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EVALUATION OF TOTAL PHENOLIC CONTENTS AND *IN VITRO* ANTIOXIDANT ACTIVITY OF *Prosopis africana* LEAF EXTRACTS

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ABSTRACT

Prosopis africana belongs to the Family Leguminoceae, is used in ethnomedicine to treat different ailments including, diarrhea, bacillary dysentery, malaria, male sterility and Cancer. Antioxidants from natural sources are effective in the treatment of many diseases including garthritis, atherosclerosis, ischemia and reperfusion injury of many tissues, gastritis, diabetics, central nervous system injury, acquired immunodeficiency syndrome (AIDS) and cancer. This work was aimed at extracting and screening the extracts for, total phenolic contents and antioxidant activity of the Prosopis africana. The plant material was extracted with absolute ethanol to obtained the crude ethanol extract which was macerated with different solvents in increasing order of polarity to obtained different fractions such as n-hexane, chloroform, ethylacetate and methanol. The results of total phenolic contents of the extract shows that methanol fraction (PA-05) showed the highest amount of phenolic compounds with the 285.92 ±0.22 mg/ml of GAE, followed by ethylacetate (PA-04) with the value of 268.49 ±0.12 mg/ml of GAE. n-Hexane fraction (PA-02) showed the lowest amounts of phenolic compounds. The results of antioxidant activity on the other hand, shows that methanol fraction (PA-05) was having the highest IC_{50} value of 6.39 µg/ml, competing with Ascorbic acid (positive control) with the IC_{50} value of 6.24 µg/ml. But, n-hexane fraction (PA-02) has the lowest IC_{50} value of 81.75 µg/ml, followed by chloroform fraction having the IC_{50} value of 76.22 µg/ml. Generally, the methanol fraction was found to be very effective in both phenolic contents and antioxidant activity among other fractions and the crude extracts.

Keywords: Ethanol extract, Fractions, Phenolics, Antioxidants, Anticancer, Inhibitions and DPPH

INTRODUCTION

Phytomedicinal research of indigenous plant parts is presently gaining more grounds than ever before as the majority of people are now patronizing herbal medicinal treatment which is considered to be more easily accessible and cheaper than orthodox medical treatment (Ajiboye *et al*., 2010). The usefulness of medicinal plants is directly linked to the wide range of chemical compounds synthesized in various biochemical pathways; which are classified as secondary metabolites (Ameyaw and Duker-Eshun G. 2009). The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body (Edoga H.O. *et al*., 2005 and Lavanya *et al*., 2007).

Oxidation process is essential to many living organisms for the production of energy to fuel biological processes. However, oxygen-centered free radicals and other reactive oxygen species (ROS) which are continuously produced *in vivo* during oxidation process may result in cell death and tissue damage. The free radicals are fundamental in modulating various biochemical processes and represent an essential part of aerobic life and metabolism (Lavanya *et al*., 2007). The most common Reactive Oxygen Species (ROS) include superoxide anion (O^{2-}), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^-) which resulted from cellular redox processes.

At low or moderate concentrations, ROS exert beneficial effects on cellular response and immune function but at high levels, these radicals become toxic and disrupt the antioxidant defense system of the body, which may lead to "oxidative stress" (Laouali A. *et al*., 2014). The oxidative stress cascade initiated by free radicals obtains stability through electron pairing with biological macromolecules such as proteins, lipids, and DNA in healthy human cells and causes damage in cell structures that include proteins and DNA along with lipid peroxidation. Moreover, the formation of free radicals shortens cells lifespan and produces changes that resemble aging. This has contributed to a wide range of diseases including coronary heart diseases, aging, neurodegenerative disorders, arthritis, diabetes, inflammation, lung damage, and cancer (Lee *et al*.,2000).

Although aging is likely to be a multifactorial process and not reducible to any one single cause but the evidence from the free radical theory detailing the impact of these free radicals on aging (Madani *et al*., 2016). Since the formation of free radicals has a great Evidence-Based Complementary and Alternative Medicine impact on the aging process, it is appropriate to examine the role of natural antioxidant as a defense system. Accordingly, plant-based natural antioxidants with free radical scavenging activity are emerging as the primary components of holistic approaches in impeding aging and many other diseases. Therefore, this study focuses on local medicinal plant as alternative source in impeding aging, cancer, and so on, namely, *Prosopis africana.*

MATERIALS AND METHODS

Collection of the Plant Samples

Small branch of *Prosopis africana* (BUKHAN 0193) was collected from Tamu in Kurfi Local Government Area of Katsina State. The plant sample was identified by Bahaudeen Said Adam in the Department of Plant Biology, Bayero University Kano and was authenticated by Prof. Bala Sidi Aliyu of Plant Biology Department, Bayero University Kano.

Solvent Extraction of the Plant Materials

The powdered sample (500g) was cold percolated using ethanol (2000cm^3) for 72hrs with constant agitation of about 20 to 30 times a day. It was decanted, filtered and concentrated using rotary evaporator (R110) at 40^{o}C to obtain the crude ethanol fraction (PA01). It was weighed and kept inside a refrigerator before the next step (Ajaiyeoba, 2006; Musa, 2015; and Haruna, 2018).

Maceration of the Crude Extract

The crude Ethanol Extract (76.42 g) was macerated sequentially using n-Hexane, Chloroform, Ethyl acetate and Methanol with 200 ml of each fraction respectively. The fractions of these solvents were dried by exposing them to air at room temperature (Musa M.F. 2015).

Total Phenolic Contents of the Plant Extracts

The total phenolic contents of the crude extract and the corresponding fractions of *Prosopis africana* leaves was determined using Folin-Ciocalteau method described by Andressa B. *et al*., 2013. Ethanol crude extract (PA-01), n-hexane (PA-02), chloroform (PA-03), ethyl acetate (PA-04) and methanol (PA-05) fractions were prepared by taking 0.1 ml of each of the samples at concentration of 5 mg/ml and added to 0.5 ml of 10% (v/v) Folin-Ciocalteau reagent. After 3-8 min, 0.4 ml of 7.5% (w/v) Sodium Carbonate solution was added to the mixture. After being kept in total darkness for 30 min, the absorbance of the reaction mixture was measured at 765 nm using a UV-VIS. Spectrophotometer. All analyses were repeated three times and the mean value of absorbance was obtained. Amounts of total Phenolic contents were calculated using a gallic acid calibration curve. The results were expressed as milligram gallic acid equivalents per gram (mg GAE/g).

Determination of Antioxidant Activity (DPPH radical scavenging)

DPPH is a stable free radical at room temperature which when accepts an electron or hydrogen radical becomes a stable diamagnetic molecule (Madani *et al*., 2016). The reduction capability of the DPPH radical is determined by the decrease in its absorbance at 517nm, induced by antioxidants. The absorption maximum of a stable DPPH radical in methanol was at 517nm. On reaction with antioxidant or free radical there is decrease in absorbance of DPPH radical because of scavenging of the radical by hydrogen donation. There is change in color from purple to yellow which is visually noticeable. Hence, DPPH is usually used as a substrate to evaluate the antioxidative property (Chang *et al*., 2002). The 0.1 mM solution of DPPH in methanol (22.2 mg in 1000 ml) was freshly prepared. Different concentrations of extract were added at an equal volume to methanolic solution of DPPH. After 30 min at room temperature, the absorbance was recorded at 517 nm. Ascorbic acid was used as standard

control. Percentage inhibition of DPPH free radical was calculated based on the control DPPH Scavenged (%) = (Acont - Atest) / Acont × 100
Where; Acont is the Absorbance of the control reaction and Atest is the Absorbance in the presence of the sample of the extracts.

Statistical Analysis
The total phenolic contents results were expressed as mean ± SD. Student's *t*-test was used to analyze level of statistical significance between groups. P< 0.05 was considered statistically significant. Whereas, antioxidant activity results were expressed in IC_{50} values for each fraction and determined at 95% confidence level or intervals by analyzing the data on a computer loaded with a "SPSS programme".

RESULTS AND DISCUSSION
Fractionation of *P. africana* Leaf Extract
The ground fine powdered *Prosopis africana* leaves (500g) was percolated and yielded 86.42g of Ethanol extract, which gives 17.28%. From the crude extract, 76.42g was macerated with different solvents (Table 1) yielded n-Hexane fraction 10.85g (14.20%, black and sticky), Chloroform fraction 1.55g (2.03%, black and sticky), Ethyl acetate fraction 6.45g (8.44%, black and sticky), Methanol fraction 53.60g (70.14%, black and sticky) and Water fraction 3.01g (3.94%, white crystalline solid).

Total Phenolic Contents of the Plant Extracts
The total phenolic contents of the extracts were calculated based on the standard and presented as Garlic acid Equivalents (GAE) per gram of dry sample.
However, based on the results obtained, as shown in Table 2, among all the fractions, methanol fraction (PA-05) showed the highest amount of phenolic compounds with the 285.92 ±0.22 mg GAE/g, followed by ethylacetate (PA-04) with the value of 268.49 ±0.12 mg GAE/g. n-Hexane fraction (PA-02) showed the lowest amounts of phenolic compounds. Observing the table of experimental result (Table 2), there was a significant difference between all five tested extract (fractions). This result is however efficient and in consistent with the reported findings of (Haq *et al*.,2011, Kasparaviciene G. *et al*., 2013 and Karimi A. & Morad M.T. 2015) of reading, which contain DPPH and distilled water without extract using the following equation:
which all of them determined the total phenolic contents of the plant's crude extracts only.

Antioxidant Activity of the Plant Extracts
The result of antioxidant or free radical scavenging activity in figure 1, showed that, at the concentrations of 250, 500, 1000 µg/ml, some fractions were significantly comparable with Ascorbic acid (positive control) of which there is no much difference in terms of scavenging capacity, whereas, at the concentrations of 125, 62.5, 31.3, 15.6 and 7.8 µg/ml of which, when comparing the activity of these extracts with the Ascorbic acid (positive control), there are significant differences as observed.
Therefore, the best scavenging among the fractions and the extract tested was achieved remarkably from the methanol fraction (PA-05) whereby at the concentration of 1000 and 7.8 µg/ml (97.1 and 61.1% respectively as shown in table 3) which was significantly higher than other fractions and the extract at many concentrations, thereby competing with the Ascorbic acid (positive control). Among all the fractions and the extract, n-hexane fraction (PA-02) showed the lowest scavenging activity at the concentrations of 250, 15.6 and 7.8 µg/ml (73.2, 9.9 and 14.8% respectively) and ethylacetate fraction (PA-04) at the concentrations of 125, 62.5 and 31.3 µg/ml (94.2, 70.4 and 48.4% respectively).
However, the result of IC_{50} values of fractions and the extract as presented in Table 3 showed that, among the tested fractions and the extract, methanol fraction (PA-05) was having the highest IC_{50} value of 6.39 µg/ml (more active), competing with Ascorbic acid (positive control) with the IC_{50} value of 6.24 µg/ml. But, n-hexane fraction (PA-02) has the lowest IC_{50} value of 81.75 µg/ml (less active), followed by chloroform fraction having the IC_{50} value of 76.22 µg/ml (also less active). The crude ethanol extract has the IC_{50} value of 17.95 µg/ml, it is therefore well understood from the experimental results involving IC_{50} value and inhibition rate that, IC_{50} value is inversely proportional to inhibition rate. The results of this study were in agreement with (Haq *et al*.,2011 and Yasoubi *et al.,* 2007) where the antioxidant activity of methanolic and water fractions were determined.

Table 1: Physical Properties and Weight of Extract Fractions of *Prosopis africana* Leaves

Solvent Extraction	Code of Fraction	Texture	Colour	Weight (g)
Ethanol (Crude)	PA-01	Sticky	Dark green	76.42
n-Hexane	PA-02	Sticky	Dark green	10.85
Chloroform	PA-03	Sticky	Dark green	1.55
Ethyl acetate	PA-04	Sticky	Dark green	6.45
Methanol	PA-05	Sticky	Dark green	53.60
Water	Residue	Crystalline	White	3.01

Table 2: Total phenolic contents evaluation of *P. africana* leaf extracts

Extract/ Fraction	Code	Total phenolic contents (mg of GAE/g)
Ethanol	PA-01	188.40 ± 0.6
n-Hexane	PA-02	14.15 ± 0.23
Chloroform	PA-03	79.74 ± 0.23
Ethylacetate	PA-04	268.49 ± 0.12
Methanol	PA-05	285.92 ± 0.22

ANTIOXIDANT ACTIVITY OF THE PLANT EXTRACTS

Table 3: Percentage Inhibition and IC_{50} Values of *P. africana* Extracts

Conc.(µg/ml) Sample & % Inhibition	1000	500	250	125	62.5	31.3	15.6	7.8	IC_{50}
PA-01	94.6	96.6	97.1	96.6	75.0	45.0	42.0	39.9	17.95
PA-02	97.3	96.3	73.2	56.6	35.9	21.8	9.9	14.8	81.75
PA-03	96.1	95.7	87.5	52.3	25.8	16.4	17.2	21.7	76.22
PA-04	95.7	94.0	95.6	94.2	70.4	48.4	40.9	34.5	20.31
PA-05	97.1	97.0	94.2	76.7	81.1	63.2	54.4	61.1	7.39
ASCOBIC ACID	98.0	98.4	97.7	97.8	97.6	97.6	94.0	75.3	6.24

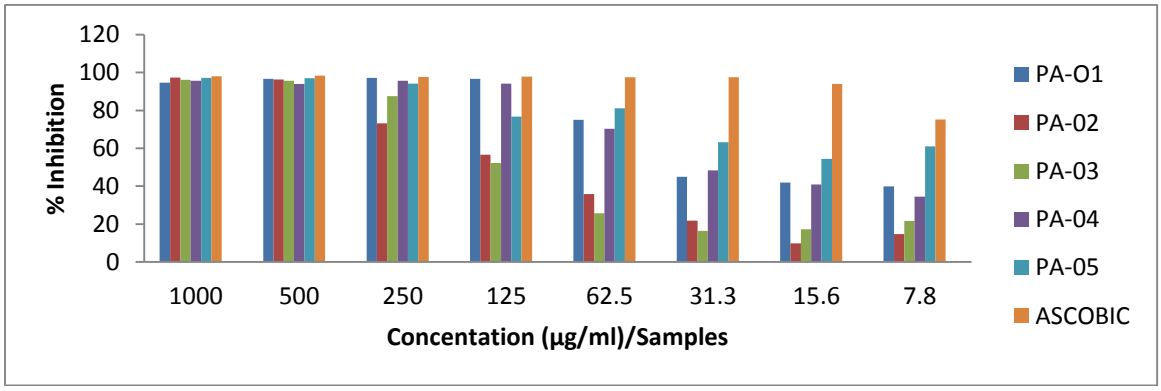

Fig 1: Antioxidant Activity (percentage (%) inhibition against concentration) of the Plant Extracts

CONCLUSION

After cold percolation and maceration processes. The results of *Prosopis africana* leaf extract and fractions show that there are significant amount of phenolic compounds and shows an effective antioxidant activity, supporting medicinal properties of the plant leaves in treatment of cancer diseases of different kinds and other oxidative stress related diseases.

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