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Functional and therapeutic potentials of oil palm sugar, aqueous infusions of T*etrapleura tetraptera* fruit, and their mixtures

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Abstract

The chemical and functional properties of aqueous solutions of palm sugar and aqueous extracts of *Tetrapleura tetraptera* fruits, as well as their mixtures, were determined as a guide to their rational utilisation as bioactive sweetener and flavouring in food, beverage, and medicinal preparations. The sugar solutions exhibited highly acidic pH values of 3.42-2.70 at concentrations of 5-40g syrup/100 mL, while the water infusions of the *T. tetraptera* fruit had lower acidity of pH 4.8-6.96 at a concentration range of 0.1-3.0 g/100 mL. Infusions of the fruit at 0.1-3 g/100 mL in 20% syrup solution, exhibited pH of 3.35-4.15. The palm sugar solutions, and the *T. tetraptera* infusions exhibited high antioxidant capacity (determined as total phenolic content and DPPH radical scavenging activity) and α-amylase inhibitory activity. The wide acid pH range, high *in vitro* antioxidant capacity, and high antihyperglycemic (i.e., high α-amylase inhibition) activity characteristic of the palm sugar and the *T. tetraptera* infusions, indicate high potential for their use as functional sweeteners and flavouring in a variety of food, beverage, and medicinal formulations for the prevention and management of type 2 diabetes mellitus.

Key words: *Tetrapleura tetraptera fruit infusions, Oil palm syrup, Antioxidant capacity, α-amylase inhibitory activity, Type 2 diabetes*

1. Introduction

Tetrapleura tetraptera is a flowering plant belonging to the Family Mimosaceae. The "winged" dark brown matured fruit of the plant is a nutritive spice (Akin-Idowu and Ibitoye, 2011), which has medicinal properties, and features in ethno-medicine for the management of diverse health conditions, including jaundice, inflammation, convulsion, fever, epilepsy and leprosy (Essien, *et al.*, 2009). An infusion of the whole fruit is taken for feverish condition, constipation, and as an enema and emetic (Burkill, 1985).

The *Tetrapleura tetraptera* fruit has culinary applications as an ingredient in soups and sauces among the people of the Southern part of Nigeria, where its sweet fragrance is highly valued, for example, in the Eastern part of Nigeria, where the fruit is an ingredient of soups prepared for mothers from the first day of delivery, to prevent postpartum contraction (Fleisher *et al.*, 2006). Reported biological properties of its extracts include,

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antimicrobial (Enabulele and Ugha, 2019), antioxidant (Adusei *et al.*, 2019; Dzah, 2022), anti-inflammatory (Onda *et al.*, 2017, Anyamele *et al.*, 2023), antidiabetic (Adesina *et al.*, 2016), antiproliferative (Aikins *et al.*, 2021), and antimalarial (Adesina *et al.*, 2016) properties, with potential antiviral activity (Erukainure *et al.*, 2017). The nutritional value, biological properties, health benefits, and applications of *T. tetraptera* are documented (Mensah *et al.*, 2024).

Oil palm syrup is the sweet, brown, viscous liquid derived from the evaporation of the sap of the oil (*Elaeis guineensis*) palm. Several authors (Ranilla *et al.*, 2008, Naknean and Meenune, 2011; Luis *et al.*, 2012; Oboh *et al.*, 2016; 2023; Oboh, 2023) have reported the composition, chemical characteristics, functional properties, and bioactive constituents of palm sugar preparations. Considered natural (unrefined) and therefore healthy, palm syrup is emerging as a major product in the health-food and supplement market. A major health claim is its low glycaemic index (GI) (Trinidad *et al.* 2010, Srikaeo and Tonga 2015), low GI foods playing an important role in the dietary management of diabetes, weight reduction, and reduction of risks associated with heart disease and hypertension (Jenkins *et al.* 1981, Foster-Powell and Miller 1995, Foster-Powell *et al.* 2002).

In this study, the chemical and functional properties of aqueous solutions of palm sugar and aqueous extracts of *T. tetraptera* fruits, as well as their mixtures were determined as a guide to their rational utilisation as functional ingredients in food, beverage, and medicinal preparations.

2. Materials and methods

2.1. Materials

2.1.1. Oil palm syrup

Palm syrup was prepared as follows: Oil palm sap was filtered through cheese cloth. The filtrate was then boiled in an open pan until it turned brown and viscous; the syrup was cooled and its volume was measured. One and a half litres (1.5 l) of the sap gave 150 ml of syrup. On cooling, the thin syrup turned into a viscous gel. Various concentrations of palm syrup (5, 10, 20, 30, and 40 g/100 ml) in distilled water were prepared and stored in the refrigerator until required for analysis.

2.1.2. T. tetraptera infusions

Finely cut winged fruits (50 g) were dried to constant weight in a ventilated oven at 40^{o}C and ground to fine powder in an electric blender. Different weights (0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 g) of the ground fruit were each added to 100 ml of water or 100 ml of a 20% syrup solution to obtain various concentrations. The mixtures were then boiled for 20 min and filtered into conical flasks, after which their mouths were covered with aluminium foil. The filtrates were cooled to ambient temperature and kept in the refrigerator until required for analysis.

2.1.3. Reagents

DPPH (2, 2-diphenyl-1-picrylhydrazyl), gallic acid, and á-amylase were obtained from Sigma-Aldrich Co. St. Louis, MO, USA. Citric acid, acetonitrile, methanol, potato starch, di-nitro salicylic acid (DNSA) acid, Folin-Ciocalteu reagent, methanol, potassium ferricyanide, ascorbic acid, BHT (butylated hydroxytoluene), trichloroacetic acid, dinitrophenylhydrazine solution (DNPH), thiourea, sodium hydroxide, monosodium phosphate, disodium phosphate, phosphoric acid, and phenolphthalein were obtained from Merck, Darmstadt, Germany. All reagents were analytical grade.

2.2. Methods

2.2.1. Analytical procedure

pH: The pH values of palm sugar and *T. tetraptera* preparations were measured using a Jenway Model 3505 pH meter (Camlab, Over, Cambridge, UK). Ten millilitre portions were read in triplicate.

2.2.2. Antioxidant capacity

This was determined as total phenolic content and DPPH radical scavenging activity.

2.2.3. Total phenolic content

Total phenolic content (TPC) was determined spectrophotometrically according to the method described by Singleton *et al*. (1999). Aliquots (100 μL each) of samples (palm syrup solutions and infusions of *T. tetraptera,* and the gallic acid standards (50, 100, and 150 up to 500 mg/l) were oxidized with 500 μL, 10% (v/v) Folin–Ciocalteu reagent and neutralised with 400 μL, 7.5% aqueous sodium carbonate. The reaction mixture was incubated in the dark for 40 min at ambient temperature and the absorbance was measured at 765 nm in a uv-visible spectrophotometer (GENESYS 10S, Thermo Fisher Scientific, Madison WI, USA). The total phenolic content was calculated from the calibration graph and expressed as ìg Gallic Acid Equivalent (GAE) /ml of sample. The determination was carried out in triplicate.

2.2.4. DPPH radical scavenging activity

The DPPH radical scavenging activities of the sugar solutions and *T. tetraptera* infusions were determined according to the method of Tang *et al.* (2001). One mL of 0.2 mM 2, 2-diphenyl-1-picrylhydrazyl in absolute ethanol was placed in a test tube containing 4 mL of the sample. A control was prepared by adding 1 mL of DPPH solution to 4 mL of 70% ethanol. Following storage in the dark for 30 min, the absorbance was read at 517 nm. The determination was carried out in triplicate.

Percentage free radical scavenging activity was calculated as follows:

$$DPPH\ Radical\ Scavenging\ Activity\ (\%) = 1 - \frac{Absorbance\ of\ sample\ at\ 517\ nm}{Absorbance\ of\ control\ at\ 517\ nm}$$

2.2.5. Antidiabetic activity

Alpha-amylase inhibitory activity: Alpha-amylase inhibitory activity was determined as follows (Kamteker, 2014): A 500 µL aliquot of the oil palm syrup solution or *T. tetraptera* infusion was incubated with 500 µL of an a-amylase solution (2 units/ml, obtained by dissolving 0.001 g of á-amylase in 100 ml of a 0.02 M sodium phosphate buffer-6.7 mM sodium chloride mixture, pH 6.9) at an ambient temperature of 32 °C for 10 min. After incubation, 500 µL of 1 % starch solution (prepared by dissolving 1 g of potato starch in 100 ml distilled water, with boiling and stirring for 15 minutes) was added and the mixture was incubated at 32° C for 10 minutes. After this, 1 ml of DNSA reagent was added to stop the reaction and the mixture was incubated in a hot water bath at 85°C for 5 min. The reaction mixture colour changed to orange-red and was removed from the water bath, cooled to ambient temperature, and made up to 5 ml with distilled water. A control was prepared by replacing the sample solution with buffer. Absorbance was measured at 540 nm. The á-amylase inhibitory activity was calculated as follows (Ranila, 2008):

$$\%\ Inhibition = \frac{A_{540}\ (Control) - A_{540}\ (Sample)}{A_{540}\ (Control)} \times 100$$

Calculation of IC_{50} values: These were calculated using MS Excel. Graphs of concentration *vs* activity were plotted, and IC_{50} values calculated from their regression equations: y= mx + c, y=50, where x is the IC_{50} value.

3. Results and discussion

Table 1 shows the pH, total phenolic content (TPC), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, and α-amylase inhibitory activity at various concentrations of the aqueous solutions of the palm syrup. The sugar solutions exhibited low and decreasing pH values, indicating increasing acidity (3.42-2.70); values for TPC (104.6-464.2), DPPH radical scavenging activity (79.5-90.5 mg GAE/mL), and á-amylase inhibitory activity (83.4-89.4) were high, and increased with increasing concentration of the solutions, all of which are within the concentrations at which the sugar is likely to be applied as sweetener in food and beverage formulations. These findings were in agreement with previous findings for oil palm syrup (Oboh *et al*. 2016; Oboh *et al*, 2021, Oboh *et al*., 2023; Oboh, 2023). In a recent study, Oboh *et al*. (2023) found that oil palm syrup exhibits adequate organic acid (including vitamin C), sugar, bioactive compounds compositions, and *in vitro* functionality, for use as a nourishing natural antioxidant, anti-inflammatory and post-prandial blood glucose-regulatory sweetener and flavouring.

Table 1: pH, total phenolic content (TPC), DPPH radical scavenging activity, and á-amylase inhibitory activity of various concentrations of the palm syrup

Conc. of palm syrup (g/100 ml distilled water) (%)	pH	TPC (mg GAE/ mL solution)	DPPH scavenging activity assay (%)	*In vitro* α-amylase inhibitory activity (%)
5	3.42	104.6±0.0	79.5±0.057	83.4±0.000
10	3.27	247.1±0.0	82.2±0.057	85.2±0.057
20	3.20	336.9±0.0	84.1±0.000	87.1±0.000
30	3.07	391.5±0.0	87.4±0.000	88.7±0.000
40	2.70	464.2±0.0	90.5±0.000	89.4±0.000

The properties of various concentrations of *T. tetraptera* fruit water infusions are shown in Table 2. The pH values were in the acid range (4.8-6.96) and increased with increasing concentration. Total phenolic content (75.9-423.6 mg GAE/ml), DPPH radical scavenging activity (28.0-87.2%, IC_{50}=4.0), and α-amylase inhibitory activity (74.5-91.6%) were high. A remarkable feature of the water infusions was their high α-amylase inhibitory activity, combined with modest TPC and DPPH radical scavenging activity values, even at very low concentrations (0.1-0.3 g/100 ml). This would enable their use in functional food and beverage preparations, where only a hint of flavour is desirable, but coupled with considerable antioxidant capacity and α-amylase inhibitory activity.

Table 2: Characteristics of *T. tetraptera* infusions in water

Conc. *T. tetraptera* (g/100 ml distilled water)	pH	TPC (mg GAE/ mL solution)	DPPH radical scavenging activity (%)	*In vitro* α-amylase inhibitory activity (%)
0.1	4.80	75.9±0.000	28.0±0.057	74.5±0.000
0.2	5.08	96.1±0.346	42.4±0.057	77.2±0.057
0.3	5.23	125.3±0.000	44.5±0.057	79.5±0.000
0.4	5.27	158.0±0.346	50.9±0.057	80.9±0.057
0.5	5.46	187.8±0.650	55.7±0.057	82.9±0.000
1.0	6.20	294.4±0.346	79.2±0.000	84.8±0.000
1.5	6.48	344.2±0.346	83.1±0.057	86.2±0.000
2.0	6.60	373.8±0.346	84.2±0.057	87.3±0.000
2.5	6.73	400.3±0.000	85.9±0.057	89.5±0.000
3.0	6.96	423.6±0.346	87.2±0.057	91.6±0.000
IC_{50} (mg/mL)	–	–	4.00	–

The characteristics of various concentrations of *T. tetraptera* infusions in 20% palm syrup solutions are shown in Table 3. They exhibited higher acidity (3.35-4.15), and TPC (223.8-563.8 µg GAE/mL), than the water infusions, coupled with high DPPH radical scavenging activity (34.8-91.2%, IC_{50}=6.2 mg/mL) and α-amylase inhibitory activity (2.2-88.6%), indicating high antioxidant capacity and anti-diabetes potential, which increased with increasing concentration. Compared with the water infusions, the sugar infusions were characterised by lower pH values, higher TPC, lower DPPH radical scavenging activity (IC_{50} 4.0 *vs* 6.2 mg/mL), and lower

α-amylase inhibitory activity, at the concentrations of the infusions studied. Similar to the water infusions, very dilute infusions in sugar solution (i.e., 0.1-0.3 g/100 mL) exhibited high TPC and high DPPH radical scavenging activity, but much lower α- amylase inhibitory activity.

Table 3: Characteristics of *T. tetraptera* infusions in palm 20% sugar solution

Conc. *T. tetraptera* (g/100 ml distilled water)	pH	TPC (mg GAE/ mL solution)	DPPH radical scavenging activity (%)	*In vitro* α-amylase inhibitory activity (%)
0.1	3.35	223.8±0.346	34.8±0.000	2.2±0.000
0.2	3.37	242.1±0.000	39.4±0.057	13.4±0.000
0.3	3.41	256.7±0.346	43.6±0.057	15.4±0.000
0.4	3.50	281.3±0.346	44.4±0.057	21.5±0.000
0.5	3.56	301.7±0.346	45.1±0.057	29.6±0.057
1.0	3.68	423.8±0.693	66.2±0.000	38.5±0.057
1.5	3.80	455.9±0.000	73.9±0.057	49.1±0.000
2.0	4.03	483.0±0.346	83.4±0.000	63.1±0.000
2.5	4.06	532.6±0.404	86.7±0.100	75.0±0.000
3.0	4.15	563.8±0.346	91.2±0.057	88.6±0.100
IC_{50} (mg/mL)	-	-	6.20	-

The beneficial biological activities and health benefits of polyphenols are generally attributed to both specific and non-specific mechanisms. The non-specific mechanisms result from a broad antioxidant activity, while the specific mechanisms arise from enzyme inhibition and interaction with key signalling proteins. Some key enzymes inhibited include the following: α-amylase and α-glucosidase (type 2 diabetes mellitus), angiotensin converting enzyme (hypertension), and pancreatic lipase (obesity) (Fraga *et al.*, 2010; Anhe *et al.*, 2013; Gonçalves and Romano, 2017).

The rising burden of type 2 diabetes mellitus is of immense concern in healthcare worldwide. In 2017, approximately 462 million individuals were afflicted by the disease, corresponding to 6.28% of the world's population, with a prevalence rate of 6059 cases per 100,000. Over 1 million deaths per annum can be attributed to this condition alone, making it the 9th leading cause of mortality. Global prevalence of type 2 diabetes is projected to increase to 7079 individuals per 100,000 by 2030 (Khan *et al.*, 2020).

Inhibition of starch digestion in the gut is a highly effective approach for the control of postprandial blood glucose concentration, and is the mode of action of the drug acarbose used for the management of type 2 diabetes mellitus. Thus, the high á-amylase inhibitory activity exhibited *in vitro* by the sugar solutions and *T. tetraptera* infusions indicate their high potential for inhibition of starch digestion *in vivo*.

Oxidative stress and free-radical mediated reactions are implicated in the pathogenesis and progression of many degenerative diseases and increase in their mortality (Kyei and Alman, 2001). Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are mostly generated from cellular metabolic reactions and could be deleterious to cells. One of the major defensive mechanisms against free-radical induced oxidative and nitrosative stress is the non-enzymatic antioxidant defence mechanism. Hyperglycaemia leads to generation of excess highly reactive free radicals, resulting in oxidative stress, which further exacerbates the development and progression of diabetic complications. An effective remedy for this is the use of natural antioxidants, the use of which is a complementary therapeutic approach in the management of type 2 diabetes mellitus (Golbidi *et al.*, 2011). The oil palm sugar and *T. tetraptera* infusions are excellent natural antioxidants, which exhibit a high potential for use in this regard.

4. Conclusion

The palm sugar solutions and the *T. tetraptera* infusions studied exhibited a wide acid pH range and a combination of high antioxidant capacity and á-amylase inhibitory activity. These properties recommend them for use in a variety of food, beverage, and medicinal preparations. In this regard, they have great potential as nutrient- and bioactive compounds-rich natural functional sweetener-flavourings, capable of imparting "tropical flavours" (i.e., those based entirely on various combinations of ingredients which are indigenous to the tropics) in functional food and beverage, as well as medicinal formulations for the prevention and management of type 2 diabetes mellitus.

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