

Antioxidant activity and Invitro Cyto-toxicity of different extracts of *Afzelia africana* bark

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Abstract

Afzelia africana bark was extracted by two methods; Macerated with distilled water and successive extraction method by soxhlet apparatus with different organic solvents in order of increasing polarity (n- hexane, ethyl acetate- butanol and methanol). The antioxidant activity was investigated using the scavenging activity on superoxide anions, 2, 2-diphenhyl-1-picrylhydrazyl (DPPH) and iron chelating activity on Fe^{2+} . On the other hand in vivo cytotoxic activity was done using Brine shrimp lethality bioassay; while Invitro cytotoxic activity was investigated using the Sulphorhodamine-B (SRB) assay against; breast carcinoma cells (MCF-7), colon carcinoma cells (HCT-116), prostate carcinoma cells (PC-3).The antioxidant activity showed variation with method; since the maximum DPPH scavenging activity was observed by ethyl acetate extract (91.3 ± 0.01), while maximum Iron chelating was shown by methanolic extract (72.7 ± 0.01). the maximum activity against the prostate cancer cell line (PC3) and breast carcinoma cells (MCF-7) was exhibited by ethyl acetate extract with (IC_{50} 12.5 μ g/ml) (IC_{50} 14.5 μ g/ml) respectively.

Key words: *Afzelia africana*, Antioxidant, Human tumor cell lines, cytotoxicity, free radical.

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Introduction

Herbal medicine is an important part of culture traditions of African people; this because herbs are generally natural, accessible and easily to be handled [7]. Medicinal plants that are effective in controlling cancer with minimal side effects are commonly used in under developed countries as alternative therapy due to limited economic situation.

Cancer continues to be one of the major causes of death worldwide and only modest progress has been made in reducing the morbidity and mortality of this disease [8]. In Africa, hundreds of plants are used traditionally for the management and/or control of different ailments and cancer is one of these diseases. Unfortunately, only a few of such medicinal plants have been scientifically validated [5]. One of the plants commonly used in Africa traditional medicine for the management of cancer is *Afzelia africana*. This plant is generally known as mahogany, widely distributed in Africa and it belongs to the family Fabaceae. It is traditionally used for treatment of different ailments such as constipation, diarrhea, GIT infection, , hernia in Nigeria [11] and as anti-malaria [10]. The antioxidant activity of the crude methanolic stem bark extract of *Afzelia africana* was investigated using different method as well as the flavonoid, proanthocyanidine and phenolic contents of the extract were determined by [1]. This study aimed to investigate the invitro Cyto-toxicity of *Afzelia africana* bark since there is no reports support this study in addition to anti-oxidant activity.

Materials and methods

Plant materials

Afzelia africana bark was collected from different areas in south Kordoufan by herb practioner during month of March 2012, then identified and authenticated by the Medicinal and Aromatic plants Research Institute, Khartoum, Sudan. The bark was cleaned, freed from dust and foreign material air-dried to constant weight, powdered and stored in an air-tight container for further use.

Preparation of extracts

The extraction of secondary metabolites from *Afzelia africana* bark was carried out in accordance to [14] with some modification by two methods; Maceration using distilled water and successive extraction method using soxhlet apparatus with different organic solvents in order of increasing polarity :(n- hexane, ethyl acetate, butanol and methanol).

Invitro Cyto-toxic assay

Sulphorhodamine-B (SRB) assay of cytotoxic activity

Cells used when 90 % confluence reached in T25 flasks. Adherent cell lines harvested with 0.025 % trypsin. Viability was determined by trypan blue exclusion using the inverted microscope). Cells were seeded in 96-well microtiter plates at a concentration of 5×10^4 - 10^5 cell/well in a fresh medium and left to attach to the plates for 24 hrs., incubated with the appropriate concentration ranges of drugs, completed to total of 200 μ l volume/well using fresh medium and incubation was continued for 24, 48 and 72 hrs. Control cells were treated with vehicle alone. For each drug concentration, 4 wells were used. Following 24, 48 and 72 hrs. treatment, the cells were fixed with 50 μ l cold 50% trichloro-acetic acid (TCA) for 1 hr. at 4 °C ;then Wells were washed 5 times with distilled water and stained for 30 min at room temperature with 50 μ l 0.4 % SRB dissolved in 1 % acetic acid. Followed by 1 % acetic acid alone. The plates were air-dried and the dye was solubilized with (121 gm of tris base was dissolved in 1000 ml of distilled water and PH was adjusted by HCl acid 2M to get Tris base 10 mM ,PH 10.5) 100 μ l/well of 10 mM tris base (ph 10.5) for 5 min on a shaker at 1600rpm. The optical density (O.D.) of each well was measured spectrophotometric ally at 564nm with an ELIZA micro- plate reader (Meter tech. Σ 960, U.S.A.). The mean background absorbance was automatically subtracted and means values of each drug concentration was calculated [6]. The experiment was repeated three times for each cell line with each extract.

Calculation of IC₅₀ values:

The percentage of cell survival was calculated automatically as follows: Survival fraction = Optical Density of. (Treated cells)/ Optical Density of. (Control cells). The IC₅₀ values were calculated.

Antioxidant activity

Free radical scavenging assay

The DPPH radical scavenging was determined according to method of [12] With some modification. In 96wells plate, the test samples were allowed to react with 2,2-Di (4-tetra-octylphenyl)-1-picryl-hydrazyl stable free radical DPPH. Ten micro-liter of each plant extracts (20mg/ml) were incubated with ninety micro-liters DPPH for half an hour at 37°C. The concentration of DPPH (purple in color) was kept as (300 μ m.), the test samples were dissolved in DMSO (20 mg/ml); While DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517nm Using multiplate reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with DMSO treated control group; propyl gallate (2mg/100 μ l) used as positive control.

Inhibition % was calculated in following way: $I\% = (A \text{ blank} - A \text{ sample}/A \text{ blank}) \times 100$ Where, A blank is the absorbance of the control reaction (containing all reagents except the extract) A sample is the absorbance of the test compound. All tests and analysis were run in triplicate.

Iron chelating assay

The iron chelating activity on Fe²⁺ was determined according to the modified method of [13]. The Fe²⁺ was monitored by measuring the formation of ferrous ion-ferrozine complex. The experiment was carried out in 96microtiter plate, ten micro-liter of each plant extracts (20mg/ml) were incubated with forty micro-liter ferrous sulphate (0.34mg/7ml) for 30min; reaction was initiated by adding sixty micro-lite ferrozine (5mM). The mixture was shaken and left at 25° for 10 min and measured at 562nm in a spectrophotometer. A lower absorbance indicates a higher chelating power. EDTA (1.8mg/100 μ l) was used as positive control. , DMSO as control; all tests and analysis were run in triplicate. Chelating activity was calculated using the following formula: Chelating activity (%) = [1 – (Absorbance of sample/Absorbance of control) x 100] Control test was performed without addition of sample. All tests and analysis were run in triplicate.

Results

Antioxidant activity

The antioxidant activity of different extracts of *Azelia africana* bark have been evaluated using two important parameters (%RSA \pm SD) free radical scavenging activity on DPPH and %Iron

chelating \pm SD. It was noticed that most of extracts showed variation in antioxidant activity according to method used except the methanolic and n-hexane extracts that showed closed activity by both methods. As indicated in fig (1) the most potent DPPH scavenging activity was observed by ethyl acetate extract (91.3 ± 0.01); while n-hexane extract exhibited no activity on DPPH as compared with (propyl gallate as positive control (95 ± 0.01)). On the other hand the maximum (%Iron chelating \pm SD) was shown by methanolic extract (72.7 ± 0.01), finally the minimum activity has been shown with n-hexane extract (16.4 ± 0.05) as compared with EDTA as positive control (91.3 ± 0.01) as represented in fig (1).

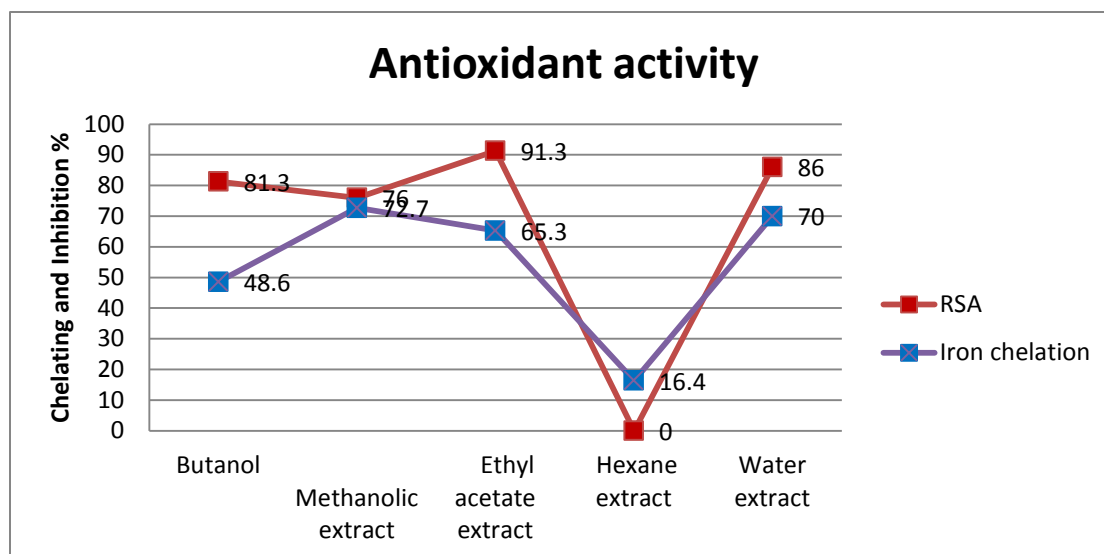


Figure (1): Antioxidant activity of *Azelia africana* bark extracts; free radical scavenging activity on DPPH (%RSA \pm SD) and the iron chelating activity on Fe^{2+} (% Iron chelating \pm SD).

Invitro Cyto-toxic assay

The Anticancer activity of different extracts of *Azelia africana* bark showed that (aqueous, ethyl acetate, n-butanol) have potent activity against colon cancer cell line (HCT-116) as compared to doxorubicin (IC_{50} 6.6 μ g/ml). However the aqueous extract (IC_{50} 23.2 μ g/ml) was noticed to have the maximum activity, but the results of two other extracts were as close as (IC_{50} 23.5) for ethyl acetate and-(23.6 μ g/ml) for n-butanol. On the other hand the maximum activity against the

prostate cancer cell line (PC3) was exhibited by ethyl acetate extract with (IC_{50} 12.5 $\mu\text{g/ml}$), while the aqueous extract showed the minimum activity (IC_{50} of 47 $\mu\text{g/ml}$). As compared to doxorubicin (IC_{50} 8.1 $\mu\text{g/ml}$). Finally the Anticancer activity of different extracts of *Afzelia africana* bark extracts were examined against Breast cancer cell line (MCF7); the maximum activity was shown by ethyl acetate extract with (IC_{50} 14.5 $\mu\text{g/ml}$), and the minimum activity was exhibited by water extract with (IC_{50} 20.6 $\mu\text{g/ml}$) on comparison with doxorubicin (IC_{50} 3.6 $\mu\text{g/ml}$). The results of anti-tumor were found to be concentration dependent. As recorded in fig (2).

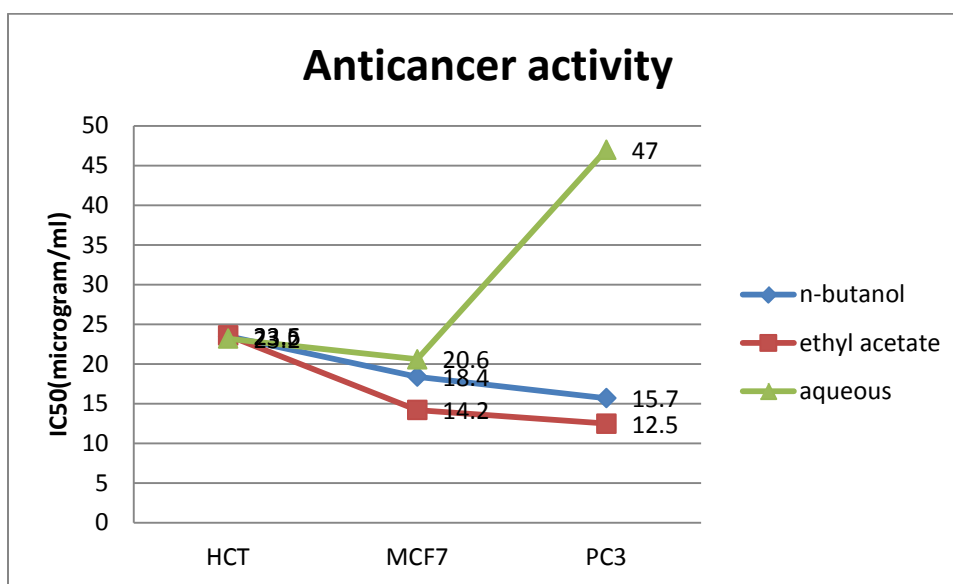


Figure (2): Anticancer activity of *Afzelia africana* bark extracts against Cell Line Carcinoma HCT-116: colon cancer cell line. MCF-7: Breast cancer cell line PC3: prostate cancer cell line. IC_{50} ($\mu\text{g/ml}$).

Discussion

Cancer is a multi-step disease incorporating environmental, chemical, physical, metabolic, and genetic factors which play a direct and/or indirect role in the induction and deterioration of cancers. Strong and consistent epidemiology evidence indicates a diet with high consumption of antioxidant rich fruits and vegetables significantly reduces the risk of many cancers, suggesting

that certain dietary antioxidants could be effective agents for the prevention of cancer incidence and mortality. Phenolic compounds in addition to their primary antioxidant activity, this group of compounds displays a wide variety of biological functions which are mainly related to modulation of carcinogenesis. Various in vitro and in vivo systems have been employed to determine the ant carcinogenic and anticancer potential of these natural phenolic compounds or extracts[9]. Natural phenolics can affect basic cell functions that related cancer development by many different mechanisms. In the initiation stage, phenolics may inhibit activation of procarcinogens by inhibiting phase I metabolizing enzymes, and also facilitate detoxifying and elimination of the carcinogens by induction of phase II metabolizing enzymes. They may also limit the formation of the initiated cells by stimulating DNA repair. Phenolics may inhibit the formation and growth of tumors by induction of cell cycle arrest and apoptosis. Since Malignant cells are characterized by excessive proliferation, inability to terminally differentiate or perform apoptosis under normal conditions, and an extended or immortalized life span. Natural phenolics have been reported induce cell cycle arrest at different cell phases: G1, S, S-G2, and G2. [9]One important aspect of carcinogenesis is recognized to be the involvement of inflammation. For instance, prostaglandins are mediators of inflammation and chronic inflammation predisposes to carcinogenesis. is believed to be associated with colon, lung, breast and prostate carcinogenesis. Natural phenolics have been reported to inhibit transcription factors closely linked to inflammation, so polyphenols exhibit anti-inflammatory properties. Anthocyanin, were tested positive in *A. africana* stem bark extract were found to suppress malignant cell migration, invasion and metastasis in vitro and in vivo[16]. In addition, phenolic compounds possess ant angiogenesis effects, which is an important aspect in the inhibition of tumor growth, invasion and metastasis. [3] reviewed the biological activities of tannins which tested positive in *A. africana* stem bark extract and observed that tannins have remarkable activity in cancer prevention and anticancer. The presence of tannins in *A. africana* may justify its in vivo and invitro Cyto-toxic activity. Alkaloid is another phytochemical compound observed in the stem bark extract of *A. africana*. Alkaloids have been associated with medicinal uses for centuries. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms and their potential use in the elimination and reduction of human cancer cell lines [4][2][17][18]. Flavonoids which is also one of the constituents of *A. africana* stem bark

extract exhibit a wide range of biological activities which are anti-microbial, anti-inflammatory, anti-angiogenic, analgesic, anti-allergic effects, cytostatic and antioxidant properties [15].

Conclusion

According to above results; invitro Cyto-toxic activities against different cancer cell lines beside antioxidant activity that supported by the results of phytochemicals screening. Suggest that *Afzelia africana* bark could be considered as good source for anticancer agent through one or more of the above mechanisms in the future.

References

- 1.Akinpelu, D.A; Aiyegoro, O.A; Okoh, A.I; (2010). *In vitro* antioxidant property of methanolic extract of *Afzelia africana*. Journal of medicinal plants research; 4(19):2021-2027.
- 2.Akinpelu, D.A; Aiyegoro,O.A; Okoh,A.I; (2008). *In vitro* Antimicrobial and phytochemical properties of crude extract of stem bark of *Afzelia africana* (smith).African journal of biotechnology, 7(20):3665-3670.
- 3.Li H, Wang Z, Liu Y (2003). Review in the studies on tannins activity of cancer prevention and anticancer. Zhong-Yao-Cai. 26(6): 444-448.
- 4.Nobori T, Miurak K, Wu DJ, Takabayashik LA, Carson DA (1994). Deletion of the cyclin-dependent kinase-4 inhibitor gene in multiple human cancers. Nature 368(6473): 753-756.
- 5.Tanko Y, Yaro AH, Isa AI, Yerima M, Saleh MIA, Mohammed A(2007). Toxicological and hypoglycemic studies on the leaves of (Menispermaceae) on blood glucose levels of streptozotocin-induced diabetic Wistar rats. Journal of Medicinal Plant Research; 2: 113-116.
- 6.Skehan,p; Storeng,R; (1990). New coloremtric cytotoxicity assay for anticancer drug screening. J Natl Cancer Inst;2:1107-1112.

7. Fennell, C.W; Lindsey, K.L; McGow, L. J; Sparg, S. G; Satafford, G. L; Elgorashi, E.E; Graee, O.M; & van Staden, J; (2004). Assessing African medicinal plants for efficacy & safety, (pharmacology screening & toxicology). *Journal of Ethnopharmacology* 94 (2-3):205-217
8. Bhanot A, Sharma R, Noolvi M (2011). Natural sources as potential anti-cancer agents. *International Journal of Phytomedicine*, 3:09-26
9. Dai, J and Mumper, R.J; (2010) Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties. *Journal Molecules*: 15, 7313-7352.
10. Asase, A; Oteng – yeboah, A-A; Odamtten, G.T & Simmonds, M.S.j;(2005). Ethnobotanical study of some Ghanaian antimalarial plant. *Journal of Ethnopharmacology*; 99 (2-3) : 273 - 279
11. Igoli, J.O; Ogaji, O.G; TorAnyiin T.A; and Igoli N.p; (2005). Traditional medicine practice amongst IGEDE people of Nigeria part II. *African journal traditional Complementary and Alternative medicines (Afr.J.Trad.CAM)*, 2(2):134-152.
12. Shimada, K; Fugikawa, K; Yahara, K; and Nakamura, K.(1992). Antioxidative properties of xanthan on the antioxidation of soybean oil in cyclodextrin emulsion. *J Agri Food Chem*: 40,945-948.
13. Dinis, T.C.B; Maderia, V.M.C; and Almeida, L.M.(1994). Action of phenolic derivate (acetoaminophen, salicylate and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxy radical scavengers. *Arch Biochem Biophys* :315,161-9.
14. Harborne J B (1984). *Phytochemical methods - A Guide to Modern Techniques of plant analysis*. 2nd edition. Chapman and Hall, New York.
15. Hodek, P., Trefil, P., Stiborova, M.(2002). Flavonoids-Potent and versatile biologically active compounds interacting with cytochrome P450. *Journal of Chemico-Biological Interaction*; 139(1): 1-21.
16. Jacobasch, G., Raab, B., Salomon, A., Pforte, H. (1999).. Anticancer formulations with flavonols or flavonoids. *Ger. Offen.*, 4.

17.Noble RL(1990): The discovery of the vinca alkaloids--chemotherapeutic agents against cancer.. Journal of Biochemistry and cell biology; 68(12):1344-

18.Da Rocha,B. A., Lopes, R., Schwartzman, G. (2001). Natural products in anticancer therapy. Current Opinion in Pharmacology; 1:364–369.