or reductive capability of each of the extracts and the standards increased with increasing concentration. There are significant differences between the reductive capabilities of the extracts and those obtained for the standards such as Butylated Hydroxytoluene (BHT), Rutin and ascorbic acid. The reductive capabilities recorded was in the following order, Vitamin C>Rutin>BHT>Methanol>Acetone>Ethanol>Aqueous which showed that vitamin C exhibiting the highest reductive capability. At the highest concentration of 0.1 mg mL^{-1} , the reductive capability of each of the extract was 0.402 (aqueous), 0.421 (ethanol), 0.453 (acetone), 0.473 (methanol extracts), 0.633 (BHT), 0.706 (Rutin) and 1.218 (vitamin C) based on their spectrophotometric absorbance at 700 nm.

According to Chang *et al.* (2002) the observed reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant property of all the plant extracts. Furthermore, there is a

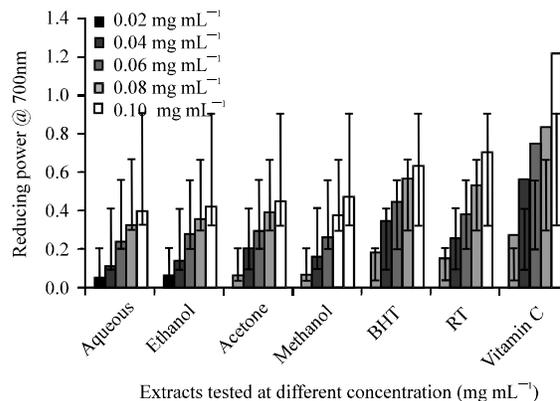


Fig. 2: Ferric reducing power determinations for the alcoholic and aqueous extracts of *A. mearnsii*. Data are presented as Means±Standard Deviation of three replicate with significant increases from all samples tested

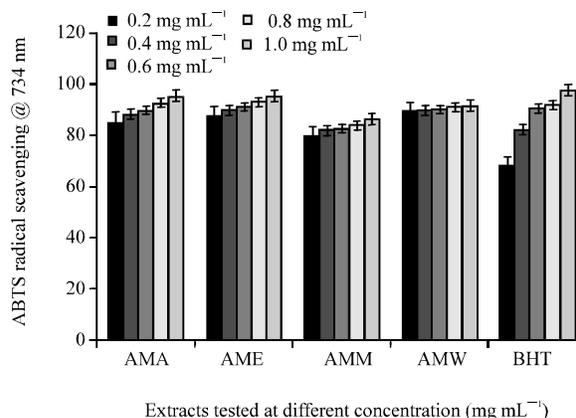


Fig. 3: ABTS radical scavenging activity of the alcoholic and aqueous extracts of *A. mearnsii*. Data are presented as Means±Standard Deviation of three replicate with significant increases from all samples tested

linear relationship between concentrations and reducing power of the different extracts. This relationship is concentration dependent and may be associated with the presence of reductones (Duh, 1998) known to exert antioxidant activity by breaking the free radical chain via donating a hydrogen atom. These findings suggest that the *A. mearnsii* extracts are capable of donating electrons, and could, therefore, react with free radicals or terminate chain reactions.

ABTS radical scavenging activity of the extract from *A. mearnsii*:

The results of the free radical scavenging activity of the different extracts of *A. mearnsii* determined

by ABTS assay are shown in Fig. 3. Almost all the extracts had strong antioxidant abilities that exceeded that of BHT at varying concentrations. There was a steady increase in the ABTS radical scavenging capacity of all the extracts of *A. mearnsii* employed in present study. At the highest concentration of the extracts, the highest percentage inhibitions were recorded for the extracts and the standards.

The ABTS assay is based on the inhibition of the absorbance of the radical cation, ABTS, which has a characteristic long wavelength absorption spectrum (Sanchez-Moreno, 2002). While phenolic compounds scavenge radicals by forming a stable ABTS-H, the explicit method to measure the antioxidant activity of phenolic compounds is the decolorization of ABTS+radical. Decolorization of ABTS⁺, in present study, reflects the capacity of an antioxidant species to donate electrons or hydrogen atoms to inactivate this radical cation in a concentration dependent manner. The scavenging activity of these extracts towards ABTS radicals is similar to those earlier reported by Miller and Rice-Evans (1997). These results showed that polyphenols from bark of *A. mearnsii* had high antioxidant activities and that the activity of ABTS⁺ radical by the extracts was significant.

DPPH radical scavenging activity of extracts from *A. mearnsii*: In this study, the extracts of *A. mearnsii* exhibited varying degree of radical scavenging activity against the DPPH. At the lowest concentration of the extracts, high level of activity was observed in acetone, ethanol, methanol and aqueous extract. The activity of BHT was observed to be greater than activities of all the extracts at the lowest concentrations. This result showed that ethanol extract of the plant had the highest activity at the lowest concentration (Fig. 4). Their scavenging activities decreased with increase in the concentration of all the extracts as characterized by a rapid decline in the absorbance at 517 nm.

The ability of the extracts of *A. mearnsii* to act as a free radical scavenger or hydrogen donor was revealed by DPPH. This method is a rapid and sensitive way to survey the antioxidant activity of specific compounds or plant extracts. In this method, the antioxidant scavenges the DPPH radicals to form stable reduced DPPH molecules. The radicals formed are stabilized through the formation of non-radical products (Argolo *et al.*, 2004). When the stable reduced DPPH molecules are formed in the presence of a free radical scavenger, the absorbance reduces and the DPPH solution is decolourised as the colour changes from deep violet to light yellow. The degree of reduction in absorbance value is indicative of

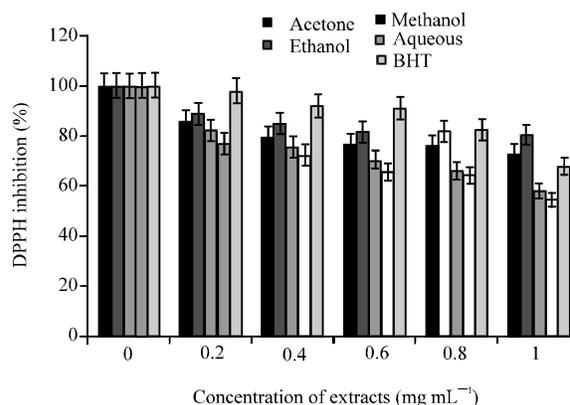


Fig. 4: DPPH radical scavenging activity of the alcoholic and aqueous extracts of *A. mearnsii*. Data are presented as Means±Standard Deviation of three replicate

the antioxidant power of the extract. More yellowish colour of DPPH shows more antioxidant activity of the compounds or extracts tested (Moein *et al.*, 2008). The observed decreases in free-radical scavenging activities with increase in concentrations of extracts are statistically significant from one extract to another. While Yu *et al.* (2002) reported that higher concentrations of extracts are more effective in quenching free radicals in the systems, Goupy *et al.* (2003) observed that the rapid reaction between antioxidants and DPPH occurs with the transfer of the most labile hydrogen atoms to the radical, while the subsequent slow steps depends on the residual H-donating capacity of antioxidant degradation products.

CONCLUSIONS

In conclusion, it is well known that free radicals are one of the causes of several diseases. The present study demonstrated that *Acacia mearnsii* had significant antioxidant and radical scavenging activities. Irrespective of the method used for the analysis, all the extracts showed antioxidant activity and free radical scavenging capability. While their activities are less than those of the commercially available synthetic antioxidants, phenolic content of this plant could be a good source of natural antioxidant substances which could help to neutralize free radicals and play a beneficial role in oxidative stress prevention.

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