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Medicinal values of *Elaeis guineensis* and *Raphia hookeri* wines

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ABSTRACT:

The medicinal values of fresh *Raphia hookeri* and *Elaeis guineensis* wines were evaluated. Face-to-face interview questionnaire-based ethno-medical survey on 1000 randomly selected families in some southeastern and southsouthern states in Nigeria on the use of the palm wines as antimicrobial agents, vehicles for antimicrobial agents, galactogogues in postpartum mothers and prophylactic agents against malaria in ethno-medicine were carried out. The presence of bioactive phytochemical and biochemical constituents with reported pharmacological activities were detected and their biochemical modes of action were proposed. In conclusion, the antimicrobial values of the wines are phytochemical and ethanol mediated, their lactogenic effects are saponin-mediated increases in serum prolactin content and their prophylactic effect is by the inhibition of the intra-erythrocytic plasmodial growth.

Keywords:
Concoction, ethno-medicine, medicinal value, palm wines, proposed mechanisms.

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INTRODUCTION

Palm wine is a sweet, effervescent drink obtained from the fermented sap of the tropical palm tree (*Elaeis guineensis*) and raphia palm trees (*Raphia* species) (Ukhun *et al*., 2005). Palm wine is used in ethno-medicine as prophylactic against malaria and also used (mainly that of *E. guineensis*) to increase milk flow in postpartum mothers (C. Ibegbulem, personal communications). Medicinal herbs can be infused in palm wine to remedy a wide variety of physical complaints (http://en.wikipedia.org/wiki/Palm_wine; Obute, 2005) and are used to drink some medicines, like the exceptionally bitter ones, such as the mixture with sequestered extracts of *Vernonia amygdalina* Del. (bitter leaf); the mixture is rubbed on the body together with drinking a glass daily in the course of curing measles, small pox and chicken pox (Obute, 2005). Wine prepared from *E. guineensis* is called *Nkwu* in Igbo while that from the *Raphia* species is called *Ngwo*.

The measurement of microbial enzyme activity is used in the assessment of ecotoxicological impacts of environmental pollutants. The most often studied groups of enzyme are oxidoreductases e.g.dehydrogenases. Total microbial dehydrogenase assays involving the reduction of 2,3,5-triphenyltetrazolium chloride (TTC) and 2-(*p*-iodophenyl)-5-phenyltetrazoliumchloride (INT) to their formazans have been used to measure microbial activity (Gong, 1997; Mathew and Obbard, 2001; Nweke *et al*., 2006).

Most plant materials used in ethno-medicine are of unknown biochemical modes of action other than that they are efficacious at treating the debilitating malady. Their phytochemical and biochemical constituents may be responsible for their acclaimed effects. This study carried out a randomized, face-to-face interview questionnaire-based ethno-medical survey on 1000 randomly selected rural families in some southeastern and southsouthern states in Nigeria on the use of *Nkwu* and *Ngwo* in ethno-medicine. The presence of some bioactive phytochemical and biochemical constituents and antimicrobial tests were carried out with these wines and their concoctions. Tests on microbial dehydrogenase activity (DHA) were run with the wines and 4-methyl hydroxyl benzoic acid (4-MHBA). The empirical and experimental evidences were then used to propose their mechanisms of action.

MATERIALS AND METHODS

Procurement of samples

The fresh palm wines used were tapped from *Raphia hookeri* G. Mann. and H. Wendl. trees and *Elaeis guineensis* trees by palm wine tappers at Orodo, Mbaitoli Local Government Area of Imo State, Nigeria. The *V. amygdalina* leaves were collected from a private garden and authenticated by Dr. F.N. Mbagwu, a taxonomist at the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria. A voucher specimen was deposited at his laboratory with voucher number: IMSUH 028.

All the chemicals used were of analytical-reagent grade and were purchased locally.

Ethno-medical survey

The survey was carried out on 1000 randomly selected rural families many of who rely on them as home-made remedies (including those of traditional medicine practitioners) in the southeastern and southsouthern states of Nigeria using face-to-face interview-based questionnaires. This method was used because most of the rural families interviewed are illiterates. There was also the need to create the atmosphere conducive for them to freely air their views without restrictions. The survey entailed gathering information on the (i) use of the samples in ethno-medicine (ii) major types of ailment treated with each sample (iii) most popular combinations (if any).

Qualitative analysis for phytochemical and biochemical constituents

Tests for the presence of tannins, flavonoids and

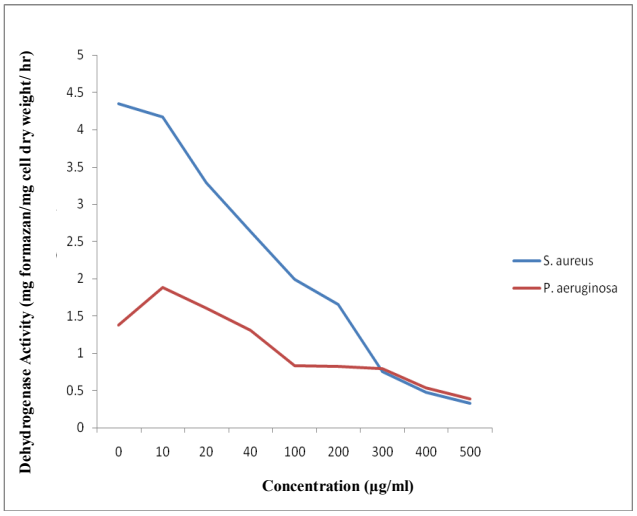

Fig 1. Effect of *Ngwo* on Dehydrogenase Activity of Microorganism

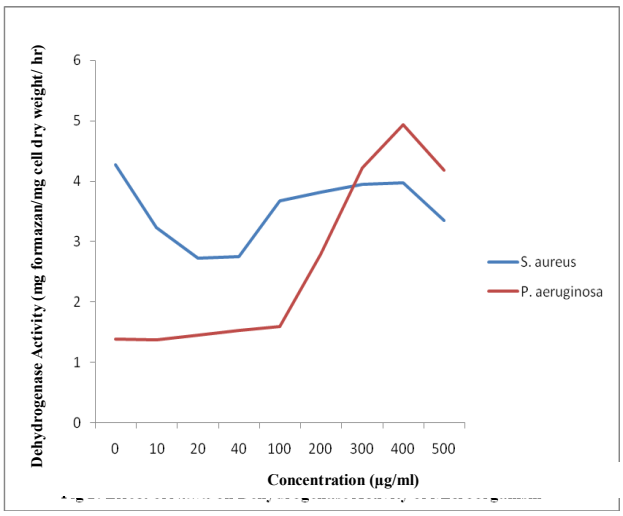

Fig 2: Effect of *Nkwu* on Dehydrogenase Activity of Microorganism

catechins were determined according to the methods of Evans (2002). The presence of tannins was confirmed using 17% Na_2CO_3 and Folin-Denis Reagent as described by Ibegbulem *et al*., (2011a). Saponins were detected using the frothing and red blood cell haemolysis tests described by Harborne (1973). Ethanol suppresses frothing (C. Ibegbulem, personal communications), so ethanol-containing samples were initially heated to 80°C to distill it off. The presence of ethanol in each sample was detected by adding 0.5 ml of Jones Reagent to a mixture of 1 ml of acetone and 0.5 ml of sample which led to formation of a green or blue-green precipitate or emulsion. Test for the presence of 4-methylhydroxybenzoic acid (4-MHBA) was carried out using the method of ASEAN (2005).

Treatment of wine and preparation of concoction

The wet *V. amygdalina* leaves were ground using mortar and pestle. 10.0 g of the ground leaves was mixed with 30.0 ml of distilled water and sieved with muslin cloth. A 50.0 ml aliquot of the respective wine was treated with 20.0 ml sequestered extract of the *V. amygdalina* leaves.

Microbial culture and sensitivity tests

The pathogenic *Pseudomonas aeruginosa* and *Staphylococcus aureus* bacteria used were obtained from degenerated wound. Isolates were purified on nutrient agar (Fluka) plates and characterizations were done using standard microbiological methods. Identification to the generic level was carried out using the methods of Holt *et al*., (1994). The microbial culture and sensitivity tests were carried out as described by Ekwenye and Ijeomah (2005) using the disc diffusion method.

Inhibition of total dehydrogenase activity of microorganism

The effects of the wines and oils on the total DHA of the test microorganisms were evaluated using 2,3,5-triphenytetrazolium chloride (TTC) as an artificial electron acceptor as described by the methods of

Table 1: Sample in ethno-medicine, ailment treated or prevented, response on ethno-medical usage and scientific data

Sample in ethno-medicine	Ailment treated or prevented	Response on Ethno-medical usage (%)*	Scientific data on usage
Nkwu or *Ngwo*	Malaria (prophylaxis)	47	-
Nkwu or *Ngwo*	Postpartum milk flow	100	-
Nkwu or *Ngwo* and *V. amygdalina* concoction	Chicken pox, small pox, measles	68	Obute (2005)

***Responses are of 1000 families (including those of traditional medicine practitioners).**

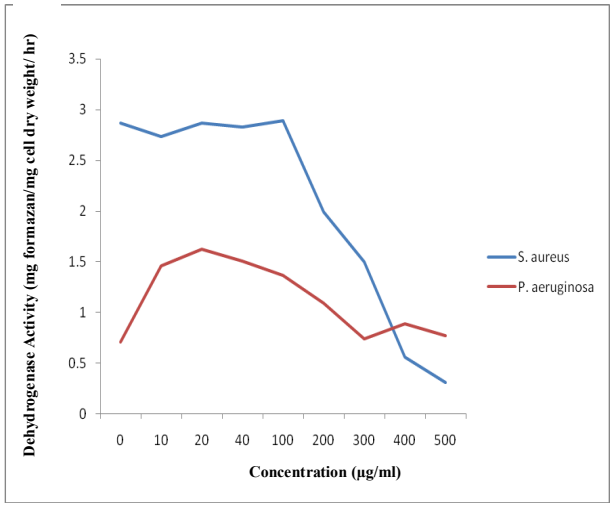

Fig 3. Effect of 4-MHBA on Dehydrogenase Activity of Microorganism

Table 2: Phytochemical and biochemical constituents of the samples*

Parameter	Sample		
	Nkwu	*Ngwo*	*V. amygdalina*
Tannins	+	+	+
Flavonoids	+	+	+
Catechins	+	+	+
Saponins	+	+	+
Ethanol	+	+	-
4-MHBA	+	+	+

***Values are mean of triplicate determinations.**
Key: + = detected, - = not detected.

Alisi *et al*., (2011). A standard antimicrobial agent, 4-MHBA (Zimmer and Huyck, 1961), was also used.

Statistical analysis

Data generated were analysed using the student's t-test of significance. Values were declared significantly different at $p<0.05$.

RESULTS AND DISCUSSION

The medicinal values of the samples were confirmed in the survey (Table 1). Researchers have also confirmed the medicinal values of some of them. Responses were also influenced by use of alternative traditional and orthodox medicines and occurrence of the ailment so treated.

The phytochemicals and biochemicals detected in the wines are presented in Table 2. The *Nkwu* however contained tannins and saponins contrary to the report of Dioha *et al.,* (2005) whose sample did not present these phytochemicals. This may be due to differences in assay methods. The phytochemical and biochemical contents of *Ngwo* corroborated that reported by Ibegbulem *et al*., (2011b).

Table 3 shows that the palm wines were potent antimicrobial agents. This may be due to their ethanol and phytochemicals contents. Ethanol is antiseptic. Tannins, saponins and flavonoids are antimicrobials (Evans, 2002). In ethno-medicine, concoctions made from these wines are more potent.

The effects of the wines and 4-MHBA on DHA of the microorganisms are shown in Figures 1-3. The *Ngwo* was a better antimicrobial agent (Fig 1) than *Nkwu* (Fig 2). The antimicrobial activity of 4-MHBA was confirmed in Fig 3. In all, the *P. aeruginosa* was a more resistant organism than *S. aureus* when the slopes of Figures 1-3 are considered. The variations in resistance may be due to differences in cell wall

Table 3: Sensitivity of microorganism to wine and concoction

Sample	Microorganism	
	Pseudomonas aeruginosa	*Staphylococcus aureus*
Nkwu	+	-
Ngwo	+	+
Nkwu and *V. amygdalina* concoction	+	+
Ngwo and *V. amygdalina* concoction	+	+
4-MHBA	+	+

Key: + = sensitive; - = insensitive.

compositions or different dehydrogenase systems. *Staphylococcus* is a Gram-positive microorganism while the *Pseudomonas* is a Gram-negative microorganism. Different microorganisms have been reported to have different dehydrogenase systems (Praveen-Kumar, 2003). The responses of the bacterial DHAs to the wines and 4-MHBA were both concentration dependent and organism dependent.

The biochemical modes of action of these wines as galactogogues and prophylactic agents are largely unknown. Even though we did not confirm the lactogenic and prophylactic effects in our laboratory, hypothetical modes of action are suggested and were however based on the suggested bioactive principles in the wines (Table 4) since most of their acclaimed effects had been confirmed in Table 1.

The biochemical basis for the lactogenic (or galactogogue) effect of palm wine in postpartum women may be mediated by their saponin contents. The presence of steroidal saponins and sapogenins in *Asparagus racemosus* had been reported to be responsible for its lactogenic effect, which increased serum prolactin content (Oketch-Rabah, 1998; Goyal *et al.,* 2003; Okasha *et al*., 2008). Flavonoids could have been mentioned as another bioactive galactogogue in the palm wines. Di Pierro *et al*., (2008) reported that silymarin (a flavonolignan) increased production of breast milk in healthy women after delivery. Silymarin is generated by the oxidative combination of a lignan and a flavonoid (Di Pierro *et al*., 2008). However, the lignan component may be needed for bioactivity. The bioactive galactogogues contained in palm wines may increase prolactin-releasing factor (PRF) which eventually increase serum prolactin (PRL) thereby resulting in increased milk production.

On the possible use of palm wines as prophylactic agents against malaria when large quantities are ingested, the hypothesized mechanism is that their ethanol contents, which diffuse freely across biological membranes, may increase membrane fluidity, altering their receptors and ion channels of the infected erythrocytes thereby impairing motor performance of plasmodia. The ethanol may even lyse the infected red blood cells thereby threatening the survival of intra-erythrocytic parasites. Chi and Wu (1991) reported that ethanol increased membrane fluidity, caused the leakage of erythrocytic potassium ions before lysing the red blood cells. Devlin (2006) posited that ethanol altered membrane receptor and ion channel activities and impaired motor performance. The mechanisms may also include other intra-erythrocytic conditions which limit the metabolism and growth of the parasites. von Brand (1966) reported that erythrocytic forms of *Plasmodium berghei*, separated from host cell, showed a much more vigorous metabolism when the K^+/ Na^+ ratio corresponded to that of the erythrocytes rather than the blood stream. Lell *et al*., (2000) on the other hand reported that ethanol inhibited the growth of *P. falciparum*, *in vitro*, adding that the growth of malarial parasites was strongly inhibited by ethanol concentrations which were attainable by extensive alcohol consumption. Neuberger (1997) had reported that the distribution of ethanol between blood and expired air was 2100:1. This meant that there is increased blood

Table 4: Suspected bioactive principle in ethno-medicine

Ethno-medicine	Ailment treated or prevented	Suspected bioactive principle*
Nkwu or *Ngwo*	Malaria (prophylactic agent)	Ethanol
Nkwu or *Ngwo*	Postpartum milk flow	Saponins
Nkwu or *Ngwo*and *V. amygdalina* concoction.	Chicken pox, small pox, measles	Ethanol, tannins, flavonoids, saponins, 4-MHBA

***Based on Table 2.**

ethanol residency time which may inhibit intra-erythrocytic plasmodial growth.

In instances when the wines are mixed with herbs to treat infections, the wines act as vehicles, their ethanol is considered antiseptic and the herbs antimicrobials because of their phytochemical contents. Many of their phytochemicals like tannins, flavonoids and saponins are both antioxidants and antimicrobials (Evans, 2002) and confer such medicinal property on the plant and its product(s).

In conclusion, the medicinal values of the wines are based on the antimicrobial effects of their ethanol and phytochemical contents, saponin-mediated lactogenic effects and their ethanol-mediated prophylactic effects.

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