PRINT ISSN 1119-8362
ELECTRONIC ISSN 1119-8362

Full-text Available Online at
https://www.ajol.info/index.php/jasem
http://ww.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 22 (11) 1849–1853 November 2018

Phytochemical Screening, Antioxidant and Antimicrobial Activities of the Stem Extracts of Woolly Bush (*Adenanthos sericeus*)

[*1]ABBA, J; [1]EKWUMEMGBO, PA; [1]DALLATU, YA; [2]MICHEAL, AM

*[*1]Department of Chemistry, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.*
**Corresponding Author Email: leujopson@yahoo.com, pat_adamma@yahoo.com; yakubudallatu@yahoo.com*

[2]Department of Science Laboratory Technology, Nigerian Institute of Leather and Science Technology, Samaru Zaria, Kaduna State Nigeria. Email: Michealpeddy1@gmail.com

ABSTRACT: Preliminary phytochemical screening of various extracts of the stem of *Adenanthos sericeus* were carried out in this work. The results obtained revealed some differences in the constituent of the aqueous, n-hexane, acetone and methanol extracts tested. Saponins, resins, anthraquinones, glycoside, alkaloids, tannins, flavonoids, steroids, phenols and terpenoid at varying proportion were detected. The free radical scavenging activity carried out using hydrogen peroxide scavenging activity to determine the antioxidant activity reveals a great antioxidant potential in *Adenanthos sericeus*. Also, the antimicrobial activities carried out using the following organisms; *Bacillus cereus*, *Staphylococcus aureus*, *Eschericia coli*, *Salmonell*a. The results obtained shows the stem extracts of *Adenanthos sericeus* has proven to have an interesting pharmacological active compounds with great radical scavenging and antimicrobial effects. As such could be used in ethnomedicine for treatment of infections and ailments.

DOI: https://dx.doi.org/10.4314/jasem.v22i11.21

Copyright: *Copyright* © 2018 Abba *et al*. This is an open access article distributed under the Creative Commons Attribution License (CCL), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Dates: Received: 16 October 2018; Revised: 19 November 2018; Accepted 30 November 2018

Keywords: *Adenanthos sericeus(s),* Antimicrobial, Phytochemicals, Organisms

Medicinal plants contain bioactive molecules which are implored to fight diseases (Harbone JB, 1973). These bioactive molecules are produced by plants during photosynthesis and are termed phytochemicals. This include tannins, saponins, reducing sugars,alkaloids, phenols (Wadood *et al*., 2013). The role of medicinal plants in human medicine is so important with respect to their therapeutic ability (Linga rao *et al*., 2012). This is so because modern drug appears no longer reliable particularly due to the adverse effect they caused in the body. Also, most organisms becomes resistant to these drugs in the course of their prolong use. There is therefore need to explore medicinal plants since they are safer (Valentina *et al*., 2013). However, nature has endowed mankind with much of these plants but not much is known. Cases where these plants are known, scientific validation on their use is not known. *Adenanthos sericeus* commonly called wooly bush, Africa never die is a plant belonging to the proteaceae family of the genus *Adenanthos*. It is native to Western Australia and naturalized in eastern Africa. It has a very bright but small obscure flowers and very soft, deeply divided hairy leaves. It belongs to the family proteaceae (Nelson, 1995). Information gathered from folklore medicine has it that *Adenanthos sericeus* has been used as stimulants, healing of wounds. There is no literature with regard to therapeutic activity of *Adenanthos sericeus*. This paper focuses on validating the claims by carrying out the antioxidant and antimicrobial screening of *Adenanthos sericeus* stem.

MATERIALS AND METHODS

Collection of plant materials, drying and pulverizing: The stem part of *Adenanthos sericeus* was collected in Zaria Kaduna State, Nigeria. It was identified and authenticated in the Herbarium section, Department of Biological Sciences, Ahmadu Bello University, Zaria with the voucher number 20775. The stem part was thoroughly washed, dried under shade for 2 weeks. It was pulverized into powder using laboratory mill (Das *et al*., 2010; Ncube *et al*., 2008).

Extraction of plant sample: The plant sample was subjected to exhaustive extraction using soxhlet extractor. The sample of 100 g and 99.5 % methanol, n- hexane, acetone and water were used in the extraction process respectively. The crude extract of the sample were each concentrated in an oven at 40°C. Thereafter, were each packed in plastic bottles with proper labeling for future use.

**Corresponding Author Email: leujopson@yahoo.com*

Qualitative determination of phytochemicals: The extracts were analyzed for the presence of alkaloids, resins, tannins, saponins, flavonoids, glycosides, phenols, anthraquinones, cardiac glycosides, steroids, phlobatannins and reducing sugars.

Test for alkaloid: The plant extract of the methanol, acetone, water and n-hexane extracts each of 0.50 cm^3 were evaporated to dryness and 2 % hydrochloric acid added to each of the residue heated on a boiling water bath. After cooling, the mixture was then filtered and treated with a few drops of Mayer's reagent. Evidence of turbidity or yellow precipitation marked the presence of alkaloid (Awoyinka *et al*., 2007).

Test for glycoside: To 0.50 cm^3 of each extracts solution in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid were added. A reddish brown colouration at the junction of two layers and bluish green colour in the upper layer was observed (Awoyinka *et al*., 2007).

Test for terpenoid and steroid: The plant extracts of 4.00 g were each treated with 0.50 cm^3 of acetic anhydride and 0.50 cm^3 of chloroform. Concentrated solution of sulphuric acid was added slowly to each extract until a red violet colour was observed for terpenoid and green bluish colour for steroids (Awoyinka *et al*., 2007).

Test for flavonoid: The plant extracts of 4.00 cm^3 were each treated with 1.50 cm^3 of 50 % methanol solution. They were warmed and metal magnesium added. To these solutions, 5-6 drops of concentrated hydrochloric acid was added. The formation of red color indicated the presence of flavonoids and orange colour, flavones (Awoyinka *et al.*, 2007).

Test for saponins: The various extracts were diluted with 20.00 cm^3 of distilled water and then agitated in a graduated cylinder for 15 min. The formation of 1.00 cm layer of foam indicated the presence of saponins. The frothing was mixed with 3 drops of olive oil and shaken vigorously. Presence of saponins resulted in formation of an emulsion (Awoyinka *et al*., 2007).

Test for tannins: To 0.50 cm^3 of each extract, 1 cm^3 of water and 1-2 drops of ferric chloride were added. Blue colour was observed for gallic tannins and green black for catecholics tannins (Sofowora, 1993).

Test for reducing sugar: To 0.50 cm^3 of each extracts, 1.00 cm^3 of distilled water and 5-8 drops of Fehling's solution were added, heated and observed for brick red precipitate (Siddiqui *et al*., 1997).

Test for resins: To 5.00 cm^3 of each extracts, a solution of copper acetate was added. The resulting solution then shaken vigorously and allowed to separate. A green coloured solution indicated the presence of resin (Harbone, 1973).

Test for anthraquinones: To 0.20 g each of the extracts, 4.00 cm^3 of benzene were added and shakened. The mixtures were then filtered and 2.00 cm^3 of 10 % ammonia solution added to the filtrate and shakened. The presence of pink, red or violet colour in the ammoniacal (lower) phase indicated the presence of free anthraquinones (Sofowora, 1993).

Test for phenols: To 0.20 g of the extracts each were dissolved in ferric chloride solution. A green or dirty green precipitate indicated the presence of phenolic compound (Harbone, 1973).

Test for phlobatannins: To 0.5 g of the extracts each, distilled water was added and thereafter filtered. The filtrate then boiled with 2 % hydrochloric acid solution. The formation of red precipitate shows the presence of phlobatannins (Soforowa, 1993).

Determination of Hydrogen peroxide scavenging activity: Hydrogen peroxide solution was prepared in PBS (Phosphate Buffer Saline). To 4.00 cm^3 of extract, 0.60 cm^3 of 4 M hydrogen peroxide was added and incubated for 10 min. The absorbance of whole mixture was measured at 230 nm using UV spectrophotometer. The age (%) of hydrogen peroxide free radical scavenging activity was estimated using the formula below (Nabavi *et al*., 2008a; Nabavi *et al*., 2008b).

$$\% H_2O_2 = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of Control}} \text{ X } 100$$

Where H_2O_2 = Percentage Hydrogen peroxide scavenging; Abs control=Absorbance of the control sample, Abs sample=Absorbance of the sample containing plant extract/ standard.

Determination of Antimicrobial activity: Test Organisms: The test organisms used for this analysis were clinical isolates of bacteria obtained from Department of Microbiology, Ahmadu Bello University Zaria. The isolates were *Bacillus subtilis (Ba), Staphylococcus aureus (Sta), Escherichia coli (Es), Salmonella (Sal).*

Determination of inhibitory activity (sensitivity test) of the extract using agar well diffusion method: The standardized inocula of the bacterial isolates was streaked on sterilized Mueller Hinton with the aid of a

sterile swab stick. Four punched wells on each inoculated agar plate with wells were properly labeled according to different concentrations of the extracts prepared such as 100, 50, 25 and 12.5 mg/ cm^3 respectively. Each well was filled up with approximately 0.20 cm^3 of the extract .it was allowed to stay on the bench for one hour to enable the extract diffuse into the agar. The plates were then incubated at 37°C for 24 hrs (plates of Mueller Hinton agar) while the plates of potato dextrose agar were incubated at room temperature for about 3-5 days. At the end of incubation period, the plates were observed for any evidence of inhibition which appeared as a clear zone that was completely devoid of growth around the well (zone of inhibition). The diameter of the zones was measured using a transparent ruler calibrated in millimeters and the result was recorded (Akinpelu and Kolawole, 2004).

RESULTS AND DISCUSSION

The preliminary phytochemical screening of the stem extracts of *Adenanthos sericeus* revealed some differences in the constituents of the aqueous, n-hexane, acetone and methanol extracts tested. From Table 1; Saponins, resins and anthraquinones were found to be highly present in the aqueous, acetone and methanol extract. Glycoside and steroid were highly present in aqueous extract. Alkaloid was found to be present in trace amount in acetone, aqueous and methanol extract.

Table 1: Different constituents of the extracts of *Adenanthos sericeus*

Chemical constituents	Aqueous extract	n-Hexane extract	Acetone extract	Methanol Extract
Alkaloid	++	-	+	+
Glycoside	-	+++	+++	+++
Terpenoid	+	-	+++	-
Steroid	++	+++	+++	+++
Flavonoid	++	+++	+++	+++
Tannins	-	-	+	-
Saponins	+++	-	+++	++
Resins	+++	-	++	+++
Reducing sugars	-	-	-	-
Anthraquinones	++	-	+++	+++
Phenols	-	++	++	++
Phlobatanins	++	-	-	-

Keys: + = Present at trace level, ++ = Moderately present +++ = Highly present , - = Absent

Tannins were present in trace amount in acetone extract. While terpenoid was highly present in acetone extract. Flavonoid was highly present in methanolic extract. In acetone, aqueous and n-hexane extract, it was moderately present. Phenols were found to be moderately present in all the extracts. Similarly, reducing sugars and phlobatanins were not detected in all the plant parts probably due to solvent effect as reported (Awoyinka *et al*., 2007). The ability of the extracts to scavenge hydrogen peroxide was determined according to the method by Nabavi *et al*., 2008a,b. In the hydrogen peroxide scavenging radical method, the percentage inhibition of n-Hexane extract was in the range of 28.1 % – 88.69%.

Table 2: The percentage inhibition (antioxidant levels) of the different concentrations of the four different extracts.

Conc. (μg/ml)	n-Hexane	Methanol	Acetone	Aqueous	α-tocopherol
20	88.69±0.5	94.8±2.69	90.9±1.6	81.6±1.2	70.6±0.08
40	87.15±10	91.5±1.3	80.1±0.4	61±0.8	52.2±20
60	83.25±10	77.9±5.7	73.1±2.8	42.1±1.5	40.9±0.8
80	52.02±1.3	32.6±1.7	51.67±1.2	28.3±0	22.7±0.4
100	28.1±0.2	27.3±0.9	30.5±0.3	27.4±10	21.3±0.4

Mean ± SD: The results for triplicate determination

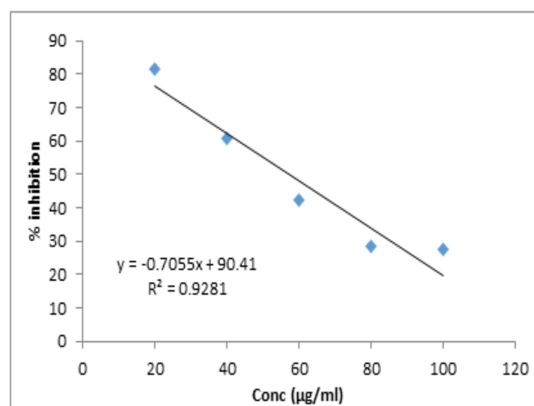

Fig. 1: A plot of % inhibition against the concentration for aqueous extract of *Adenanthos sericeus* showing the IC_{50}

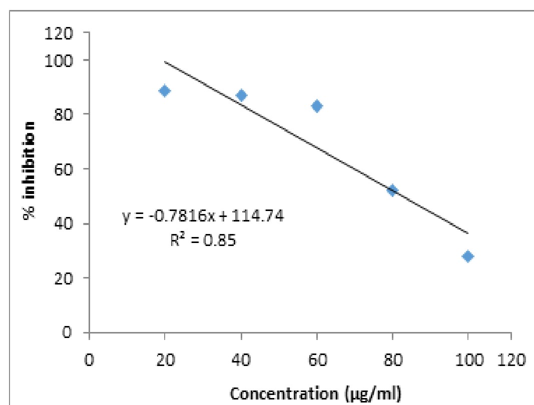

Fig. 2: A plot of % inhibition against the concentration for n-hexane extract of *Adenanthos sericeus* showing the IC_{50}

The lowest concentration (20 $\mu g/ml$) showed the highest percentage inhibition value (88.69 %) as shown in table 2. There is a characteristic increase in inhibition as the concentration decreases. The methanolic extract also showed similar trend as

revealed in Table 2 (27.3 % at 100 $\mu g/ml$, 32.6 %, 77.9 %, 91.5 % at 80 $\mu g/ml$, 60 $\mu g/ml$, 40 $\mu g/ml$ respectively, and 94.8 % at 20 $\mu g/ml$). The acetone extract followed similar trend, showing 30.5 % at 100 mg/ml and 51.67 %, 73.1 %, 80.1 % at 80 $\mu g/ml$, 60$\mu g/ml$, 40 $\mu g/ml$ respectively and 90.9 % at 20 $\mu g/ml$ as shown in Fig. 3. The aqueous extract followed similar trend also with 27.4% at 100 $\mu g/ml$, 28.3 %, 42.1%, 61% at 80 $\mu g/ml$,60 $\mu g/ml$,40 $\mu g/ml$ respectively and 81.6% at 20 $\mu g/ml$ At the lowest concentration of 20 $\mu g/ml$ the percentage inhibition was the highest for all the extracts.

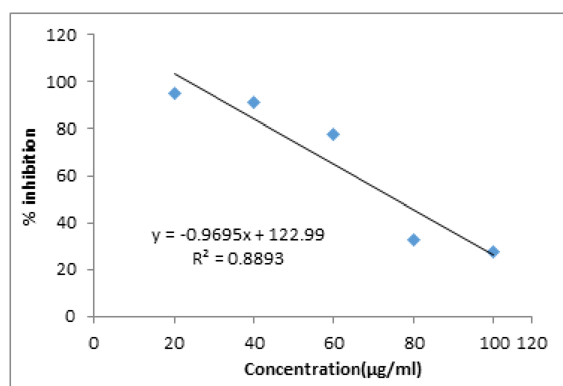

Fig. 3: A plot of % inhibition against the concentration for methanolic extract of *Adenanthos sericeus* showing the IC_{50}

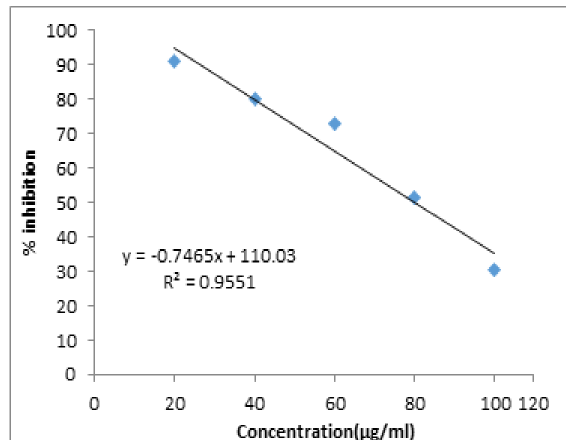

Fig. 4: A plot of % inhibition against the concentration for acetone extract of *Adenanthos sericeus* showing the IC_{50}

However, the α-tocopherol used as standard showed a better inhibition. The IC_{50} derived from plots in Fig. 1-5 revealed that the synthetic antioxidant (α-tocopherol), showed the best antioxidant effectiveness with IC_{50} of 46.8$\mu g/ml$. However, the test samples; aqueous, methanol, acetone and n-hexane extracts had IC_{50} of 57.3 $\mu g/ml$, 75.2 $\mu g/ml$, 80.0 $\mu g/ml$ and 82.8 $\mu g/ml$ respectively. In the order of decreasing antioxidant effectiveness, the trend: α-tocopherol >aqueous> methanol > acetone> n-hexane reveals high inhibition efficiency for α- tocopherol used as standard in figure 5, though the test samples are good antioxidant.

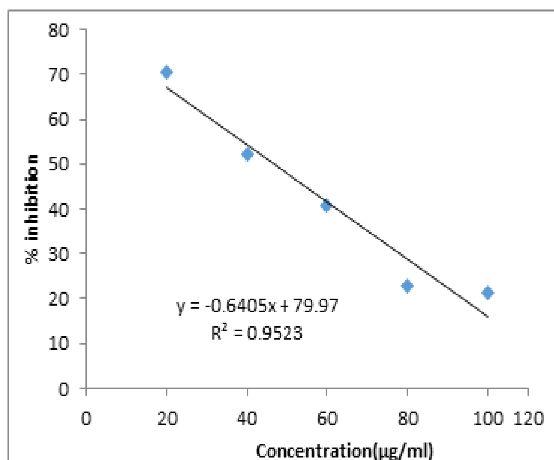

Fig. 5: A plot of % inhibition against the concentration for α-tocopherol (control) showing the IC_{50}

Table 3: The zones of inhibition of the isolates at different concentrations compared to control drug

Conc.(mg/ml)	Ba	Sta	Es	Sal.
Acetone extracts				
100	16	16	16	-
50	13	14	-	-
25	12	13	-	-
12.5	11	12	-	-
Aqueous extracts				
100	18	16	18	-
50	16	14	16	-
25	14	13	14	-
12.5	12	12	12	-
Methanolic extracts				
100	18	15	10	16
50	16	13	-	14
25	14	12	-	12
12.5	12	11	-	11
n-Hexane extracts				
100	-	-	-	-
50	-	-	-	-
25	-	-	-	-
12.5	-	-	-	-
Ciproflaxacin	35	40	25	35

The antimicrobial activity shown in table 3, is largely due to the presence of bioactive principles responsible for the antibacterial activities of most medicinal plants (Shimbe *et al.*, 2014). Absence of some bioactive secondary metabolites could be responsible for non-inhibitory activity of the n-hexane extract. *Bacillus* and *Staphylococcus aureus* were the most inhibited.

Conclusion: The phytoconstituents, their antioxidant and antimicrobial activities were studied in these research. All the extracts screened contain bioactive metabolites which has good antioxidant and antimicrobial effect. This can be purified and harnessed for pharmaceutical use.

Acknowledgement: The authors are thankful to the laboratory technologists, Department of Science Laboratory Technology, Nigerian Institute of Leather and Science Technology and Department of Pharmaceutical sciences, Ahmadu Bello University, Samaru Zaria.

REFERENCES

Harbone, JB (1973). Phytochemical Methods. A Guide to Modern Technique of Plant Analysis. Chapinan and Hall, London.

Wadood, A; Ghufran, M; Jamal, SB; Naeem, M; Khan A; Ghaffar, R (2013). Phytochemical a nalysis of medicinal plants occurring in Local area of Mardan. Biochem anal Biochem: 2:14.

Linga rao, M; Savithamma, N (2012). Quantification of primary and secondary metabolites of *Svensonia hyderobadensis –A Rare Medicinal Plant. Int J Pharm Sci:* 4:519-521.

Valentina, P; Kiruthiga, B; Parimala, MJ. (2013). Preliminary Phytochemical Analysis and Biological Screening of Extracts of Leaves of *Melia dubia Cav. Int. J. Res. In Ayurveda Pharm:* 4(3): 417-418.

Nelson, EC. (1995). “Adenanthos”. In Mc Carthy, Patrick. Flora of Australia. Collingwood, Victoria: CSIRO publishing / Australian biological resources study. 314-342 ISBN 0-643-05692-0.

Das, K; Tiwan, RK; Shivasteva, DK (2010). Techniques for Evaluation of Medicinal Plant products as Anti-microbial agent: Current Methods and Future Trends. *J. Medic. Plants Res.:* 4: 104 – 111.

Ncube, NS; Afolayan, AJ; Okoh, AT (2008). Assessment techniques of anti-microbial properties of natural compounds of plant origin: current methods and future trends. *Afr. J. Biotechnol.* (12): 1797 – 1806.

Awoyinka, OA; Balogun, IO; Ogunnowo, AA (2007). Phytochemical Screening and in vitro Bioactivity of *Cnidoscolus aconitifolius (Euphorbiaceae)*. J. Med. Plants Res. 1:63-65.

Siddiqui, A; Ali, M (1997). Practical pharmaceutical Chemistry, 1st edition, CBS publishers and Distributors, New Delhi: 126-131.

Sofowora, A (1993). Medicinal Plants and Traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria: 191-289.

Nabavi, SM; Ebrahimzadeh, MA; Abavi, SF; Hamidinia, A; Bekradnia, AR (2008a). Determination of antioxidant activity, phenol and flavonoids content of Parrotia Persica mey. Pharmacology online: 2:560-567.

Nabavi, SM; Ebrahimzadeh, MA; Nabavi, SF; Jafari, M (2008b). Free radical scavenging activity and antioxidant capacity of Eryngium cancasicum Trantv and Froripia Subpinnata. Pharmacology online: 3:19-25.

Akinpelu, DA; Kolawole, DO (2004). Phytochemical and antimicrobial activity of leaf extract of *piliotigmathonning* (schum). *Sci. Focus J.* 7: 67-70.

Shimbe, RY; Tor-Anyiin, TA (2014). Phytochemical and antibacterial studies of hymenocardia acida roots. *J. Chem. Soc. Nig.* 39 (2):119-122.