International Journal of Research (IJR)
e-ISSN: 2348-6848, p- ISSN: 2348-795X Volume 2, Issue 3, March 2015
Available at http://internationaljournalofresearch.org

In Vitro Human Red Blood Cell Membrane Stabilizing Potentials and Phytochemical Screening of Water Extract of Zingiber Officinale (Ginger) Rhizome

Adebayo Ayub-Eniola Ayodele[1a*]; Haruna Baba[2]; Adelowo Kayode Adekunle[3]; Halima Idris[4]; Fatima Baba Sanda[1b]& Maryam Yusuf Musa[1c]

[1a]Department of Chemical Pathology, University of Maiduguri Teaching Hospital, PMB 1414, Maiduguri. Nigeria. Email: ayodeleadebayo95@yahoo.com

[1b]Department of Chemical Pathology, University of Maiduguri Teaching Hospital, PMB 1414, Maiduguri. Nigeria. Email: afnansanda@yahoo.com

[1c]Department of Chemical Pathology, University of Maiduguri Teaching Hospital, PMB 1414, Maiduguri. Nigeria. Email: mairomym@gmail.com

[2]Department of Medical Laboratory Sciences, College of Medical Sciences, University of Maiduguri PMB 1069, Maiduguri. Nigeria. Email: hba92@yahoo.com

[3]Department of Immunology, University of Maiduguri Teaching Hospital, PMB 1414, Maiduguri. Nigeria. Email: kemkaypm@gmail.com

[4]Department of Haematology, University of Maiduguri Teaching Hospital, PMB 1414, Maiduguri. Nigeria. Email: halimaidris2010@yahoo.com

Corresponding Author: Adebayo Ayub-Eniola Ayodele[1*]

Department of Chemical Pathology, University of Maiduguri Teaching Hospital, PMB 1414, Maiduguri. Nigeria. Email: ayodeleadebayo95@yahoo.com

Abstract

Zingiber Officinale rhizome popularly call Ginger, native to Maiduguri, Northern Nigeria was assessed for water extractable bioactive phytochemicals and stabilization potentials of human erythrocyte membrane. Four water extractable secondary metabolites, flavonoids, terpenoids, cardiac glycosides, and saponnins were observed. The presence of these bioactive phytochemicals in the water extractable zingiber officinale (ginger) rhizome confirmed the folkloric use of the rhizome in the treatment of various disease states. Haemolytic activity of any herbal formulation is an indicator of cytotoxicity, antioxidant potentials and other bioactivities that can be used in pharmacological applications. Haemolytic activities on human erythrocyte showed concentration dependent partial haemolysis with 5.2% at the highest concentration of 1000µg/ml, an indication of its less toxicity.

Keywords: Zingiber Officinale; Phytochemicals; Human Erythrocyte; Haemolysis

International Journal of Research (IJR)
e-ISSN: 2348-6848, p- ISSN: 2348-795X Volume 2, Issue 3, March 2015
Available at http://internationaljournalofresearch.org

Introduction.

Zingiber Officinale popularly call Ginger, holds an important place in food spices and has been universally used throughout history for its health benefits Chopra et al 1982. It originated from China and India over 4000 years ago, where it liked for its sharp and spicy flavour in cooking (Akram et al 2011). Zingiber Officinale products are made from fresh or dried ginger rhizome or steam distillation of the oil. Zingiber Officinale is a well known herbal medicine all over the world Akram et al (2011), in Nigeria, it is known by different tribes as, Atale in Yoruba, Chitta meyasha in Hausa and Chimbaduwan in Phabur. Zingiber officinale has many phytonutrients and has aromatic and pungent taste, the rhizome of Zingiber officinale is commonly used in herbal prescriptions, it is said to contains Essential oils especially gingerol and zingiberene and pungent principles: zingerone, gingerol and shogaol (Yamahara, 1985).

For centuries, zingiber officinale has been prescribed for the treatment of headache, nervous diseases, rheumatoid arthritis, gout, nausea and vomiting without any side-effects (Badreldin el al, 2008). In Northern Nigeria, aqueous extract of Zingiber officinale is a popular beverage drink among the indigene particularly during dry season where the drink is referred to as 'Magani Rhana' (Medicine for heat).

An herbaceous rhizomatous perennial, reaching up to 90cm in height under cultivation. Rhizomes are aromatic, thick lobed, pale yellowish, bearing simple alternate distichous narrow oblong lanceolate leaves. The herb develops several lateral shoots in clumps, which begin to dry when the plant matures. Leaves are long and 2-3cm broad with sheathing bases, the blade gradually tapering to a point. Inflorescence solitary, lateral, radical pedunculate oblong, cylindrical spikes. Flowers are rare, rather small, calyx superior, gamosepalous, three toothed, open splitting on one side, corolla of three subequal oblong to lanceolate connate greenish segments (Kawai, 1994).

Traditional medical herbs used for therapeutic purposes are known to have many phytochemicals for human consumption, however, environmental conditions such as soil type, rainfall, agricultural activity, vicinity and type of industry influence the level of their bioavailability in plants (Choudhury and Garg 2007).

Herbal medication is becoming increasingly popular world-wide, however, the indiscriminate use of herbal preparations by 70 – 80% of world's population (Lesniewicz et al 2006) without regards to the proportion of various active constituents and dosage of a particular formulation has been a major challenge facing traditional herbalists or healers. Toxicity of active molecule is a key factor in drug designing and dosage formulation, phytochemical and haemolytic screening represents a useful starting point (Eric Da Silva 2004) where primary information on the interaction between molecules and biological entity at cellular level is provided. The aim of this study is to investigate and document the water extractable phytochemical constituents and human erythrocyte membrane stabilization potentials of Zingiber officinale rhizome found in Maiduguri, Northeastern Nigeria which to our knowledge has not been documented before despite the popular use of the plant in tradomedical formulations.

METHODS AND MATERIALS

Plant:

Fresh Zingiber officinale rhizomes were purchased at Baga Road Fruit Market in Maiduguri. Borno State, Nigeria in the month of July 2014. Maiduguri (lat 11^0 24'N, long

International Journal of Research (IJR)
e-ISSN: 2348-6848, p- ISSN: 2348-795X Volume 2, Issue 3, March 2015
Available at http://internationaljournalofresearch.org

11^0 8'E) is in Sahel belt with raining season spanning the period between June/July and September/October. Zingiber officinale rhizome was authenticated by experts from Forestry Department of University of Maiduguri, Maiduguri, Borno State, Nigeria

Aqueous extraction:

The fresh Zingiber officinale rhizomes were washed thoroughly in tap water, followed by distilled water and then deionized water. The 500g of rhizomes were sliced and blended with 1000ml of deionized water using stainless domestic blender(Master Chef® China). The slush was filtered using filter cloth, followed by Number 1 filter paper. The filterate was freeze dried using lyophilizer (FreeZone® 4.5 Liter Freeze Dry System: Labconco, U.S.). The dried extract was kept in air tight containers and store at 4^0C for further use.

Phytochemical screening:

Phytochemical screening of aqueous water extract of Zingiber officinale rhizome was carried out for carbohydrates, soluble starch, Tannins, Phlobatannins, Glycosides, Anthraquinone, Terponoid, Cardiac Glycoside, Alkaloid and Saponnins using standard protocols Trease and Evans (2002), Sofowora (1993), Makham (1982).

Haemolytic activity:

Preparation of erythrocyte suspension. In Vitro haemolytic activity was performed by modified Spectrophotometer method WHO (1998). Five milliliter of blood was collected from healthy subject into plain specimen tube containing glass beads, swirling for about five minutes to remove fibrinogen. The defibrinated blood was centrifuged at 1500rpm for five minutes, the plasma discarded and the harvested pellet was washed three times with sterile phosphate buffer saline solution (pH 7.2) by centrifugation at 1500rpm for five minutes. The washed erythrocytes were re-suspended in normal saline solution to a concentration of 0.5%.

A freshly prepared stock solution of extract in 100ml volumetric flask of 10mg in 100ml PBS was made. Graded concentrations of 1000µg/ml; 500µg/ml; 250µg/ml; 125µg/ml; 62.5µg/ml and 31.3µg/ml were made. 1.0ml of graded concentrations were mixed with 1.0ml of suspended erythrocytes (0.5%) in separate test tubes, phosphate buffer saline and distilled water tubes as minimal and maximal haemolytic controls, incubated at 37^0C for 1-2 hours, centrifugation at 1500rpm for five minutes, the absorbance of the supernatants were measured at 540nm (Chem – 7: Erba Diagnostics Mannheim, Germany).

The percentage haemolysis by the graded concentration of the extract was calculated using

$$\% \text{ haemolysis} = (A_t - A_a / A_c - A_a) \times 100$$

Where A_t = absorbance of test sample; A_a = absorbance of minimal haemolytic sample (PBS) 0.0%; A_c = absorbance of maximal haemolytic sample (Distilled Water) 100%.

International Journal of Research (IJR)
e-ISSN: 2348-6848, p- ISSN: 2348-795X Volume 2, Issue 3, March 2015
Available at http://internationaljournalofresearch.org

Results.

Table 1: Phytochemical screening of aqueous extract of ginger rhizome

Key: + = Present; - = Not present

Phytochemicals	**Tests**	**Aqueous Extract**
Carbohydrates		
	Molisch Test (General Test)	-
	Fehling Test (Free reducing Sugar)	-
	Combind reducing Sugar	-
	Ketoses	-
	Pentoses	-
Soluble starch		-
Tannins		
	Ferric Chloride	-
	Lead Acetate	-
Phlobatannins		-
Glycosides		
	Test for free Anthraquinone (Banbger Test)	-
	Test for Combined reducing Sugar	-
Flavonoids		
	Shinoda Test	+
	Ferric Chloride	-
	Lead Acetate	-
	Sodium Hydroxide	-
Terponoids		+
Saponnins		
	Fridthrig Test	+
Cardiac Glycosides		
	Salkowski Test	+
	Leiberman-Burchard Test	+
Alkaloid		
	Dragendroff's Reagent	-
	Mayer's Reagent	-

Table I displayed the results of phytochemical screening of the water extract of ginger rhizome to contain four bioactive chemicals, flavonoids, terponoids, saponnins and cardiac glycosides.

International Journal of Research (IJR)
e-ISSN: 2348-6848, p- ISSN: 2348-795X Volume 2, Issue 3, March 2015
Available at http://internationaljournalofresearch.org

Table 2: Haemolytic activity of aqueous extract of ginger rhizome.

Aqueous Extract (µg/ml)	Percentage Haemolysis (%)
1000	5.2
500	2.8
250	2.2
125	1.7
62.5	1.5
31.3	1.3

Table 2 showed the result of haemolytic screening of the ginger rhizome , the partial haemolysis of 5.2% at the highest concentration of 1000µg/ml and the least haemolysis of 1.3% at concentration of 31.3 µg/ml

Discussion

Plants which have one or more of its organ containing substances that can be used for therapeutic purpose, are referred to as medicinal plants [Sofowara 1993]. The medicinal value of any plants lies in the phytochemical substances that produce a definite physiological action on the human body. Screening of medicinal plants for bioactive phytochemicals is important in finding potential new compounds for therapeutic use that are effective, safe with little or no side effects and ate relatively cheap. Several phytochemicals surveys have been published in litratures, Oyedepo et al 2010, Sanjeeb 2011). Our present study assessed the qualitative phytochemical composition of crude extract of Zingiber officinale rhizome native to Maiduguri, Northeastern Nigeria as evidence abound in literature implicating the influence of environmental conditions such as soil type, rainfall, agricultural activity, vicinity and type of industry on the level of their bioavailability in plants (Choudhury and Garg 2007).

Using commonly employed precipitation and coloration reaction to identify the major natural chemical groups Trease and Evans (2002), Sofowora (1993), Makham (1982), water extractable flavonoids, terponoids, saponnins and cardiac glycosides were observed (Table 1). Flavonoids referred to as Vitamin P from the mid-1930s Benthsath (1937), Mobh, Shiro (1938) to early 50s probably because of the effect they had on the permeability of vascular capillaries), but the term has since fallen out of use McNaught (1997) belong to the larger group of beneficial plant phytochemicals known as polyphenols, Hooper el at (2008), they are rich in medicine and constitute one of most of the valuable drugs. They are well-known for their multi-directional physiological effect on animals including anti-diabetic efficacy. Akram et al (2011).

Terponoids are a large class of natural phytochemical secondary metabolites primarily found in plants, marine organisms and some insects Breitmaier (2008). The bioactivity of treponoids are diverse, antimalerial, antitumor, anti-inflammatory and lead compounds for new drugs Breitmaier (2008).

Saponnins has been known for some time as compounds that can affect the integrity of biological membranes. In fact this property altering membrane potentials and increase permeability has been used as the basis for a range of assays for these substances and employed

in their therapeutic applications. However, the degree to which disruption occurs varies markedly and appears to be related to their structure Price et al., (1987).

Cardiac glycosides act physiologically on mem-Branes through ATPase Na/K pump.Erythrocyte is biconcave disc shaped, this shape provides a surface area to volume ratio and specialized cytoskeletonmade of cholesterol and phospholipids that is optimal for gas exchange tolerance to high amounts of shear force, mechanical stability and flexibility, Yang et al 2005 In hypertonic and hypotonic solutions, erythrocyte transform to various shapes which is employed in screening.

Haemolytic screening of water extractable Zingiber officinale rhizome in our study showed concentration dependent partial haemolysis of human erythrocytes (Table 2). The percentage haemolysis at the highest concentration of 1000 µg/ml employed in this study was 5.2% an indication that the Zingiber officinale rhizome is relatively safe therapeutically for humans as erythrocytes resemble the mechanism of other cells. Assessing hemolytic activity of any herbal formulation is important as it is an indicator of cytotoxicity, antioxidant potentials and other bioactivities that can be used in pharmacological applications.

Reference

[1.] Chopra RN, Chopra IC, Handa KL, Kapur LD (1982). Indigeous Drugs of India. 2nd Ed. (Reprinted). Academic Publishers, New Delhi. India.

[2.] Akram M, Ibrahim Shah, Khan Usmanghan, Mohiuddin E, Abdul Sami, Asif M, Ali Shah SM, Khalil Ahmed and Ghazala Shaheen. (2011). Zingiber officinale Roscoe (A Medicinal Plant). Pakistan Journal of Nutrition. 10(4): 399 – 400. 2011

[3.] Yamahara J. (1985). Cholagogic effect of ginger and its active constituents. J. Ethnopharmacol. 13: 217 – 225.

[4.] Badreldin HA, Gerald B, Musbah OT, Abderrahim N. (2008). Some phytocemical, pharmacological and toxicological properties of ginger (Zingiber officinale Roscoe): A review of recent research. Food and Chemical Toxicology. 46: 409 – 420.

[5.] Kawai T. (1994). Anti-emetic principles of Magnolia obovata Bark and Zingiber officinale rhizome. Planta Medica. 60: 17 – 20.

[6.] Choudhury RP, Garg AN. (2007). Variation in essential, trace and toxic elemental contents Murraya Koenigii – A spice and medicinal herb from different India states. Food Chem. 104: 1454 – 1463.

[7.] Lesniewicz A, Jaworska K, Zyrnicki W. (2006). Macro- and micro-nutrients and their bioavailability in Polish herbal medicaments. Food Chem. 99: 670 – 679.

[8.] Eric Da Silva, Patrick Shahgaldian, Anthony W Coleman. (2004). Haemolytic properties of some water-soluble *para*-sulphonato-calix-[*n*]-arenes. International Journal of Pharmaceutics. 273: pp. 57 – 62.

[9.] Trease GE and Evans WC. (2002). Textbook of Pharmacognosy, 14th Edition. WB Saunders Company London.

13 -53, 117 – 139, 227, 293 – 334, 471 – 511.

[10.] Makham KK. (1982). Technique of flavonoids identification. Academic Press, New York. USA. 1 – 113.

[11.] Sofowara A. (1993). Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd, Ibadan. Nigeria. 289.

[12.] Quality control methods for medicinal plant materials. (1998). World Health Organization. Geneva.

[13.] Oyedepo OO, Akinpelu BA, Akinwunmi KF, Adeyinka MO and Sipeolu FO. (2010). Red Blood Cell membrane stabilizing potentials of extracts of Lantana camara and its fraction. International Journal of Plant Physiology and Biochemistry. 2(4): 46 – 51.

[14.] Sanjeeb Rao. (2011). Phytochemical composition and in vitro Hemolytic activity of Lantana Camara L. (Verbenaceae) Leaves. Pharmacologyonline. 1: 59 -67.

[15.] Benthsath, A.; Rusznyak, S. T.; Szent-Györgyi, A. (1937). "Vitamin P". *Nature* **139** (3512): 326–327.

[16.] Mobh, Shiro (1938). Research for Vitamin P. *The Journal of Biochemistry* **29** (3): 487–501.

[17.] McNaught Alan D; Wilkinson, Andrew; IUPAC (1997). IUPAC Compedium of Chemical Terminology. (2nd ed.). Oxford: Blackwell Scientific.

[18.] Hooper L, Kroon PA, Rimm EB, Cohn JS, Harvey I, Le Cornu KA, Ryder JJ, Hall WL, Cassidy A. (2008). Flavonoids, flavonoid-rich foods and cardiovascular risk: a meta-analysis of randomized controlled trials. American Journal of Clinical Nutrition. 88: 38-50.

[19.] Breitmaier E (2008). In: Terpenes; Flavors, Fragrances, Pharmaca, Pheromones. (1st Ed.) Wiley-VCH, Verlag GmbH & Co. Germany.

[20.] Price KR, Johnson 1. T. and Fenwick G. R. (1987) The chemistry and biological significance of saponins in foods and feeding stuffs. Critical Reviews in Food Science and Nutrition 26: 27-135.

[21.] Yang ZG, Sun HX, Fang WH. (2005). Haemolytic activities and adjuvant effect of Astragalus membrenaceus saponin (AMS) on immune responses to ovalbumin in mice. Vaccine. 23(44): 5196 – 5203.