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Original Article

Phytochemical Constituents and Antioxidant Activities of Crude Extracts from *Acacia Senegal* Leaf Extracts

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ABSTRACT

Background: *Acacia senegal (Fabaceae)* Wild is a leguminous tree with economic values, but its leaves are under-utilised. **Objective:** To investigate the phytochemical constituents and antioxidant potential of crude extracts from *A. Senegal's* leaves. **Methods**: Methanol and acetone crude extracts of leaves of *A. senegal* were prepared by maceration using organic solvents, methanol and acetone respectively. Qualitative and quantitative phytochemical analysis of the crude extracts were evaluated using Association of Agricultural and Chemist (AOAC) protocols. Antioxidant activities of the crude extracts were determined using 2, 2′-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) respectively. **Results**: The crude extracts (acetone and methanol) showed vary quality of phytochemical constituent including flavonoid, alkaloids, carbohydrate, saponins, tannin, steroids, and terpenoids. Acetone crude possessed significant ($P < 0.05$) higher total flavonoid and proanthocyanidin content in comparison with methanol extracts. Whereas, methanol crude extract possessed significant higher total phenol content compared with acetone crude extract. The crude extracts showed antioxidant activities as evidence in scavenging ABTS and DPPH radicals. However, acetone crude with lower IC_{50} of 0.09 mg/mL possessed significant higher ABTS scavenging ability compared to methanol (0.07 mg/mL) and ascorbic acid (0.07 mg/mL). **Conclusion**: The crude extracts could serve as a promising natural antioxidant agent in management of oxidative stress diseases. For further studies, bioactive compounds need to be ascertained.

Key words: ABTS, *Acacia Senegal*, Antioxidants, Crude Extract, DPPH, Free Radicals.

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INTRODUCTION

Medicinal plants have been the basis of traditional medicines from ancient times, and continue to provide new remedies for a large spectrum of diseases.[1] Medicinal Plants consist of range of compounds known as secondary metabolites, which help to protect plants from predators.[2] Secondary metabolites including polyphenols, flavonoids, steroids, saponins, tannins, terpenoids, alkaloids, anthraquinones, glycosides, and other endogenous metabolites are beneficial to mankind.[3] Biological activities such as analgesic, antioxidant, anticancer, antibacterial, anti-inflammatory, antiviral, and antitumor of most medicinal plants have been attributed to their secondary metabolite constituents.[2]

Oxidative stress is an imbalance between reactive oxygen species (ROS) and antioxidant agents.[4] Oxidative stress is implicated in most life-threatening diseases including cancer, diabetes, heart attack, stroke and neurodegeneration.[4,5] The use of synthetic antioxidant agents such as butylated hydroxytoluene (BHT), and butylated hydroxyanisole (BHA) in the management of oxidative stress diseases are associated with side effects such as toxicity and carcinogenic.[6] Hence, the search for alternative remedy became paramount. Medicinal plants have wider range of biological activities such as antioxidant, anti-diabetic, anti-inflammatory, anti-helminths and anti-cough coupled their no or little side effects.[1]

Acacia senegal is a leguminous tree which belongs to the family of Fabaceae. It is endemic in sub-Saharan Africa, and also found in other Africa countries, such as Sudan, Kenya, Nigeria, Chad, Ethiopia, Tanzania, Cameroun, South Africa, Zimbabwe and Senegal.[7] The tree produces gum arabic, a substance widely used as an adhesive, microencapsulating agent and an emulsifier. It is also used in confectionaries, pharmaceuticals, cosmetics, lithography, and in textile industries.[8,9] Gum arabic, when used as a food supplement, has been shown to reduced chronic renal failure and improve renal function by increasing the release of faecal nitrogen and the production of urea in the body.[10,11] Additionally, gum arabic possessed antioxidant and antimicrobial properties.[9] However, the literature on Acacia *senegal's* leaves for medicinal purpose is still paucity. Therefore, this study investigated the phytochemical constituents and antioxidant potential of crude extracts of *A. senegal* leaves.

MATERIALS AND METHODS

Chemicals

The chemicals used in this study were of analytical grade and were purchased from Sigma-Aldrich, USA. These include; ascorbic acid, aluminium chloride, catechin, 2,2′-azino-bis-(3-ethylbenzothiazoline-

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6-sulphonic acid (ABTS), 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4,4′-disulfonic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, ferrous chloride, Folin-Ciocalteu reagent, potassium persulfate, quercetin, sodium carbonate, tannic acid, and sodium acetate.

Plant materials

Fresh leaves of *Acacia senegal* were collected from Rubber Research Institute of Nigeria (RRIN), Edo State, Nigeria (6.339185° S, 5.617447° E). The plant was authenticated by Chief Botanist at Department of Botany, University of Benin, Nigeria. The specimen with voucher specimen number (UBH_A379) was deposited at the University of Benin's s herbarium.

Preparation of crude extracts

The fresh leaves of *A. senegal* were thoroughly washed and air-dried at room temperature. Dried leaves were pulverized using Perten Laboratory Mill 3303, The pulverized sample (25 g) was then macerated using 200 mL of acetone and methanol respectively for 24 hours. The solution was filtered using Whatman filter paper 1, and the filtrate concentrated using a rotary evaporator (40 °C, 60 rpm). The crude extracts were stored at room temperature (25 °C) until required for use.

Phytochemical analysis

Phytochemical screening including flavonoids, alkaloids, carbohydrates, saponins, tannins, steroids, terpenes, anthraquinones and cardiac glycosides of each crude extract was determined using the standard protocol as described by Harborne.[12] and Sofowara.[13]

Total phenolic content

Total phenolic content of methanol and acetone crude extracts was determined using the method described by Wolfe and co-workers.[14] with slight modifications. The prepared concentrations (0.1mg/ml, 0.2 mg/mL, 0.4 mg/L, 0.6 mg/L, 0.8 mg/L and 1.0 mg/mL) of crude extracts along with the standards were mixed with Folin-Ciocalteau reagent (5 mL; 10 %) and sodium carbonate (4 mL; 7.5 %). The mixture was vortexed for 15 seconds and incubated for 30 minutes to allow for colour changes. The absorbance of the mixture was read at 765 nm using a Synergy HT microplate reader. The total phenolic content was expressed in mg/mL of tannic acid equivalent.

Total proanthocyanidin content

The total proanthocyanidin content of methanol and acetone crude extracts was determined using the method described by Sun and co-workers.[15] Crude extracts (0.5 mL) with concentration ranges of 0.2 mg/mL to 1.0 mg/mL were mixed with Vallin-methanol solution (3.0 mL; 4 %) and concentrated hydrochloric acid (1 mL) The resultant mixture was then incubated at room temperature for 15 minutes, and absorbance read at 500 nm. The total proanthocyanidin contents were expressed in mg/mL of catechin equivalent.

Total flavonoid content

The total flavonoid content of methanol and acetone crude extracts was determined using a modified method described by Ordonez and co-workers.[16] Aluminium chloride solution in ethanol (5 ml; 2 %) was mixed with various concentrations ranging from 0.2 mg/mL to 1 mg/mL of the crude extracts. The mixtures were incubated for 1 hour to develop a colour change to yellow. Quercetin was used as a positive control. The absorbance was read at 420 nm using a Synergy HT microplate reader. Total flavonoid content was calculated as quercetin equivalent in mg/mL.

Antioxidant studies

2,2-Diphenyl-1-picrylhydrazyl (DPPH)

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging potential of the crude extracts was determined using method described by Brand-Williams and colleagues.[17] The solution of DPPH (0.5 ml; 0.3 mM) was added to various of crude extracts (2 mL) with concentrations ranging from 0.1 to 1.0 mg/mL. The mixtures were vortexed for 15 minutes and incubated for 1 hour in a dark room at room temperature. The absorbance was read at 517 nm using a Synergy HT microplate reader. Ascorbic acid was used as positive control. The following formula was used to calculate DPPH radical scavenging activity:

$$\% \text{ DPPH radical scavenging} = [(A_0 - A_1)/ (A_0)] \times 100.$$

Where $A_{0,}$ is the absorbance of negative control and A_1 is the absorbance for the treatment.

2′-Azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay

2′-Azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay was used determined antioxidant potential of acetone and methanol crude extract, following the method described by Re and co-workers.[18] ABTS stock solution (7 mM ABTS and 2.4 mM potassium persulphate) was prepared, and incubated in a dark room for 16 hours. A portion (1mL) of the stock solution was then pipetted into a test tube containing methanol (60 mL) and mixed thoroughly. Afterwards, 1 mL of the solution was mixed with different concentrations (0.1 to 1.0 mg/mL) of the crude extracts (1 mL). The mixtures were incubated for 7 minutes, and absorbance was read at 734 nm using a Synergy HT microplate reader. Ascorbic acid served as positive control. The percentage inhibition was calculated as follows;

$$\% \text{ ABTS radical scavenging activity} = [(\text{Abs control} - \text{Ab sample})/ (\text{Abs control})] \times 100$$

Where $A_{0,}$ is the absorbance of negative control and A_1 is the absorbance for the treatment.

Data analysis

The experiments were performed in triplicate and data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) and post-hoc Tukey's test was carried on the data using Graph Pad Prism version 5.03. The significant statistical value was considered at $P < 0.05$.

RESULTS

Phytochemical constituents of A. senegal leaf extracts

The results revealed that methanol and acetone crude extract from *A. senegal* leaves consist of vary quality of flavonoid, alkaloids, saponins, tannin, steroids, and terpenoids, anthraquinones and cardiac glycoside as their phytochemical constituents (Table 1). However, steroid and tannin were more present in methanol extract than acetone extract. Whereas, in acetone extract, the flavonoid is more present when compared to methanol crude extract (Table 1).

Total flavonoid, phenol and proanthocyanidin content in acetone and methanol crude extracts

Acetone crude extract showed higher significant ($p < 0.05$) total flavonoid (1.602 ± 0.922 mg/ml) and total proanthocyandin (0.089 ± 0.035 mg/ml) content in comparison with methanol crude extract (Table 2). Interestingly, methanol crude extract (0.942 ± 0.413 mg/ml) showed better significant total phenol content than acetone crude extract (0.779 ± 0.313 mg/ml) (Table 2).

DPPH radical scavenging activity against acetone and methanol crude extracts

Acetone crude extracts significantly ($p < 0.05$) scavenged DPPH radical in dose-dependent manner. Whereas, methanol crude scavenged DPPH radical in irregular pattern. The highest scavenging inhibitory activity for the crude extracts (acetone and methanol) was observed at 1 mg/ml (Figure 1). Acetone crude extract showed significant better scavenging activity at concentration of 2 mg/ml and 4 mg/mL than methanol crude extract (Figure 1). Likewise, acetone crude extract with lower IC_{50} values of 1.22 mg/mL showed significant better scavenging ability than methanol crude extract (IC_{50} values of 1.44 mg/mL) (Table 3).

ABTS radical scavenging activity against acetone and methanol crude extracts

The crude extracts scavenged ABTS radical in irregular patterns. The highest scavenging activity was observed at 1 mg/ml for both extracts (Figure 2). However, methanol crude extract showed significant better scavenging potential than acetone as evident with lower IC_{50} of 0.07 mg/mL (Table 3). In addition, methanol crude also showed similar IC_{50} value in comparison with ascorbic acid (IC_{50} of 0.07 mg/mL), the positive control.

DISCUSSION

Medicinal plants are used in the treatment of a wide range of diseases among developing countries.[19] Medicinal potentials of these plants depend solely on their phytochemical constituents including; steroids, saponins, flavonoids, phenols, tannins, alkaloids terpenoids and anthraquinones. Phytochemicals composition of plant varies among different parts of the plant such as the leaves, fruits, bark and flowers.[20]

Table 1: Phytochemical *constituents* of Acetone and Methanol crude extracts of A. *senegal* leaves.

Phytochemicals	Methanol	Acetone
Flavonoids	+	++
Alkaloids	++	++
Carbohydrates	-	-
Saponins	-	-
Tannins	++	+
Steriods	+++	+
Terpenoids	++	++
Anthraquinones	+	+
Cardiac glycosides	++	++

Sign notation: - Absent; + slightly present; ++ Present, +++ highly present

Table 2: Total flavonoid, phenol and proanthocyanidin content of acetone and methanol crude extract from *A. senegal* leave. Data expressed as mean ± SD. Values with different alphabets (a, b) indicate significant differences ($p < 0.05$).

Extract	Total flavonoid (quercetin mg/mL)	Total Phenol (Tannic acid mg/mL)	Total Proanthocyanidin (catechin mg/mL)
Acetone	1.6026 ± 0.922^{b}	0.779 ± 0.313^{a}	0.089 ± 0.035^{b}
Methanol	0.6876 ± 0.773^{a}	0.842 ± 0.413^{a}	0.056 ± 0.012^{a}

Table 3: The IC_{50} values of acetone and methanol crude extracts against ABTS and DPPH radicals. Values with different alphabets (a, b, c) indicate significant differences ($p < 0.05$).

Extracts	ABTS (mg/mL)	DPPH (mg/mL)
Acetone	0.09^{a}	1.22^{a}
Methanol	0.07^{b}	1.44^{b}
Ascorbic	0.07^{b}	0.06^{c}

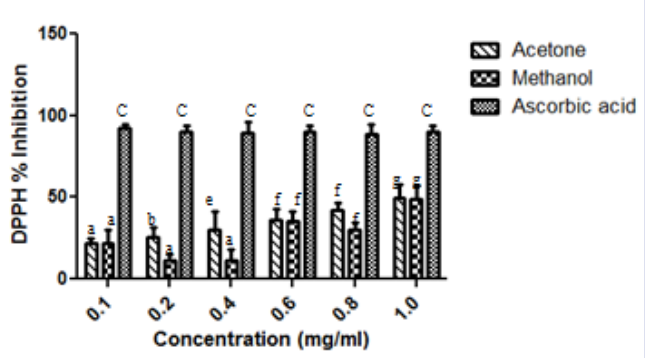

Figure 1: DPPH radical scavenging activities of acetone and methanol extracts of *A. senegal* leaves. Data expressed as mean ± SD. Values with different alphabets (a, b, c, e, f) indicate significant differences ($p < 0.05$).

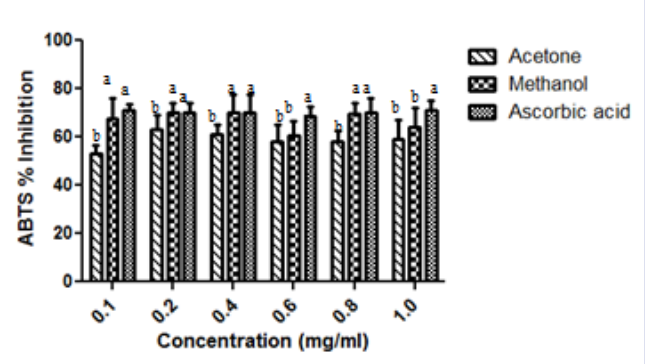

Figure 2: ABTS radical activities of acetone and methanol extracts of *A. senegal* leaves. Data expressed as mean ± SD. Values with different alphabets (a, b) indicate significant differences ($p < 0.05$).

This present study revealed vary quality of phytochemicals composition including; flavonoid, alkaloids, saponins, tannin, steroids, terpenoids, anthraquinones and cardiac glycoside between acetone and methanol extract. The disparity in phytochemicals' quality could be linked to differences in polarity of organic solvents used for the extraction. This finding supported the report of Molly *et al.*[20] on variation of phytochemicals constituent of crude extracts using different organic solvents. Likewise, this finding also explained the disparity in quantitative phytochemical composition of total flavonoid, phenol and proanthocyanidin between acetone and methanol crude extract. The phytochemicals identified in this study possessed antioxidant activity, thus the crude extracts might be a promising antioxidant agent. Phenols, flavonoid and anthraquinones were reported to possess tremendous antioxidant, anti-inflammatory, anticancer and antimicrobial activities.[21,22] Antioxidant potential of alkaloid, tannin, terpenoids and steroids have also been reported.[23]

Free radicals are generated in the body during metabolism. This enhanced physiological signalling which attenuates microbial activities during infections. However, uncontrolled free radicals due to lack antioxidant regiments could lead to oxidative stress.[24] Medicinal plants have been demonstrated to possessed antioxidant potential by interruption-free radical chain reaction.[25] This study reported the scavenging potential of acetone and methanol crude extracts against DPPH and ABTS radicals. Previously, DPPH and ABTS were used as reliable test for antioxidant studies.[26]

DPPH radical reacts with scavenging agents to give yellowish colouration by losing electron.[26] In this present study, the crude extracts from *Acacia senegal* showed antioxidant activities by scavenging DPPH

radical. Previously, crude extracts from some medicinal plant displayed antioxidant ability.[20] The higher antioxidant activity of acetone crude extract in comparison with methanol crude extract could be associated with the cumulative effects of high level of total flavonoid and anthraquinones. Flavonoid possessed high oxygen affinity, thus easily donate electrons to quench free radicals.[26]

ABTS radical is commonly used to investigate the antioxidant potential of compounds. ABTS loss is blue-green to become colourless when reacted with antioxidant agents.[27] The antioxidant ability of crude extracts in this study was further confirmed by the scavenging potential against ABTS radical. The higher scavenging activity of methanol crude compared to acetone crude extract in the study could be linked to high level of phenol compound as well as the quality of steroid and tannin. This finding corroborated with previous of Oyedemi, *et al.*,[28] reports on crude extract from *Strychnos henningsii* scavenging ABTS radicals. High phenolic content was reported to show positive correction with scavenging ability.[29] Phenolic compound easily loss electron due to its hydroxyl moiety, thus reduce free radical activity.[26] Minimum inhibition concentration at 50 % (IC_{50}) is defined as the minimum concentration of compound or extract to inhibit particular activity. Lower values of IC_{50} of compound denotes better activity, and *vice-versa*.[30] The similarity in the IC_{50} of methanol crude extract when compared with ascorbic acid, the positive control indicated that methanol crude extract possessed high antioxidant potential. Interestingly, the finding from this study also negated popular opinion that crude extracts with ABTS radical activity may not inhibit DPPH scavenging activity because of the method of preparation and solubility.[1]

CONCLUSIONS

The crude extracts possessed vary quality of phytochemical composition which could be associated with various organic solvents used for extraction. Likewise, the crude extract showed different quantity of phytochemicals. Acetone possessed higher level of total flavonoids and anthraquinones compared with methanol extract. Whereas, methanol crude extract showed higher level of phenol compared with acetone crude extract. The crude extracts possessed antioxidant ability, as evident in scavenging DPPH and ABTS radical with different threshold. Acetone extract scavenged DPPH radical higher than methanol, whereas methanol crude extracts scavenged ABTS better than acetone. Therefore, the crude extracts could be a lead therapy for natural antioxidant agent in the treatment of oxidative stress diseases. Bioactive compounds from the crude extract need to be isolated for future studies.

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CONFLICTS OF INTEREST

The authors declared no conflict of interest.

ABBREVIATIONS

ABTS:2′-Azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid); DPPH: 2,2-Diphenyl-1-picrylhydrazyl; BHT: butylatedhydroxytoluene; RRIN: Rubber Research Institute of Nigeria; C_{50}: Inhibitory Concentration.

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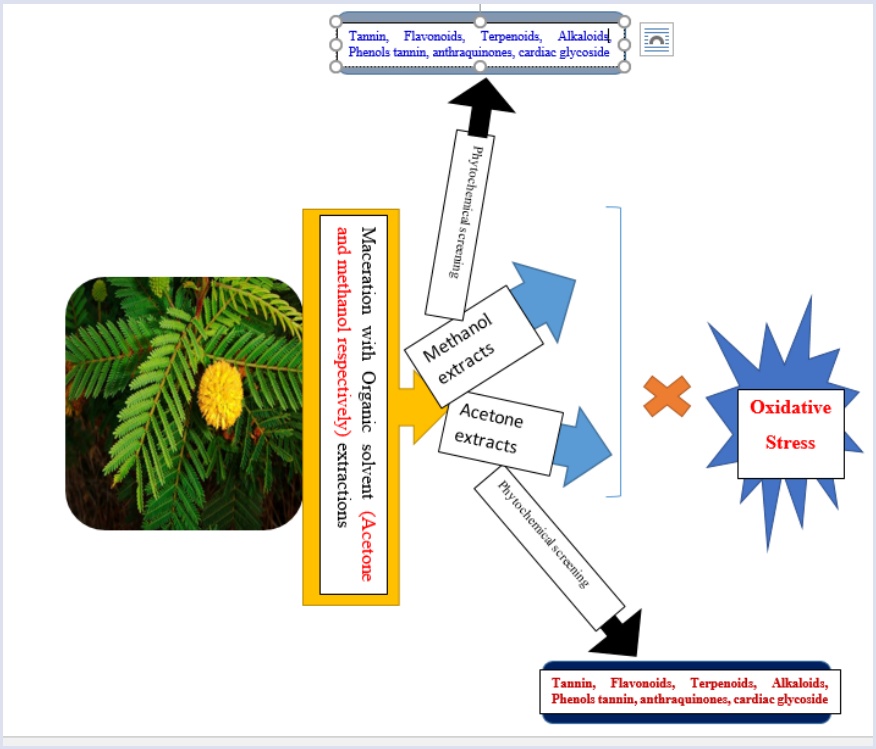
GRAPHICAL ABSTRACT



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