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DETERMINATION OF ANTIOXIDANT CAPACITY AND ANTIMALARIAL ACTIVITIES OF UNRIPE COCONUT WATER IN BLOOD SERUM

*Onyeyilim EL[1], Ezema BE[1] and Alumona TN[1]
Research on Natural Product and Medicinal Chemistry, Department of Pure and Industrial Chemistry
University of Nigeria, Nsukka, 41002, Nigeria

ABSTRACT

The aim of the work is to investigate the antioxidant capacity and antimalarial activities of unripe coconut water. Wistar Albino mice of either sex weighing 20–34 kg were housed in separate cages, acclimatized for one week and then divided into five groups of five mice each. Group 1 (positive control): was inoculated with malaria parasite (Mp^+) and treated with 5 mL/kg body weight of normal saline. Group 2 (normal control) was not inoculated with malaria parasite (Mp^+) and treated with 5 mL/kg body weight of normal saline. Group 3 (standard control) was inoculated with malaria parasite (Mp^+) and treated with 5 mg/kg body weight of Artesunate (standard drug) whose mechanism of action is known. Group 4 was inoculated with malaria parasite (Mp^+) and treated with 200 mL/kg body weight of the unripe coconut water. Group 5 was inoculated with malaria parasite (Mp^+) and treated with 300 mL/kg body weight of the unripe coconut water. It was observed that unripe coconut water has antimalarial activities since it produced similar effect with Artesunate drug which served as the standard reference drug. Unripe coconut water was observed to have good antioxidant capacity in the serum part of the blood.

Keywords: Abino mice, unripe coconut water, malaria parasite (Mp^+), vitamin c and vitamin e.

INTRODUCTION

A natural product is a chemical compound or a substance produced by a living organism that is found in nature. Within the field of organic chemistry, the definition of natural products is usually restricted to mean purified organic compounds isolated from natural sources (Bhagya *et al*., 2010). Natural products sometimes have pharmacological or biological activity that can be of therapeutic benefit in treating diseases. As such, natural products are active components not only for most traditional medicines but also for many modern medicines (Antherden, 1999).

Unripe coconut water is one of the world's most versatile natural products with increasing scientific evidence that support its role in health and medicinal application (Sofowora, 1984; Ghasemzadeh *et al*., 2010). Despite the use of the various parts of coconut palm in herbal medicine, reports on antimalarial activities and antioxidant capacity of unripe coconut water are rather scanty. Hence it is imperative to examine its antimalarial activities and its antioxidant capacity in blood serum.

MATERIALS AND METHODS

Sample collection

Samples of unripe coconut were collected from the department of biochemistry in the University of Nigeria, Nsukka campus, authenticated at the Department of Plant Science and Biotechnology. The mesocarp was carefully removed to get the endocarp which habours the clear liquid of unripe coconut water. This clear liquid was extracted from the endocarp with the aid of a syringe into a clean container.

Inoculation of the Parasitaemia

Parasitaemia was maintained in the laboratory by the method of David *et al*. (2006) and Ochei and Kolhatkar (2007). Ten drops of the parasitized blood obtained with the aid of a capillary tube through the ocular region of the mice, was diluted with normal saline (1 mL). Thereafter diluted parasitized blood (0.2 mL) was used to infect the three mice that served as the host from where other experimental animals were infected.
Group 1 (positive control): was inoculated with malaria parasite (Mp^+) and treated with 5 mL/kg body weight of normal saline.

*Corresponding author e-mail: onyeyilimebuka@gmail.com

Group 2 (normal control): was not inoculated with malaria parasite (Mp^+) and treated with 5 mL/kg body weight of normal saline

Group 3 (standard control):was inoculated with malaria parasite (Mp^+) and treated with 5 mg/kg body weight of Artesunate (standard drug)
Group 4**:** was inoculated with malaria parasite (Mp^+) and treated with 200 mL/kg body weight of the unripe coconut water
Group 5: was inoculated with malaria parasite (Mp^+) and treated with 300 mL/kg body weight of the unripe coconut water.

Determination of malaria parasite (Mp^+)
The determination of the malaria parasite (Mp^+) was carried out according to the method of Dacie and Lewis (2006). A pair of scissors was used to cut the tail which was squeezed gently to obtain a small drop of blood that was placed on the centre of a microscopic slide. Immediately the thin film was spread using a smooth edged slide spreader. The slide was labeled with a black lead pencil. This method was repeated for different infected mice. The slide with the blood was stained using Leishman's stain (in order to differentiate the parasite from the red blood cell when viewed under microscope), sprinkled with water after 2 min of the stain and air–dried in horizontal position.

The Haematological test
Blood was collected from the animal into an EDTA tube to prevent coagulation of the blood sample. The collected blood samples were put in a capillary tube which were centrifuged for 5 min at the speed of 4000 rpm (revolution per minute) to separate the blood from the serum.

Determination of Total white blood cell count
Reagents: Turks solution, Acetic acid, Gentian Violet (G.V) and distilled water. Turks solution was prepared by mixing distilled water (49 mL) with acetic acid (1 mL) and with a drop of G.V. Turks solution destroys red blood cells within a blood sample and stains the nuclei of the white blood cells, making it easier to see and count.

Procedure
Turks solution (380 µL) was pipetted into an empty EDTA tube and the blood sample (20µL) was also pipetted into the same tube and shaken. The count was done with the aid of a microscope.

Determination of total red blood cell count
Reagents: Normal saline, EDTA tube and distilled water. Normal saline was prepared by dissolving NaCl (9g) in distilled water and making up to $1000cm^3$ with distilled water.

Procedure
Normal saline (2 mL) was pipetted into an empty EDTA tube and blood sample (4 mL) was added into the tube and shaken. The count was done with the aid of a microscope.

Determination of Haemoglobin (Hb) concentration
Reagents: EDTA tube, Drabkin's solution and distilled water.

Procedure
Drabkin's solution (4 mL) was added into an empty EDTA tube after which blood sample (20 mL) was also added to the same tube and shaken. The mixture was allowed to stay for 10 min before taking the absorbance at the wavelength of 540 nm.

Determination of Superoxide Dismutase (SOD)
Reagents: a) Phosphate buffer (0.05 M) pH 7.8. This was prepared by dissolving K_2HPO_4(6.97 g) and KH_2PO_4(1.36 g) in distilled water and making up to 1000 mL with distilled water. The pH was adjusted to 7.8
b) Adrenaline solution (0.059 %)
This was prepared by dissolving adrenaline (0.01 g) in distilled water (17 mL)
c) The blank was prepared with adrenaline (0.3 mL) in buffer (25 mL)

Procedure for the determination of SOD
The post mitochondrial fractions were properly diluted. Each of the diluted sample (2 mL) was added to phosphate buffer (2.5 mL, 0.05 M) pH 7.8. The mixture was equilibrated in the spectrophotometry before adding adrenaline solution. The reaction started with the addition of freshly prepared adrenaline solution (0.3 N) to the mixture followed by quick mixing by inversion in the cuvette. The reference cuvette therefore contains buffer (2.5 mL), adrenaline (0.3 mL) and extract (0.2 mL). Increase in absorbance was taken at 450 nm for 150 sec at 30 sec interval.

Determination of Vitamin E
Reagents: Ethanol, ferric Chloride (0.2 %) in ethanol and distilled water

Procedure
Extract (0.1 mL) was added to ethanol(2 mL). Solution (1 mL) of ethanol and liquid extract was pipetted and ferric Chloride (1 mL, 0.2%) in ethanol was added. The solution was diluted to 5 mL with distilled water and the absorbance was measured at the wavelength of 520 nm.

RESULTS AND DISCUSSION

Percentage Parasitaemia

Table 1. Four Days After Inoculation of Malaria Parasite.

	GRP 1(UN)	GRP 2 (NC)	GRP 3 (SC)	GRP4 (200 mL/kg)	Grp5 (300 mL/kg)
1	3^+	0	5^+	4^+	5^+
2	4^+	0	6^+	4^+	7^+
3	3^+	0	4^+	5^+	4^+
4	2^+	0	7^+	7^+	6^+
5	2^+	0	3^+	3^+	2^+
AP	2.8^+	0	5.6^+	4.6^+	4.8^+

Table 2. Seven Days Treatment of Malaria Parasite.

S. No.	GRP1(UN)	GRP 2 (NC)	GRP 3 (SC)	GRP4(200mL/kg)	Grp5 (300 mL/kg)
1	5^+	0	2^+	2^+	2^+
2	5^+	0	2^+	1^+	4^+
3	6^+	0	1^+	3^+	1^+
4	4^+	0	3^+	4^+	3^+
5	5^+	0	2^+	1^+	0^+
AP	5^+	0	2^+	2.2^+	2^+

An understanding of the life cycle of malaria parasite is the fundamental method of treatment and eradication of the disease. Malaria is a mosquito-borne, caused by unicellular protozoan parasites of the genus plasmodium and transmitted only by the female Anopheles mosquito (Bremen, 2001).

After inoculation of parasitaemia, the parasite enters into the bloodstream of the host in the form of haploid sporozoite (Trape, 1985). It immediately moves to the liver and invades the liver cell (hepatocytocyte) where it reproduces by mitosis (Wander *et al*., 2009). The sporozoite transforms into schizont which contains thousands of haploid cells called merozoits. The merozoits that are released from the ruptured hepatocyte or liver cell into the blood stream which quickly invade erythrocytes stage of malaria (Farnsworth, 1984). The parasites matures into trophozoites that feed on the haemoglobin found in the erythrocytes resulting in high increase of average parasitaemia from 2.8^+ to 5^+ as recorded in GRP1 (UN) of Table 1 and 2, respectively.

Most antimalarial drugs and natural products with antimalarial activities are stage-specific blood schizonticides (Gen and Lin, 1986), since they act principally on the mature trophozite stage of parasite development. Unripe coconut water is believed to be one of the natural products with antimalarial properties because it has the ability to lower the level of malaria parasite in the blood and also produced similar effect with Artesunate which served as the standard reference drug. This is illustrated in Table 2 in Groups 3,4 and 5 above.

Table 3. Total White Blood Cell count (mL/mg).

GRP1(UC)	GRP2(NC)	GRP3(SC)	GRP4(200mL/kg)	GRP5(300 mL/kg)
10800	10300	10200	10200	10600
10600	10100	10400	10600	10100
10400	10200	10300	10400	10100
10600	10400	10100	10100	10000
10700	10200	10500	10000	10400
Av=10620	Av=10240	Av=10300	Av=10260	Av=10240

White blood cells are also known as leukocytes or immune cells. They are cells which form a component of the blood. They help to defend the body against infectious disease and foreign materials as part of the immune system (Gutteridge, 1989). GRP1 in Table 3 showed an increase in the number of white blood cells when compared to GRP2, the normal control. The reason may be due to the fact that more white blood cells are produced in a bid to defend the body system against diseases and infection that was caused by the parasite in the blood. Alternatively, GRPS 3, 4 and 5 showed a decrease in the number of white blood cells when compared to GRP 1**.** This decrease may be due to the death of the parasite that caused the malaria.

Table 4. Total Red Blood Cell Count (mL/mg).

GRP1(UC)	GRP2(NC)	GRP3(SC)	GRP4(200 mL/kg)	GRP5(300 mL/kg)
5.31	8.14	6.66	6.45	10.61
4.61	10.31	10.28	7.92	8.45
4.93	7.69	9.08	8.92	10.42
5.73	7.54	7.49	9.45	7.41
4.80	6.72	8.36	7.66	6.35
Av=5.08	Av=8.08	Av=8.37	Av=8.08	Av=8.65

Table 5. Haemoglobin Concentration (mL/mg).

GRP1(UC)	GRP2(NC)	GRP3(SC)	GRP4(200m/Lkg)	GRP5(300 mL/kg)
9.357	11.923	16.817	10.121	15.442
7.269	11.371	15.714	14.205	14.867
9.049	10.755	11.872	13.750	17.749
8.650	12.458	10.366	10.463	11.457
9.748	11.546	10.457	11.578	10.248
Av=8.815	Av=11.611	Av=13.045	Av=12.023	Av=13.953

Haemoglobinis a component of the red blood cell. It is the iron-containing substance in the red blood cells that transports oxygen from the lungs to the rest of the body. It consist of a protein (globin) and haem (a porphyrin ring with an atom of ironat its centre (Epstein et al., 1998).When the parasite that causes malaria infects a red blood cell, it consumes haemoglobin within its digestive vacuole which results in decrease in the number of red blood cells and haemoglobin concentration as seen in Tables 3 and 4 for GRP1 (UC). As the parasites that consume haemoglobin in the red blood cell die as a result of antimalarial effect exerted by the standard drug and the unripe coconut water, the number of red blood cells and haemoglobin concentration increased above the normal control (GRP2). This effect is most pronounced in GRP5 in Tables 3 and 5.

Table 6. Superoxide Dismutase (µmol/L).

GRP1(UC)	GRP2(NC)	GRP3(SC)	GRP4(200 mL/kg)	GRP5(300 mL/kg)
26.375	89.370	89.376	66.926	72.427
30.337	76.397	79.387	74.344	70.046
34.391	50.677	82.397	70.404	78.593
32.814	56.805	65.452	67.655	69.667
28.976	48.967	70.667	65.789	63.578
Av=30.579	Av=64.443	Av=69.024	Av=69.023	Av=70.862

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SOD is an enzyme that alternatively catalyze the partitioning of superoxide (O_2^-) radical to either O_2 or H_2O_2 (Mc cord, 1969, 1998). Groups 4 and 5 in Table 6 showed increase in SOD activities when compared to group 2. This shows that the unripe coconut water has good SOD activities in the blood serum. Alternatively Group 1 in Table 6 showed a decrease in SOD activities when compared to groups 2, 3, 4 and 5. This agrees with the investigation of (Mc cord, 1998). Where this decrease may be attributed to the exhaustion of SOD in a bid to scavenge excess production of reactive oxygen caused by oxidative stress of the malaria parasite.

Table 7. Vitamin E (µmol/L).

GRP1(UC)	GRP 2 (NC)	GRP3(SC)	GRP4(200 mL/kg)	GRP5(300mL/kg)
0.409	0.665	0.554	0.476	0.532
0.476	0.632	0.620	0.576	0.587
0.404	0.654	0.609	0.643	0.576
0.413	0.644	0.509	0.587	0.622
0.402	0.661	0.600	0.698	0.501
Av=0.421	Av=0.651	Av=0.578	Av=0.596	Av=0.564

Vitamin E is an important antioxidant that protects unsaturated oil from being destroyed in the body by oxygen and also a potent water-soluble antioxidant in humans. Group 1 in Table 7 showed a decrease in vitamins E concentration when compared to GRP 2. This agrees with the investigation of (Das et al., 1996) where these decrease may be attributed to the exhaustion of these antioxidants in a bid to scavenge excess production of reactive oxygen caused by oxidative stress of the malaria parasite. Groups 4 and 5 in Table 7 showed increase in vitamin E concentration when compared to group 2. These increase show that the unripe coconut water has appreciable vitamins E activity in the blood serum.

CONCLUSION

Unripe coconut water is believed to be one of the natural products with antimalarial properties because it has the capacity to reduce the level of malaria parasite in the blood serum and also sproduced similar effect with Artesunate which served as the standard reference drug. This unripe coconut water is seen to have good antioxidant capacity in the serum part of the blood.

REFERENCES

Antherden, LM. 1999. Textbooks of Pharmaceutical Chemistry. (6th edi.). Oxford University Press London. 0-814.

Bremen, J. 2001. The ears of the hippopotamus: manifestation, determinants, and estimates of the malaria burden. Am. J. Trop. Hyg. 64(1, 2 S):1-11.

Bhagya, D., PremaL, RT. 2010. Beneficial effect of tender coconut water on blood pressure and lipid levels in experimental hypertension. Journal of Cell and Tissue Research. 10(1):2139-2144.

Das, BS., Thurnham, DI. and Das, DB. 1996. Plasma alpha-tacopherol, retinol and carotenoids in children with Falciparum malaria. Am. J. Clin Nutr. 64:94-100.
David, K. *et al*. 2006. Inoculation and Maintenance of Parasitaemia. Bioorg Med Chem. 14:875-874.

Dacie and Lewis. 2006. Evaluation of antimalarial effects of medicinal plants extracts in male and female albino rats. Asian J Pharm Clin Res. 6:217-220.

Epstein, FH. and Hsia, CCW. 1998**.** Respiratory Function of Haemoglobin. New England Journal of Medicine. 338(4):239-247.

Farnsworth, NR. 1984. The role of medicinal plants in drug development. 17-30

Ghasemzadeh, A., Jaafar, HZE. and Rahmat, A**.** 2010. Antioxidant activities, total Phenolics and flavonoids content in two varieties of Malaysia Young Ginger (*Zingiber officinale* Roscoe). Molecules. 15:4324-4333.

Gen, XP. and Lin, FS. 1986. Traditional antiparasitic drugs in china. Parasitol Today. 2:353-355.

Gutteridge, WE. 1989. parasite vaccines, versu anti-parasite drugs rivals. Parasitol. 98:587-597.

Mc cord, JM Fridovich. 1969. Superoxide dismutase An enzyme function for erythrocuprein. The Journal of Biological Chemistry. 224(22):6049-55.

Mc cord, JM, Fridovich. 1998. Superoxide dismutase: The first twenty years (1968-1988). Free Radical Biology and Medicine. 5(5-6):363-9.

Ochei, J. and Kolhatkar, A. 2007. Care and use of experimental animals. In: Medical Laboratory Science Theory and practice. Tata McGraw-Hill India. 1213-1232.

Sofowora, A. 1984. Medicinal Plants and Traditional Medicine in Africa. John Wiley and Sons. Ltd, London. 100-102.

Trape, JF. 1985. Rapid evaluation of malaria parasite density and standardization of thick smear examination for epidemiological investigations. Trans. R. Soc. Trop. Med. Hyg. 79(2):181-184.

Wander, K., Shell-Duncan, B. and McDade, TW. 2009. Evaluation of health of the liver and kidney deficiency

as a nutritional adaptation to infectious disease: An evolutionary medicine perspective. Am. J. Hum. Biol. 21:(2):172.

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