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SYNERGETIC ASSESSMENT OF THE ELEMENTAL PROFILE, PHYTOCONSTITUENTS, ANTIOXIDANT AND ANTIBACTERIAL EFFICACY OF THE METHANOLIC EXTRACTS OF *Persea americana* AND *Daucus carota* FRUITS

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ABSTRACT

This study is undertaken to evaluate the chemical composition, antioxidant and antibacterial efficacy of *Persea americana* and *Daucus carota* synergetically. The results of elemental analyses of samples A (*Persea americana*), B (*Daucus carota*) and C (the formulation) in mg/100g revealed that the samples contained: Sodium (261.50, 362.50 and 808.50), Potassium (121.50, 432.30 and 558.00), Calcium (434.00, 1362.50 and 1774.50), Magnesium (212.00, 786.50 and 921.50), Iron (14.99, 25.27 and 43.17), Phosphorus (585.00, 286.30 and 2237.60), Zinc (2.04, 6.77 and 2.29) and Chromium (0.03, 0.05 and 0.07) respectively. Results of the qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins and total phenols. Quantitative phytochemical screening of the samples A, B and C in mg/g showed; Alkaloid (1.51, 1.05 and 0.53), Flavonoid (5.06, 5.89 and 4.17), Total phenol (39.50, 41.09 and 42.42), Tannin (9.57, 7.07 and 6.85), Saponin (2.34, 0.43 and 0.43), and Cardiac glycoside (1.39, 2.26 and 2.12) respectively. Antioxidant activity of the samples using DPPH showed that sample C has higher activity (IC_{50} of 295.60 µg/mL). The samples exhibited antibacterial activity against *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Streptococcus pyogenes and Bacillus subtilis*. However, sample C revealed highest activity at MIC value of 0.20 µg/mL, at the zone of inhibition ranging from 6 – 18. This implies that, sample C is bacteriostatic due to the high sensitivity of the extract on the test organisms. The results showed that the samples are good source of minerals. However, sample C could play a vital role in phytomedicine.

Keywords: Antioxidant, Antibacterial, synergy, chemical composition, *Persea Americana, Daucus carota*

INTRODUCTION

Interest in the nutritional value of fruits has been increasing among the populace, due to the recent findings on the high level of obesity and other diet-related health problems in adults and children (Diane, 2017). A fruit is defined as the edible part of a plant that consists of the seeds and surrounding tissues. Fruits are important sources of micronutrients and dietary fibres and are components of a healthy diet, which help in preventing major diseases (Ifeoluwapo, 2019). Because fruits possess health-promoting properties, they contribute to dietary guidance (Ifeoluwapo, 2019).

Fruits and vegetables are a major source of macronutrients such as fiber and micronutrients such as minerals and vitamins C, thiamin, riboflavin, B6, niacin, folate, A, and E. Phytochemicals in fruits and vegetables, such as polyphenolics, carotenoids, and glucosinolates may also have nutritional value. While many fruits and vegetables are consumed primarily in their fresh state, some product such as tomatoes, snap beans, corn, peaches, nectarines, and pineapples are also consumed to a significant degree in their processed state (ERS, 2004). Benefits obtainable from the consumptioof fruits and vegetables are a greater life span (Bellavia *et al*., 2013), improved mental health (Conner *et al*., 2017), better cardiovascular health (Oyebode *et al*., 2014), reduced risks of some cancers (Boffetta *et al*., 2010), and weight management (Rolls *et al*., 2016). In a study conducted in the USA, a lower risk of obesity was observed among healthy middle-aged women who consume fruits and vegetables (He *et al*., 2004).

Specifically, fruits contain sufficient potassium, which is needed to reduce the effect of bone loss and the occurrence of kidney stones (USDA, 2009). Fruits assist in the proper functioning of the brain as it stimulates memory recall and supplies the human body with fibre needed for a healthy digestive system (USDA, 2009; Ifeoluwapo, 2019). Fruits are also rich in dietary nutrients such as potassium, antioxidants and folic acid (Ifeoluwapo, 2019).

Carrot (*Daucus carota*) is high in consumption both in raw and processed form. It has been reported to be a good carrier of microelements in the human diet. Carrot contains; carbohydrate (53.19 %), dietary fiber (4.45 %), proteins (27.21 %), fat (3.72 %), and minerals (7.63 %) including mainly, calcium, iron and phosphorus (Dauda *et al*., 2020). Furthermore, carrot is a good source of carotenoids, including beta-carotene, and many bioactive compounds, such as luteolin, quercetin, and kaempferol which are examples of flavonoids. In addition, it is also a rich source of cinnamon derivatives such as chlorogenic, p- hydroxybenzoic and caffeic acid (Dias, 2014).

The fruit of *Persea americana*, commonly known as avocado, is an edible fruit from Central America which is easily adaptable in tropical regions including Nigeria. The species belong to the family Lauraceae (Leite *et al*., 2009). Dauda *et al*. (2020), also reported that avocado contains; carbohydrate (57.36 %), dietary fiber (4.82 %), proteins (27.15 %), fat (2.30 %), and minerals (4.05 %). It has been reported in literature that avocado contains phytosterols, triterpenes and fatty acids. Literature have reported that avocado possesses several biological activities such as antioxidant, antihypertensive, larvical, fungicidal, hypolipidemic, and recently, amoebicidal and giardicidal activities (Anaka *et al*., 2009). Diets high in fruits and vegetables are widely recommended for their health-promoting properties**.** However, this research is aimed at evaluating the elemental profile, phytoconstituents,

antioxidant and antimicrobial efficacy of *Persea americana* and *Daucus* ***carota*** synergetically.

MATERIALS AND METHODS

Sample collection

The fruits of *Daucus carota* (carrot) and *Persea americana* (avocado) were purchased from Anyigba in Dekina Local Government Area of Kogi State, Nigeria. The samples were then transported to the laboratory in a polythene bag.

Preparation of carrot (Daucus carota) sample

The carrot fruits were washed under a running tap for cleansing and removal of extraneous dirt. The clean carrots were peeled manually with a knife and cut into slices (at about 5 mm size). The sliced carrots were dried under the sun and stored in air-tight food-grade plastic containers until used.

Preparation of avocado (Persea americana) sample

The fruits of the avocado were washed to remove dirt. The clean avocados were sliced and the pastes were extracted using a spoon. The extracted pastes were spread out, and sun-dried. The dried avocado pastes were then stored in an air-tight polythene bag, until further analysis.

Preparation of carrot and avocado formulation

Fifty grams (50 g) each of the dried sliced carrots and avocado pastes were mixed (that is, ratio 1:1). The formulation was then stored in an air-tight plastic container until further analysis.

Extraction of Samples

Fifty grams each of the pulverized samples (carrot, avocado and the formulation) was extracted with 300 mL of methanol using maceration for 2 hours. The percentage yield of the extract was calculated.

Mineral Analyses

Digestion of sample

Mineral analyses were determined from the solution obtained by first dry-ashing two grams (2 g) of each of the samples at 550 °C and then dissolving the ash in 10 % HCl, filtered and make-up to 50 ml in a volumetric flask using deionized water and the elements (except Na and K) determined by Atomic Absorption Spectrophotometer (AOAC, 2010). Sodium and potassium were determined using the Photometric method by interpolation on a standard curve (AOAC, 2010).

Analysis of phosphorus (molybdate method)

Phosphorus in each sample was determined by the molybdate method using hydroquinone as a reducing agent. To 0.5 ml of the mineral digest are added 1.0 ml of ammonium molybdate, 1.0 ml sodium sulphate and 1.0 ml of hydroquinone. The mixture is agitated and allowed to stand for 30 minutes. The blue colour that develops is quantitated using a colorimeter at 660 nm against a standard curve (AOAC, 2010). The concentration of phosphorus (P) was calculated using the equation below;

$$\text{P}\ (mg/dl) = \frac{Absorbance\ x\ conc, of\ std.(5\ mg/dl)}{Absorbance\ of\ standard}$$

Phytochemical Screening

Qualitative phytochemical screening was performed on the samples using standard procedures described by Sofowara (1996) and Mbatchou and Kosoono (2012), to identify the presence or absence of secondary metabolites.

Qualitative phytochemical analyses of the samples

Test for saponins

Froth test: 2.0 mL of each of the samples was taken in a test tube and shaken vigorously with a small amount of sodium bicarbonate and water. The formation of a stable froth indicates the presence of saponins.

Test for phenolic compounds

Ferric chloride test: 2 mL each of the samples was taken in a test tube and 2 mL of ferric chloride solution ($FeCl_3$) was added and observed. The formation of a deep bluish-green solution shows the presence of phenol. Lead acetate test: To 2 mL of the syrup (PHS), 2 mL of lead acetate solution was added and observed. The formation of a precipitate indicates the presence of phenolic compounds.

Test for flavonoids

a) Shinoda test: To 2 mL of the sample, three drops of concentrated hydrochloric acid and 0.5 g magnesium ribbon were added and observed. The formation of pink or magenta colour indicates the presence of flavonoids.
b) Alkaline reagent test: 2 mL each of the samples was taken in a test tube and a few drops of sodium hydroxide solution was added and observed. The formation of intense yellow colour which turns colourless with the addition of a few drops of dilute HCl indicates the presence.

Test for tannins

Gelatin test: A small quantity of gelatin solution was added to 2 mL each of the samples in a test tube and observed. Formation of white precipitate indicates the presence of tannins.

Test for glycosides

a) Legal test: Few drops of pyridine and sodium nitroprusside solution are added to 2 mL of the samples. The mixture was made alkaline with a few drops of NaOH solution. The formation of a pink or red colour indicates the presence of glycosides.
b) Borntrager's test: 1 mL of benzene was added to 2 mL each of the samples, and to the mixtures, 0.5 mL of dilute ammonia solution was added. The formation of pink or red colour indicates the presence of glycosides.

Test for alkaloids

a) Wagner's test: Few drops of Wagner's reagent were added to 2 mL of the samples and observed. The formation of a reddish-brown precipitate indicates the presence of alkaloids.
b) Hager's test: Few drops of Hager's reagent were added to 2 mL of the samples and observed. The formation of a yellow precipitate or precipitate indicates the presence of alkaloids.

Quantitative Phytochemical Analyses

Quantitative phytochemical analyses were carried out on the samples using standard procedures.

Determination of total alkaloid

The method reported by Akenga et al. (2005) was used. 50.0 mL of 20 % acetic acid was added to 5.0 g of sample in a 250 mL beaker and covered, and allowed to stand for 4 hours. The mixture containing solution was filtered and the volume was reduced to one-quarter using a water bath. To the sample, concentrated ammonium hydroxide was added drop-wise until the precipitate was completed. The whole solution was

allowed to settle and a precipitate was collected by filtration and weighed. The percentage of total alkaloids was calculated using equation 1.

$$\%\ Alkaloid = \frac{W2 - W1}{Weight\ of\ sample}\ x\ 100 \quad (1)$$

Where; W_2 = Weight of sample, W_1 = Weight of precipitate

Determination of total saponin content

Total saponin content was determined by the method described by Makkar *et al.* (2007), based on vanillin-sulphuric acid colorimeter reaction. A 50 µL of sample extract was added to 250 µL of Vanillin reagent. Then 2.5 mL of 72 % sulphuric acid was added and it was mixed well. The solution was kept in a water bath at 60 ^{O}C for 10 minutes, it was cooled and the absorbance was taken at 544 nm. The total saponin content was measured using equation 2.

$$Y = 0.0005X - 0.0052 \quad (2)$$

Where; Y= Absorbance. X= Concentration.

Determination of total phenolic content

Total phenolic content was determined by the Follin-Ciocalteu method reported by McDonald et al. (2001). 20 µL aliquot of extract solution was mixed with 1.16 mL distilled water and 100 µL of Follin-Ciocalteu reagent followed by addition of 300 µL of 20 % Na_2CO_3 solution. Subsequently, the mixture was shaken and the absorbance was measured at 760 nm. The total phenolic content was determined using equation 3.

$$Y = 0.0155X - 0.2673 \quad (3)$$

Where; Y= Absorbance. X= Concentration.

Determination of total flavonoid

The method reported by Chang *et al.* (2002) was used. Total flavonoid content was measured by Aluminum Chloride Colorimetric Assay. 1.0 g of sample was dissolved in 10.0 mL of 95 % ethanol for 1 hour. 1.0 mL of the extract and 4.0 mL of distilled water were measured into a 10 mL volumetric flask, 0.03 mL of 10 % Aluminum Chloride was added. The mixture was treated with 1.0 M Sodium Hydroxide, and diluted to 10 mL with distilled water. The absorbance for test and standard solutions were determined at 510 nm with UV/Visible Spectrophotometer. The total flavonoid was determined using equation 4.

$$Y = 0.009X - 0.006 \quad (4)$$

Where; Y= Absorbance. X= Concentration.

Determination of total tannins content

Total tannin content was determined by Follin-Ciocalteu method, a procedure reported by Prabhavathi *et al*. (2016). About 0.1 mL of the sample was added to a 10 mL volumetric flask containing 7.5 mL of distilled water, 0.5 mL of Folin-Ciocalteu phenol reagent and 1 mL of 35 % Na_2CO_3 solution. The mixture was diluted to mark with distilled water. The mixture was shaken well and kept at room temperature for 30 minutes. The absorbance was determined using equation 5.

$$Y = 0.057X - 0.071 \quad (5)$$

Where; Y= Absorbance. X= Concentration.

Free Radical Scavenging Analysis.

Quantitative determination of free radical scavenging activity

The free radical scavenging activity of the samples against 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined by the method reported by Adesegun *et al.* (2008). Various concentrations of the syrup were prepared in methanol (25, 50, 100, 200, 400, 800, 1600, 3200 µg/mL), 3200 µg/mL (0.032 g) of the samples were weighed and dissolved in 100 mL of methanol. The samples of the different concentrations were mixed with 3.0 mL of prepared DPPH**.** The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 minutes. **A**scorbic acid (Vitamin C) at various concentrations (25, 50, 100, 200, 400, 800, 1600, 3200 µg/mL) was used as standard. The absorbance was read against reagent blank at 517 nm, and inhibition of free radical by DPPH in percent (I %) was calculated as follows:

$$I\ (\%) = \frac{A_{blank} - A_{sample}}{A_{blank}}\ x\ 100 \quad (6)$$

Where; A_{blank} = Absorbance of blank
A_{sample} = Absorbance of sample

Antibacterial Analysis

Collection and identification of test organisms

The microorganisms tested with the samples for antibacterial activity were pure clinical isolates of *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Streptococcus pyogenes and Bacillus subtilis*. The bacterial isolates were obtained from the Department of Microbiology, Kogi State University, Anyigba.

Antibacterial screening of the samples

The susceptibility tests were determined using the agar well diffusion method which has been recommended as a standard protocol for conducting antibacterial screening (Collins *et al*., 1995; Mukthar and Okafor, 2004). The microorganisms were cultured with nutrient broth and incubated at a temperature of 37 ^{O}C for 24 hours. The cultured microorganisms were subcultured again for another 24 hours to obtain purer microbial colonies. Mueller-Hinton agar powder (19 g) was prepared in 500 mL distilled water and sterilized in an autoclave for 15 min at a temperature of 120 ^{O}C to get rid of any possible contamination of the agar. The cultures were diluted with distilled water to achieve an optical density corresponding to 2.0×10^6 colony-forming units per ml (CFU/ml). 100 mL of the prepared Mueller-Hinton agar was poured into the petri dish containing the various microorganisms and allowed to solidify, and this was repeated for the controls; negative control (distilled water) and positive control (ciprofloxacin). The samples (100 mg to 200 mg) were dissolved in 10 mL of distilled sterile water. A 100 µL of each concentration was transferred to the hole that was made on the Mueller-Hinton agar plate. Before that, the agar plate was spread uniformly using a sterile cotton swab with each type of bacteria. The agar plates were inverted and incubated for 24 hours at 37 ^{O}C in an incubator. The antibacterial activities of the samples were monitored by observing the various zones of inhibition after the incubation by measuring the diameter of the zones of inhibition.

Determination of minimum inhibitory concentration (MIC)

The estimation of MIC for the samples was carried out using the method of Akinpelu and Kolawole (2004). 0.5 mL of varying concentrations of the samples (100, 150 and 200 mg/mL) were dispensed into each test tubes containing nutrient broth, inoculated with a loopful of each test microorganism. 0.5 Mcfarland turbidity standard was adopted. The MIC was taken from the tube with the least concentration, showing no visible turbidity after 24 hours of incubation at 37 ^{O}C. Therefore, growth was examined by observing turbidity.

RESULTS AND DISCUSSION

Minerals composition

Table 1 shows the elemental composition of sample A, sample B and sample C. The elements analyzed were Na, K, Ca, Mg, Fe, P, Zn and Cr. All results are presented as the mean and standard error of the mean of triplicate measurement.

Table 1: Mineral Composition of the Methanolic Extracts of the Samples

	Concentration (mg/100g)		
Element	**Sample A**	**Sample B**	**Sample C**
Sodium	261.50 ± 2.12	362.50 ± 2.12	808.50 ± 0.71
Potassium	121.50 ± 0.71	432.30 ± 0.35	558.00 ± 1.41
Calcium	434.00 ± 2.83	1362.50 ± 2.12	1774.50 ± 2.12
Magnesium	212.00 ± 1.41	786.50 ± 3.54	921.50 ± 0.01
Iron Phosphorus	14.99 ± 0.21	25.27 ± 0.14	43.17 ± 0.14
Zinc	585.00 ± 3.54	286.30 ± 1.77	2237.60 ± 0.01
Chromium	2.04 ± 0.06	6.77 ± 0.08	2.29 ± 0.21
	0.03 ± 0.01	0.05 ± 0.01	0.07 ± 0.01

Mean ± SEM of three replicates.
Sample A (*Persea americana*), Sample B (*Daucus carota*) and Sample C (Formulation)

The results of elemental composition (Table 1) revealed that phosphorus is the most abundant in the formulation (sample C) (2237.60 mg/100g), followed by calcium, magnesium, sodium, potassium and iron with the values in mg/100g of 1774.50, 921.50, 808.50, 558.00 and 43.17 respectively. The values are higher when compared with samples A and B. Concentrations of the mineral elements in the formulation are higher when considering the recommended daily allowance (FND, 2002). This implies that the formulation (sample C) is a good source of mineral elements. However, the concentration of mineral elements in samples A, B and C are in the order of sample C > sample B > sample A.

Phytochemical analysis

The results of the qualitative phytochemical screening of the samples were recorded in Table 2. The parameters examined are; Tannins, Flavonoids, Saponins, Alkaloids, Cardiac glycosides and Total phenol, Table 3 show the concentration of some selected phytochemicals present in the samples.

Table 2: Phytochemical constituents of the Samples

S/N	Tests	Sample A	Sample B	Sample C
1	**Alkaloids Test**			
	Wagner's test	+	+	+
	Hager's test	+	+	+
2	**Glycosides Test**			
	Legal test	-	-	-
	Borntrager's Test	-	-	-
3	**Flavonoids Test**			
	Shinoda test	+	+	+
	Alkaline reagent test	+	+	+
4	**Tannins Test**			
	Gelatin solution test	+	+	+
5	**Saponins Test**			
	Froth/foam test	+	+	+
6	**Phenolic compds Test**			
	Ferric chloride test	+	+	+
	Lead acetate test	+	+	+

- = Below detection limit (BLD); + = Present
Sample A (*Persea americana*), Sample B (*Daucus carota*) and Sample C (Formulation)

Table 3: Concentration of some phytoconstituents of the methanolic extracts of the Samples.

Phytochemicals	Concentration (mg/g)		
	Sample A	**Sample B**	**Sample C**
Alkaloid	1.51 ± 0.007	1.05 ± 0.071	0.53 ± 0.035
Flavonoid	5.06 ± 0.078	5.89 ± 0.156	4.165 ± 0.078
Total Phenols	39.50 ± 0.240	41.09 ± 0.120	42.42 ± 0.354
Tannin	9.57 ± 0.014	7.07 ± 0.028	6.85 ± 0.014
Saponin	2.34 ± 0.028	0.43 ± 0.007	0.43 ± 0.014
Cardiac glycoside	1.39 ± 0.007	2.26 ± 0.007	2.12 ± 0.007

Mean ± SEM of three replicates.
Sample A (*Persea americana*), Sample B (*Daucus carota*) and Sample C (Formulation)

The results summarized in Table 2, revealed the presence of phytoconstituents in the samples. Samples A, B and C revealed the presence of alkaloids, tannins, saponins, phenols and flavonoids. Cardiac glycosides were absent in the samples.

The results summarized in Table 3 revealed the concentration of some selected phytochemicals in the samples. The results revealed that the phytochemicals contained in the samples are in varying amounts. It was observed that the concentration of the phytochemicals was synergized in the formulation (sample C). The phytochemical with the highest concentration was total phenols, followed by tannins, flavonoids, saponins, cardiac glycosides and alkaloids respectively.

Saponins have been shown to play an important role in lowering blood cholesterol levels by forming complexes with cholesterol and bile acid, preventing them from being absorbed through the small intestine and thus lowering cholesterol levels in the blood and liver (Shereen, 2011).

Saponins also act as antioxidants, preventing DNA degeneration and reducing colon damage.

They help to boost our immune system and lower the risk of human cancers by preventing cancer cells from growing (Umaru *et al*., 2007). Total phenol compounds have also been shown to play an important role in regulating various metabolite functions in plants, including structure and growth, pigmentation, and resistance to various pathogens (Naumovski, 2015).

The presence of phenols and flavonoids, on the other hand, suggests that the syrup will be beneficial in the management of cardiovascular diseases and oxidative stress because flavonoids and phenols are biological antioxidants. Alkaloids are fundamental essential medicinal agents with analgesic, antispasmodic, and bactericidal properties (Edeoga and Eriata, 2001). Alkaloids have also been used as central nervous system stimulants, ophthalmic topical anesthetics, and anti-puretics. As a result, the presence of alkaloids in the syrup suggests that it could be used as a powerful pain reliever.

Flavonoids have been reported to be essential secondary metabolites with anti-oxidant properties that are only slightly toxic when consumed and have the inherent ability to modify the body's reaction to allergens, viruses, and carcinogens (Lotito and Frei, 2006). Flavonoids also protect against these diseases by contributing to the human body's overall antioxidant defense system. Tannins and glycosides are antibacterial and antifungal (Dangoggo *et al*., 2001; Shittu *et al*., 2007). The presence of tannins in the syrup may explain its antibacterial properties.

Antioxidant potential of the samples

The results for antioxidant activity and IC_{50} composition of the samples are presented in Tables 4 – 5.

Table 4: Quantitative analysis of free radical scavenging activity using DPPH

		Zone of inhibition (%)			
S/N	**Conc. (µg/mL)**	**Sample A**	**Sample B**	**Sample C**	**Ascorbic acid**
1	100	23.00	38.00	48.00	33.00
2	200	38.00	43.00	51.00	42.00
3	400	44.00	49.00	59.00	68.00
4	800	57.00	52.00	65.00	75.00
5	1600	84.00	67.00	82.00	85.00
6	3200	91.00	78.00	87.00	96.00

Sample A (*Persea americana*), Sample B (*Daucus carota*) and Sample C (Formulation)

Table 5: IC_{50} Values of the Extracts

Extracts	**IC_{50} (µg/mL)**
Sample A	750.14
Sample B	684.92
Sample C	295.60
Ascorbic acid	107.49

The presence of free radical scavenging compounds in the methanolic extracts of *Daucus carota*, *Persea americana* and their formulation was analyzed and detected. The extracts exhibited different degrees of free radical scavenging activity as revealed in Table 4. Table 4 revealed the zone of inhibition of the antioxidant activity of the extracts (samples A, B and C). From the table, sample C (i.e. the formulation) has the highest percentage of antioxidant activity in all concentrations, except when compared with sample A at 1600 and 3200 µg/mL. This implies that the extracts are concentration dependent. From the results obtained from this research work, it was found that sample C shows the highest antioxidant potency. **Table 5** shows the IC_{50} of the extracts and the result confirmed that the formulated extract (sample C) has the highest antioxidant potency when compared with other extracts. The lower the IC_{50} value the higher the antioxidant activity of the extracts. From the table, sample C possessed the lowest IC_{50} (295.60 µg/mL). This ascertains the antioxidant potency of the formulation (sample C) over the other extracts.

Antibacterial Potency of the Samples

Tables 6-11 show the antibacterial activities and minimum inhibition concentration (MIC) of the samples on pure clinical isolates of *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Streptococcus pyogenes and Bacillus subtilis.*

Table 6: Antibacterial Activity of the Methanolic Extract of Sample A on Test Organisms

Test organisms	Concentration (mg/mL)/Zone of inhibition (mm)				
	100 mg/mL	150 mg/mL	200 mg/mL	Positive control	Negative control
S. typhi	0 mm	11 mm	19 mm	12 mm	0 mm
S. pyogenes	6 mm	0 mm	0 mm	15 mm	0 mm
B. subtilis	0 mm	6 mm	15 mm	6 mm	0 mm
S. aureus	6 mm	10 mm	15 mm	20 mm	0 mm
E. coli	7 mm	11 mm	14 mm	10 mm	0 mm

Table 7: Minimum Inhibitory Concentration (MIC) of the Methanolic Extract of Sample A on Test Organisms

Test organisms	Concentration (mg/mL)				
	100	150	200	+ve control	-ve control
S. typhi	G	G	NG	G	G
S. pyogenes	G	G	G	NG	G
B. subtilis	G	G	NG	G	G
S. aureus	G	G	NG	NG	G
E. coli	G	G	NG	NG	G

Key: NG = No growth, G = Growth

Table 8: Antibacterial Activity of the Methanolic Extract of Sample B on Test Organisms

Test organisms	Concentration (mg/mL)/Zone of inhibition (mm)				
	100 mg/mL	150 mg/mL	200 mg/mL	Positive control	Negative control
S. typhi	6 mm	12 mm	15 mm	14 mm	0 mm
S. pyogenes	0 mm	0 mm	0 mm	15 mm	0 mm
B. subtilis	6 mm	13 mm	17 mm	21 mm	0 mm
S. aureus	6 mm	13 mm	18 mm	12 mm	0 mm
E. coli	0 mm	11 mm	15 mm	22 mm	0 mm

Table 9: Minimum Inhibitory Concentration (MIC) of the Methanolic Extract of Sample B on Test Organisms

Test organisms	Concentration (mg/mL)				
	100	150	200	+ve control	-ve control
S. typhi	G	G	NG	NG	G
S. pyogenes	G	G	G	NG	G
B. subtilis	G	NG	NG	NG	G
S. aureus	G	NG	NG	G	G
E. coli	G	G	NG	NG	G

Key: NG = No growth, G = Growth

Table 10: Antibacterial Activity of the Methanolic Extract of Sample C on Test Organisms

Test organisms	Concentration (mg/mL)/Zone of inhibition (mm)				
	100 mg/mL	150 mg/mL	200 mg/mL	Positive control	Negative control
S. typhi	6 mm	14 mm	16 mm	15 mm	0 mm
S. pyogenes	6 mm	6 mm	13 mm	15 mm	0 mm
B. subtilis	6 mm	14 mm	18 mm	17 mm	0 mm
S. aureus	6 mm	15 mm	14 mm	22 mm	0 mm
E. coli	6 mm	11 mm	15 mm	15 mm	0 mm

Table 11: Minimum Inhibitory Concentration (MIC) of the Methanolic Extract of Sample C on Test Organisms

Test organisms	Concentration (mg/mL)				
	100	150	200	+ve control	-ve control
S. typhi	G	NG	NG	NG	G
S. pyogenes	G	G	NG	NG	G
B. subtilis	G	NG	NG	NG	G
S. aureus	G	NG	NG	NG	G
E. coli	G	G	NG	NG	G

Key: NG = No growth, G = Growth

Tables 6-11 show the antibacterial activities of the methanolic extracts of samples A, B and C on the test organisms (*Staphylococcus aureus, Escherichia coli, Salmonella typhi, Streptococcus pyogenes and Bacillus subtilis*). The zone of inhibition expressed by various concentrations of the samples on the test organisms ranges from 6 – 19 mm for *Persea Americana*, 6 – 18 mm for *Daucus carota* and 6 – 18 mm for the formulation. According to Mukhtar and Okafor [24], 0 mm zone of inhibition indicates no effects, less than 8 mm zone of inhibition indicates low sensitivity, and more than 8 mm zone of inhibition indicates high sensitivity. All the test organisms are sensitive in the samples at the highest

concentration (200 mg/mL) except for *Streptococcus pyogenes*, which is resistant against samples A and B at the same concentration. Also, all the test organisms are resistant against all the extract at the least concentration (100 mg/mL), this implies that, there was sign of turbidity for all the test organisms at this concentration.

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial (antibiotic or bacteriostatic, antifungal) drug that will inhibit the visible growth of a microorganism after overnight incubation (Tripathi, 2013). Tables 7, 9 and 11 show that sample B inhibits growth at 150 mg/mL (0.15 µg/mL) and, didn't show any sign of turbidity or growth for *Staphylococcus aureus* and *Bacillus subtilis.* Therefore, the value 150 mg/mL (0.15 µg/mL) was taken as the minimum inhibitory concentration. However, sample A inhibits growth at 200 mg/mL (0.20 µg/mL) and, didn't show any sign of turbidity or growth for *Salmonella typhi*, *Eschericia coli*, *Staphylococcus aureus* and *Bacillus subtilis.* Therefore, the value 200 mg/mL (0.20 µg/mL) was taken as the minimum inhibitory concentration whereas, the extract of the formulation (sample C) inhibit growth also at 150 mg/mL (0.15 µg/mL) and this value was taken as the minimum inhibitory concentration. However, turbidity at the highest concentration for the formulated extract (sample C) only, was not observed for all the test organisms. This implies that, the samples are bacteriostatic. However, due to synergy, sample C revealed higher efficacy when compared to samples A and B. Furthermore, sample C could be effective in treating infectious diseases caused by the aforementioned microbes.

CONCLUSION

In this study, elemental composition, phytoconstituents, antioxidant and antibacterial efficacy of *Persea americana* and *Daucus carota* were synergetically assessed. The results analyzed reveal that the samples are a good source of minerals, as revealed in Table 1. The presence of calcium, magnesium and phosphorus in the samples, indicates its consumption could be good source of minerals. The samples were found to contain significant quantities of bioactive agents which are responsible for medicinal benefits. The formulations contain alkaloids, tannins, phenols, saponins and flavonoids. The quantitative phytochemical analysis of the samples estimates the presence of tannins, saponins, flavonoids and alkaloids. The antioxidant activities of the samples were evaluated. However, sample C (the formulation) exhibits a significant amount of antioxidant potency, which makes it a promising therapeutic agent with the ability to quench free radicals. The result of antibacterial screening ascertain a promising antibacterial potency of the samples against *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Streptococcus pyogenes and Bacillus subtilis* isolates used in this study. However, sample C showed higher antibacterial efficacy towards the bacteria isolates. The minimum inhibitory concentration (MIC) of sample C at 200 mg/mL showed that the sample is bacteriostatic**.** This is an indication that sample C (the formulation) could be used in the treatment of sicknesses and diseases arising from the aforementioned microorganisms. However, further analyses like toxicological profiles and other pharmacological assays are required.

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