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 varying 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 evident in scavenging ABTS and DPPH radicals. However, acetone crude with lower IC₅₀ 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.

**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, anthraquiones, 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.⁴

Oxidative stress is an imbalance between reactive oxygen species (ROS) and antioxidant agents.¹⁵ Oxidative stress is implicated in most life-threatening diseases including cancer, diabetes, heart attack, stroke and neurodegeneration.¹⁶ The use of synthetic antioxidant agents such as butylated hydroxytoluene (BHT), and butylated hydroxianisole (BHA) in the management of oxidative stress diseases are associated with side effects such as toxicity and carcinogenicity.¹⁷ 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.¹⁸

*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.¹⁹ The tree produces gum arabic, a substance widely used as an adhesive, microencapsulating agent and an emulsifier. It is also used in confectioneries, pharmaceuticals, cosmetics, lithography, and in textile industries.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.³ 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-
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.\textsuperscript{12} and Sofowara.\textsuperscript{13}

**Total phenolic content**

Total phenolic content of methanol and acetone crude extracts was determined using the method described by Sun and co-workers.\textsuperscript{14} with slight modifications. The prepared concentrations (0.1 mg/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 Re and co-workers.\textsuperscript{18} was used determined antioxidant potential of acetone and methanol crude extract, following the method described by Re and co-workers.\textsuperscript{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 (1 mL) 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. Ascobic acid served as positive control. The percentage inhibition was calculated as follows:

\[
\% \text{ ABTS radical scavenging activity} = \left(\frac{A_0 - A_{sample}}{A_0}\right) \times 100.
\]

Where \(A_0\) is the absorbance of negative control and \(A_{sample}\) 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 \textit{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 proanthocyanidin (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.

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

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Methanol</th>
<th>Acetone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

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 leaf. Data expressed as mean ± SD. Values with different alphabets (a, b) indicate significant differences (p < 0.05).

<table>
<thead>
<tr>
<th>Extract</th>
<th>Total flavonoid (quercetin mg/mL)</th>
<th>Total Phenol (Tannic acid mg/mL)</th>
<th>Total Proanthocyanidin (catechin mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>1.6026 ± 0.9226</td>
<td>0.779 ± 0.3136</td>
<td>0.089 ± 0.0356</td>
</tr>
<tr>
<td>Methanol</td>
<td>0.6876 ± 0.7736</td>
<td>0.842 ± 0.4136</td>
<td>0.056 ± 0.0126</td>
</tr>
</tbody>
</table>

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).

<table>
<thead>
<tr>
<th>Extract</th>
<th>ABTS (mg/mL)</th>
<th>DPPH (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>0.09*</td>
<td>1.22*</td>
</tr>
<tr>
<td>Methanol</td>
<td>0.07*</td>
<td>1.44*</td>
</tr>
<tr>
<td>Ascorbic</td>
<td>0.07*</td>
<td>0.08*</td>
</tr>
</tbody>
</table>

This present study revealed vary quality of phytochemicals composition including; flavonoid, alkaloids, saponins, tannins, 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.

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. Medicinal plants have been demonstrated to possessed antioxidant potential by interruption-free radical chain reaction. 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.

DPPH radical reacts with scavenging agents to give yellowish colouration by losing electron.

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Uzunuigbe, et al.: Phytochemical Constituents and Antioxidant Activities of Crude Extracts from Acacia Senegal Leaf Extracts
radical. Previously, crude extracts from some medicinal plant displayed antioxidant ability.\textsuperscript{26} 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.\textsuperscript{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.\textsuperscript{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, \textit{et al.},\textsuperscript{28} reports on crude extract from \textit{Streycnos henningsii} scavenging ABTS radicals. High phenolic content was reported to show positive correlation with scavenging ability.\textsuperscript{29} Phenolic compound easily loss electron due to its hydroxyl moiety, thus reduce free radical activity.\textsuperscript{30} Minimum inhibition concentration at 50 % (IC\textsubscript{50}) is defined as the minimum concentration of compound or extract to inhibit particular activity. Lower values of IC\textsubscript{50} of compound denotes better activity, and vice-versa.\textsuperscript{31} The similarity in the IC\textsubscript{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 antioxidant ability. \textsuperscript{20} The higher antioxidant activity of acetone crude extract from \textit{Acacia Senegal} is greatly due to the presence of polyphenols in the gut and impact on health. Biomed Pharmacother. 2015;8(3427):145-9. Moreover, the antioxidant and radical scavenging potential of extracts of \textit{Spondias mombin} and \textit{Polyalthia longifolia} was studied by Jordan J Biol Sci. 2013;2(7):10-16. Polyanthia longifolia Extracts of \textit{Spondias mombin} for its antioxidant activity and nutritional value. Food Chem. 2006;97(3):452-58.

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 activity, as evident in scavenging DPPH and ABTS 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\textsubscript{50}: Inhibitory Concentration.

REFERENCES


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**GRAPHICAL ABSTRACT**

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**Dr. Edwina Olohirere Uzunuigbe** obtained her PhD in 2018 from the department of Biochemistry and Microbiology, University of Zululand, South Africa. Her past research work focuses on Plants biochemistry and she has co-authored four articles from her previous work. Her current research work focuses on green synthesis using green Nano-biotechnology and their biomedical applications. She is presently a research scientist in a Research Institute. She is a member of the South African Society of Biochemistry and Molecular Biology (SASBMB), Nigerian Society of Biochemistry and Molecular Biology (NSBMB) and Nigerian Society of Experimental Biology (NISEB).

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**Prof. Andrew R Opoku** obtained his PhD in 1977 from the University of Manchester, United Kingdom. He had academic position in various University across South Africa. Currently, he is a Professor Emeritus in the University of Zululand, South Africa with lots of publications.
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