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PRODUCTION AND CHARACTERIZATION OF NON-CARBONATED DRINKS WITH EXTRACT OF LOCAL HERBAL MATERIALS

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ABSTRACT

Weighed portion, 100g of *Persea americana* leaves, *Mangifera indica* leaves, *Azadirachta indica* leaves respectively were boiled to obtain extract of specific gravity of phenolics 0.04 that simulate local traditional medicine practitioner. 20ml of respective extract were used to compound drinks in the manner of composition specified for Ribenna^{Tm}. Standard methods were then used to assess the phenolic and potassium content of the respective mixture. Mean value for triplicate analysis were calculated. These were subjected to one way analysis of variance (ANOVA) and Turkey-Kramner multiple composition Post-hoc test using Graphical prism statistical software (version 6). Differences were considered statistically significant at 95% confidence level ($P<0.05$). *Azadirachta indica* leaf extract compounded drink recorded the highest sodium ion content (9.35±0.29mg). Potassium ion content observed in corresponding samples were lower than sodium ion in the sample. *Mangifera indica* leaf extract drinks recorded the highest phenolic content, 4.07±1.73mg. Evidence from the study suggest capability of the local variety of herbs to serve purpose of herbal inclusion in Added Value Water as have been previously reported for various international accepted products.

KEYWORDS: Boil, Added Value Water, Simulate, Traditional, Herbal and Local variety.

INTRODUCTION

Soft drink composition has increased dramatically over the years (O'Connor et. al., 2006; Tahmassebi and BaniHani 2020). It improves "mouth feel" and quench thirst. As people demand more innovative drinks to quench thirst, this led to the production of different soft drinks (Darkwah et. al., 2020). These are classified into carbonated and non-carbonated drinks (Abdelazim et. al., 2017). Non-carbonated drinks are without carbon dioxide and do not have sparkling taste (Ashurst and Hargitt, 2009.). The list include juice drinks, sport drinks, tea, coffee and water such as flavoured or nutritionally enhanced water with herbal extract which have been receiving attention because of their contribution to wellness/health (Plaskova and Mlcek, 2023). Exploring phenolic content of sour sop bark is essential in evaluating plant potentials in medicinal application. Mexico is the world's leading producer of avocados supplying nearly 30% of the global harvest in the year 2020. Guava leaves extract are believed to contain various phytochemicals including phenolic compounds that have been associated with potential health benefits (Kumar et. el, 2021). The extract physiologically act as antioxidants by neutralizing harmful free radicals (Carr and Frei, 1999; Purba and Farang, 2022) including cardiovascular malfunctions or cancer (Pallari and Lewison (2022). Nutrients like vitamins, minerals, and bioactive compounds support functions by enhancing the immune system, mental wellbeing and protecting against oxidative damage (Mitra et. al., 2022). Considering these, this study investigated the use of aqueous extracts of local *Persea americana*, *Mangifera indica* and *Azadirachta indica* leaves to compounding soft non-carbonated drink. The sodium, potassium and phenolic content in the respective drinks were assessed. The possible role in meeting physiological demand was then discussed. The major focus was using locally sourced materials to meet challenging demand of foreign exchange savings.

Apparatus/Equipment

Plastic containers, weighing balance (Shimadzu, TX323L, England), muslin cloth, funnel, syringe,

refrigerator, spatula, spectrophometer (721 visible spectrophometer, PEC medicinal U.S.A.) and test tubes.

Reagent
Sodium carbonate, distilled water, Folin-Ciocalteu and all other chemicals and reagents used were of analytical grade.

Collection of sample
Fresh leaves of Zobo (*Hibiscus sabdariffa)*, Pawpaw (*Carica papaya*), Dogoyaro (*Azadirachta indica)*, Mango (*Mangifera indica)* and Avocado (*Persea americana)*, bark of Dogoyaro, and Avocado seed were collected from a private garden in Ekpoma, Esan West Local Government Area, Edo State, Nigeria. The samples were air dried to constant weight. Proper identification were done in Botany department, Ambrose Alli University, Ekpoma by a Taxonomist.

Processing of Sample
Weighed portion 30g of respective clean samples were boiled in 200ml to a specific density. This is to simulate how Trado-Medical Practitioner here obtain bioactive components of plant for medicinal uses.

Compounding the Drink
Ribenna ^{TM} energy drink template was used to formulate the drink with composition for calculated 2 litres as shown below

Constituent	Amount
Sugar	243.68 g
Glucose	35.11 g
Citric Acid	2.14 g
Malic Acid	2 g
Sodium Benzoate	4 mg
Carboxymethyl Cellulose	2 g
Extract	20 ml
Water	2000ml

Chemical Analyses of Drink
Determination of Potassium
The procedure reported in Beacon diagnostics for potassium determination in fluid was adopted (Henry et. al, 1974). In this, the principle involve potassium content in sample reaction with sodium tetra phenol boron in a buffer to form a colloidal suspension. The amount of the turbid produced is proportional to the concentration of potassium in the sample. The procedure required three test tubes for standard, blank and test samples. To each is added 2µml of standard or sample in test tube containing 1ml of reagent. Absorbance was read at 630nm in a spectrophotometer against blank and value obtained via the calculation.

Potassium in Mg/L = 5 × Absorbance of test Absorbance of standard

Determination of Sodium
The level of sodium in the drink was determined enzymatically via sodium dependant beta-galacosidase activity with O-nitrophenly B-D galactopyranose as substrate (Norbert, 1982), into three different test tubes with added 1ml of sodium reagent each to tubes containing 10ml of water as blank or test sample or prepared sodium standard solution respectively. Absorbance were read at 630nm in a spectrophotometer against the blank with result calculated from

Concentration of sodium in Mg/L = Absorbance of test x 150 Absorbance standard

Determination of Phenol
Total phenolic content (TPC) was determined using the Folin-Ciocalteu assay. The sample (300µl triplicate) was introduced into the test tubes followed by 1.5ml of Follin-Ciocalteu reagent (10 times dilution) and 1.2ml of sodium carbonate (7.5% w/v). The tubes were allowed to stand for 30mins before absorbance at 765 nm were measured. TPC was expressed as gallic acid equivalent (GAE) in mg per 100g of dry material. The calibration equation for gallic acid was y = (0.011x00148). Where y is absorbance and x is concentration of gallic acid in mg GAE/ml (Singleton and Rossi, 1965).

Statistical Analysis
Mean value of each triplicate analysis were subjected to one way analysis of variance ANOVA and Turkey-Kramer multiple comparison Post-hoc test using graph and prism statically software (version 6). Results were recorded as mean ± standard error of mean and difference considered at 95% confidence level i.e. P <0.005

RESULT
The result of analysis of the compounded drinks is presented below

Extract Portion	Phenolic Content Eq GAE/ml	Sodium Content (Na) mg/L	Potassium Content (K) mg/L
Pawpaw Leaf (*Carica papaya)* extract	1.47 ± 0.00	6.99 ± 0.49	0.30±0.02
Dogoyaro bark (*Azadirachta indica)* extract (Neem bark)	0.039 ± 0.00	7.89 ± 0.29	1.07±0.29
Zobo leaf (*Hibiscus sabdariffa)* extract	3.12 ± 0.02	7.14 ±0.49	1.06 ± 0.49
Mango leaf (*Mangifera indica)* extract	4.07 ±1.73	7.83 + 034	3.67 + 0.01
Dogoyaro leaf (*Azadirachta indica*) extract (Neem leaf)	0.03± 0.00	9.35 ± 0.29	0.391 + 0.08

Avocado leaf (*Persea americana)* extract	2.31±0.16	3.67 ±036	0.96 ± 0.05

Values = Mean ± SD of Mean of Triplicate Analysis

The potassium content observed in the represented samples were lower compared to sodium content in the respective sample. Higher potassium content was observed in sample of mango leaf extract compounded drink. Comparable potassium content was observed in pawpaw leaf extract and the extract of dogoyaro leaf extract compounded drink. Sodium content observed in dogoyaro leaf extract compounded drink was the highest, comparable value was observed in compounded drinks of zobo leaf and mango leaf. This value was significantly ($P<0.05$) higher than observed in pawpaw leaf extracted drink. Avocado leaf extract compounded drink recorded the least sodium content. The least phenolic content was observed in compounded extract of dogoyaro bark but compare to value in dogoyaro leaf extract compounded drink. The highest phenolic content was observed in drink compounded with mango leaf extract. Higher phenolic content was observed in drink extract compounded with Avocado leaf extract compare to pawpaw leaf extract compounded drink.

DISCUSSION

Sodium and potassium ions are important electrolytes for proper body function to regulate body pH, balance osmotic pressure, regulate blood volume and pressure (Espinosa et. al., 2009; WHO, 2012). The potassium content observed in drink compounded with Neem bark extract compare to value in soft drinks available in Pakistan, 0.1-0.2 ± 0.00mg/L (Mussarat et. al., 2022). The amount of sodium observed in Neem leaf extract compounded drink is higher than reported for some drinks available in Pakistan (Mussarat et al, 2022). The amount of sodium observed in the product compound with Avocado leaf extract compare to medium daily intake in drink of German men and women, 3. 4 - 4 (Klenow et. al., 2016). However, the drink compounded with avocado leaf extract contain significantly lower than 3900 – 4300mg intake per day observed in German men and women drinks respectively (Klenow et. al, 2016). Evidence to date suggests that high sodium intake affect health adversely yet the role of population level strategy to reduce sodium intake is often contested (Ghandeer et. al., 2021). A notable finding from this study is the high sodium content observed in drink compounded with zobo leaf. This is significant considering the potential implication for consumer's health. Sodium is vital and cannot be synthesized by the body but it is an important component in several biological functions that are crucial for health (Airaodion et. al., 2021). The level observed compared to potassium is consistent with that reported in an earlier investigation (Airaodion and Anabam 2022). Foline et. al., 2011, reported higher potassium content in zobo drink compared to sodium. The potassium content in Neem bark compounded extract compare to value reported for some minerals and spring waters in average Polish diet (Krystyna et. al., 2020). The level observed can meet the median daily sodium intake of German women and men which is 3.4 - 4g respectively (Sebastian et. al., 2022) though higher than recommended daily intake by international organization (Klenow et al, 2016). It however compare with the level observed in drinks sold in Brazil (Sandra et. al., 2013). The potassium content is also lower than reported for some carbonated drinks sold in Mexico 128.0-112 mg/L (Guillermo et. al., 2009). Potassium analyte in food or beverage is becoming important for phenolic health guidelines policy management in specific diseases (Guillermo et. al., 2009). The level of phenolics observed in pawpaw leaf or neem bark extract compounded drink is low compare to value reported in different varieties of fruit juice drink. The use of herbal extract in compounded soft drink is not yet regulated, however International Herbal Water Foundation proposed a draft standard in 2008 for Value Added Water based beverage specification. The main essence is to take advantage of the role of physiological effect i.e. antioxidant consequences applicable in

1. Hypoglycemic and hypolipidemic (Agada et. al., 2020)
2. Antisicking (Adeayo et. al, 2020)
3. Antimicrobial (Callite et. al., 2020)
4. Anti- inflammatory (Singh et. al, 2020)
5. Antioxidative (Rahmani and Aly, 2015)
6. Anticancer (Otsuki et. al., 2010).

This study showed that local herbs can be sourced for compound value added water product/ beverage. The mineral content in some of the drinks compared to some soft drinks sold in international market. Phenolic in the products or drinks is likely to be of benefit as reported for phenolic extracts ability in management of reduce risk of several diseases like hepatitis, cardiovascular disorder, diabetes or urinary tract infection (Aldiab et. al., 2018). The usage of the plant material that is presently considered can serve foreign exchange spent on purchasing packaged leaf as tea obtained from foreign countries. It will contribute to help reduce cost of health care management and provide economic employment.

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