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In Vitro Antioxidant Activity of Aqueous Root Extract of *Parkia biglobosa, Anogeissus ledocarpus* and *Moringa oleifera* on Erythrocytes Exposed to Oxidative Stress

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Abstract
The in vitro study aimed at determining the antioxidant, scavenging activity and lipid peroxidation of aqueous root extract of *Pakia biglobosa, Anogeissus leiocarpus and Moringa oleifera* on oxidatively stressed human erythrocytes. The investigation shows that *Pakia biglobosa* aqueous root extract at varying concentrations (1.5mg/ml, 3mg/ml and 6mg/ml) shows significant antioxidant activity (13.94±0.05, 23.65±0.07 and 20.69±0.17) respectively compared to the control sample (11.80±0.10). *Anogeissus leiocarpus* aqueous root extract malondialdehyde formation at concentrations (3mg/ml and 6mg/ml) also shows scavenging activity and antioxidant potential in protecting the cell from oxidative stress (19.49±0.13 and 31.61±0.07) respectively while at 1.5mg/ml concentration of the extract it shows no significant effect compared to the control sample. Analysis of *Moringa oleifera* aqueous root extract shows protective effect at 6mg/ml (17.46±0.13) only while concentrations (1.5mg/ml and 3mg/ml) showed no antioxidant and scavenging activity thus rendering the cell vulnerable to oxidative stress. Hence root extract of these medicinal plants have been used traditionally and are employed for the treatment of metabolic and nonmetabolic diseases.

Keywords: Antioxidant, *Pakia biglobosa, Anogeissus leiocarpus, Moringa oleifera*

1. Introduction
Oxidative stress refers to the phenomena in which over-production of free radicals, reactive oxygen species (ROS) or inactivation of the antioxidant usually shift antioxidant balance/ROS in favor of stress (Wiernsperger, 2003) [23]. Oxidative stress is a factor involved in the pathogenesis of many diseases including hypertension, cardiovascular diseases, diabetes mellitus, and other related metabolic syndromes (Ceriello and Motz, 2004; Takayanagi *et al.,* 2000) [4, 18].

Medicinal plants and herbs are known for their wide variety of phenolic compounds such as flavonoids that act potentially as an antioxidant to scavenge free radicals, ROS and inhibit lipid peroxidation (Kumawat *et al.,* 2012) [7]. Hence the benefit of medicinal plants therapeutically has been attributed to its antioxidant properties (Nayak *et al.,* 2006) [14] and as a result they have been utilized economically as antioxidant additives or nutritional supplements or are explored for novel antioxidants.

These however explains the use of medicinal plants for the treatment of various diseases and why it continued to be an important component of the healthcare delivery system most especially in Africa where over 5400 plants were reported to have over 16,300 medicinal properties and uses (van Wyk, 2008) [22].

Parkia biglobosa (Jacq.) (Mimosaceae) called the African locust bean tree is native to Nigeria and other West African countries. The different parts of *P. biglobosa* are used by traditionalist and herbal medicine healers to treat several metabolic and some nonmetabolic disorders like haemorrhages, hypertension and dermatosis (Tokoudagba *et al.,* 2010; Udobi and Onaolapo, 2009) [19, 21]. Recent survey on the ethno-pharmacology carried out in the northern parts of Nigeria revealed that the stem bark extract was among the commonly used plants used for the treatment of diabetes mellitus by traditional healers (Etuk *et al.,* 2010) [5]. Antidiarrheal (Agunu *et al.,* 2005) [2], antibacterial (Millogo-Kone *et al.,* 2008) [10] and wound healing (Adetutu, *et al.,* 2011) [1] activities of extract from different parts of the plant was ascertain.

Anogeissus leiocarpus belongs to the family Combretaceae (common name: Axle-wood tree).

Anogeissus leiocarpus is employed medically in the treatment of gonorrhea, body pain, asthma, blood clots, coughing, tuberculosis and ascaricide (Mann *et al.,* 2008) [8]. In Nigeria, the plant is used as an antimicrobial agent against some bacterial infections. In eastern part of Nigeria, the leaves are used externally as decoction for the treatment of skin diseases and itch of psorisis. The powdered bark mixed with 'green clay' and applied as an unusual face mask for serious blackheads. The powdered bark is also applied to wounds, sores, boils, cysts and diabetic ulcers for good results (Mann *et al.,* 2008) [8]. Traditionally *Anogeissus leiocarpus* is effective in treating infectious wounds in man and animals.

Moringa oleifera Lam. is a member of the family Moringaceae, a small-medium sized tree 10-15m tall. It is widely distributed in West Africa, East and Southeast Asia and the West Indies. *M. oleifera* various parts are have been reported to possess pharmacological properties. (Ghasi *et al.,* 2000; Mehta *et al.,* 2003) [6, 9] reported that the leaves and fruits possess hypocholesterolemic activity in Wister rats and rabbits respectively. Additionally the leaves are rich source of beta-carotene, vitamin C, E and polyphenolics (Nambiar Seshadri, 2001) [13]. In Thailand, hot water extract of the dried roots was taken orally as a cardiotonic and as a stimulant against fainting while the tender pods, fruits and leaves are consumed as vegetables for more than 100years.

These plants were selected because of their medicinal properties and continued traditional use in the treatment of several diseases. Thus, the aims of the present study are to determine the *in vitro* protective effect and antioxidant activities of aqueous root extract of *Pakia biglobosa, Anogeissus leiocarpus and Moringa oleifera* on oxidatively stressed erythrocytes.

2. Materials and Methods

2.1 Preparation of crude extracts: Root barks of *Pakia biglobosa, Anogeissus leiocarpus and Moringa oleifera* were collected from around Sangere village, Girei Local Government Area, Adamawa State of Nigeria. The plants were first identified by a botanist from Modibbo Adama University of Technology, Yola before the root were collected. They roots were washed, chopped into small pieces shade dried and pulverized with laboratory mortar and pestle, followed by sieving with Endicott's test sieve to obtain a fine powder. 50g of the fine powder was extracted in a soxhlet with 200ml MeOH for 24 hours. It was then filtered; the filtrate was evaporated in a vacuum below 40 ^{0}C on a rotary evaporator. The final dry weight of the solid extract was used to estimate the yield (g/kg) of each plant, the main values was presented and also stock solution was prepared from these plant extracts by dissolving the dried solid extracts in dimethyl sulfoxide (DMSO) final concentration not exceeding 0.2% prior to being diluted with phosphate buffered saline (PBS), all solutions were stored in a refrigerator at 4^{0}C until use.

2.2 Cell separation: Blood samples from healthy human volunteers were collected in sterile heparinised glass tubes. Erythrocytes were separated by centrifugation (3000 rpm for 10 min) at 4 °C. The red cells were then washed three times with 5 volumes of phosphate buffered saline (PBS). Isolated erythrocytes were divided into appropriate aliquots for various treatment schedules. The blood was stressed by applying oxidative reagent.

2.3 Study design: The erythrocyte fraction was divided into four groups; in each group three samples were processed. Group I - Control (untreated erythrocytes); Group II - Erythrocytes treated with 1.5mg/ml of root extract for 20 min. at 37 °C; Group III - Erythrocytes treated with 3mg/ml of root extract for 20 min. at 37 °C and Group IV - Erythrocytes treated with 6mg/ml of root extract for 20 min. at 37 °C (Trotta, 1982) [20]. The various parameters were examined in the haemolysate.

2.4 Biochemical assay: Lipid peroxides in terms of malondialdehyde (MDA) were determined by thiobarbituric acid reaction as described by (Ohkawa *et al.,* 1979) [15]. The reduced glutathione (GSH) Moron *et al.,* (1979) [12], superoxide dismutase (SOD) (Misra and Fridovich, 1972), catalase (CAT) Bergmeyer *et al.,* (1974) [3] and glutathione peroxidase (GPx) Rotuck *et al.,* (1973) [16]. Calculated data were statistically analysed and compared with that of the student's t-test, taking $P<0.05$ as significant.

3. Result

The antioxidant activity of *Pakia biglobosa, Anogeissus leiocarpus and Moringa oleifera* (table 1) shows that the levels of lipid peroxidation (malondialdehyde formation) are significant and possess antioxidant potentials at various concentration of the root extract. *Pakia biglobosa* aqueous root extract at concentrations (1.5mg/ml, 3mg/ml and 6mg/ml) shows protective effect and antioxidant activity compared to the Control sample (untreated). *Anogeissus leiocarpus* shows antioxidant activities at concentration (3mg/ml and 6mg/ml) while the lipid peroxidation shows no significant effect at concentration (1.5mg/ml) compared to the untreated sample. *Moringa oleifera* root extract compared to the control sample proofs to have significant effect and antioxidant activity only at the highest concentration (6mg/ml) while concentrations (1.5mg/ml and 3mg/ml) shows no significant levels of malondialdehyde formation.

Table 1: Malonyldiahyde Formation in Human erythrocytes (nmol/h) at Concentration 1.5mg/ml, 3mg/ml and 6mg/ml of Aqueous Root Extract

Plant Extract	Untreated Cell (Control)	Treated Cell (1.5mg/ml)	Treated Cell (3mg/ml)	Treated Cell (6mg/ml)
Parkia biglobosa	11.80±0.10	13.94±0.05	23.65±0.07	20.69±0.17
Anageissus ledocarpus	17.69±0.08	12.42±0.14	19.49±0.13	31.61±0.07
Moringa oleifera	13.31±0.08	6.31±0.14	10.53±0.10	17.46±0.13

Values are in mean ± SEM, n=3

4. Discussion

Erythrocytes are model for assessing the potentiality of drugs and are very sensitive to toxic influences. This is because of the high oxygen tension, presence of polyunsaturated lipid and suitability components to oxidative stress. They present study assessed the antioxidant potential of the aqueous root extract of *Pakia biglobosa, Anogeissus leiocarpus and Moringa oleifera* on erythrocytes explosed to oxidative stress. *Pakia biglobosa* aqueous root extract malondialdehyde formation exact antioxidant activities at the various concentration of the extract (1.5mg/ml, 3mg/ml and 6mg/ml) compared to the control cell concentration (11.80±0.10). This may be due to the high antioxidant activities and content of *parkia biglobosa extract* in treating different metabolic diseases as reported by (Udobi and Onaolapo, 2009) [21]. Reports in literatures (Mann *et al.,* 2008) [8] showed that *Anogeissus leiocarpus* leaf extract (decoction) have were used in the treatment skin diseases and

itch of psoriasis. The result showed that malonyldiahyde formation aqueous root extract of the plant possess antioxidant activity at concentrations of 3mg/ml and 6mg/ml of the extract while extract at 1.5mg/ml showed no protection compared to the untreated cell (17.69±0.08).

Moringa oleifera malondialdehyde formation showed that erythrocyte was protected only at concentration (6mg/ml) of the extract and malondialdehyde formation (17.46±0.13) while extract concentration (1.5mg/ml and 3mg/ml) and malondialdehyde formation (6.31±0.14 and 10.53±0.10) respectively showed no protection and antioxidant activity compared to the untreated cells (13.31±0.08). Several reports showed that *M. oleifera* has pharmacological actions, particularly the aqueous root extract was reported to have been used extensively in treating inflammation and cardiovascular diseases and hot water extract of the dried roots was taken orally as a stimulant against fainting. The plant named 'wonder tree' in Thailand was due its therapeutic potentials against cancer, diabetes and other diseases (Rukumnuaykit, 2004) [17].

5. Conclusion

The present study evaluates the antioxidant activity in aqueous root extract of three medicinal plants. The result of the malondialdehyde formation shows that the tested medicinal plants have potent antioxidant activity and free radical scavenging activity at varying concentrations. Although the great benefit of the antioxidant potential will be from the consumption of the plants parts either as vegetables or its decoction. However, more detailed analysis may be carried out to determine the exact chemical compositions and to characterize them as biological antioxidants which are beyond the scope of this study.

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