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The Synergistic Interaction of Phenolic Compounds in Pearl Millets with Respect to Antioxidant and Antimicrobial Properties

Ogunsina Olabode Isaiah*

Department of Biochemistry, Faculty of Science, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria

Abstract

Pearl millet (*pennisetum glaucum*) is one of the major cereals that are good source of nutrients, containing phytochemicals and antioxidants molecules that are known for disease prevention. In order to understand how interaction of individual phenolics contributes to the total antioxidant and antimicrobial capacity, the new study quantitatively measured antioxidant and antimicrobial capacity of various phenolics in different combinations, using HPLC-UV, *in vitro* antioxidant potential and antimicrobial properties of phenolics compounds in pearl millet. The result of the Phytochemical screening revealed the presence of alkaloids, flavonoids, terpenoids, saponins, and tannins, but steroids and cardiac-glycosides were absent. Selected phenolics compounds included in this study were those found in pearl millets such Protocatechuic acid, Vanillic acid, p-hydroxybenzoic acid, Caffeic acid, Syringic acid, Ferulic acid. The combine and mixture phenolics compound demostrated higher antioxidant and antimicrobial potential while individual phenolics showed their characteristic antioxidant and antimicrobial capacities in moderate manner. This study could be concluded that pearl millet phenolics compound has antioxidant activities with a high level of radical scavenging action. The antioxidant and antimicrobial activities of the combination of the major phenolic compound with some selected minor compounds showed a synergistic interaction. It is therefore recommended that the phenolic compounds in Pearl millet could be used as food additives in order to have a much better utilization of this phytoconstituents in health care and prevention.

Keywords

Pearl Millet, Phytochemical, Phenolic Compounds, Antioxidant, Antimicrobial

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1. Introduction

In recent years, nutritional biochemist or nutritionists and the general public have come to regard foods as more than sources of energy and essential nutrients. Certain minor components of foods are now recognized for their health-promoting properties, in particular for their roles in preventing or alleviating the effects of some of the chronic diseases such as cardiovascular disease and certain cancers [1]. These include vitamin E, vitamin C, lignans, flavones, phenolic compounds, carotene and certain trace elements. Evidence for important roles of vitamin E and C is strong, whereas the roles of other classes of antioxidants are still being elucidated [1]. Vegetables, fruits and grains are the most important sources of these antioxidants. However, grains have largely been ignored as important contributors of dietary antioxidants, despite the fact that they are the staple dietary component for most of the world's population. Antioxidants are found in whole grain foods, and phytochemicals (phytates and phenolic compounds), which are responsible for the high antioxidant activity of whole grain foods [2].

* Corresponding author
E-mail address: metabolitebode@yahoo.com

There are nine species of millet cultivated around the world but the most widely grown species in Africa is pearl millet (*Pennisetum glaucum*) [3]. In many African countries, millet is often the main component of many meals and is essentially consumed as steam-cooked products (couscous), thick porridges (Tô) and thin porridges (Ogi), which can be used as a complementary food for infants and young children. It is a staple food in many developing countries and constitutes the major source of essential nutrients in India, semi-arid and arid regions of Africa. Pearl millets grow under difficult ecological conditions; tolerate poor soils and a certain degree of drought better than any other cereal crop. It is generally accepted that pearl millet originated in Africa and was subsequently introduced into India and are been grown since prehistoric times.

Phenolics are secondary metabolites synthesized by plants, both during normal development and in response to stress conditions such as infection, wounding and UV radiation, among others. These compounds are a much diversified group of phytochemicals derived from phenylalanine and tyrosine. Phenolic acids and flavonoids are present in cereals in the free and conjugated forms. Dietary polyphenols exert beneficial biochemical properties such as free radical scavenging, metal chelation and inhibition of lipid peroxidation. Polyphenols are also useful in management of several physiological disorders such as diabetes mellitus, hypertension, vascular fragility, hypercholesterolemia, prevention of oxidation of low-density lipoproteins (LDLs) and also improvement of the health of gastrointestinal tract [4].

The antimicrobial agents normally used for preservation of foods and treatment of microbial infection are mostly synthetic chemicals. However, in recent time there is greater interest in natural products with antimicrobial properties for food preservation and also for the treatment of number of diseases that are related to microbial infections. The antimicrobial activity of phenolics and flavonoids are also well documented [5, 6]. The mechanisms responsible for phenolic toxicity to microorganisms include: adsorption and disruption of microbial membranes, interaction with enzymes, and metal ion deprivation [7, 6]. Due to their antibacterial, antifungal and antiviral activity, phenolic compounds and antioxidant biomolecules were the subject of anti-infective research for many years [8, 9, 7, 10]. These activities suggested that phenolic compounds can be used as chemotherapeutic agents, food preserving agents and disinfectants [11]. They can affect the growth and metabolism of bacteria, activating or inhibiting the microbial growth according to their constitution and concentration [13, 14]. Hence, this investigation seeks to evaluate the interaction of antioxidant and antimicrobial effect of polyphenolics compounds in pearl millets (*pennisetum glaucum*).

2. Materials and Methods

2.1. Experimental Samples

Pearl millets (*Pennisetum glaucum*) were bought from Kano markets, Kano state of Nigeria. Authentication of sample was done by Dr Ajayi Ademola of the Department of Agronomy, Federal College of Agriculture Akure, Ondo State. The chemicals were analytically graded.

2.2. Extraction Method

Finely grinded samples of Pearl millet (0.3 g) were extracted with 30 ml acidified methanol (1% conc. HCl in methanol) in three phases as follows: 10 ml solvent was added to flour sample in a conical flask and completely covered with aluminium foil. The sample was stirred magnetically (magnetic stirrer) for 2 hours, centrifuged in a 40 ml plastic centrifuge tube at 1900 rpm for 10 min (25°C) and decanted, keeping the supernatant. The residue was re-suspended in 10 ml of the solvent stirred for 20 min; centrifuged and decanted, keeping the supernatant, and this process was repeated a third time. The supernatant was combined and stored in a glass bottle covered with aluminium foil and kept in a cold room at 4°C before analysis.

2.3. Preparation of Phenolic Compounds

The phenolics standard was prepared in two forms, the selected single phenolic compounds identified as major and minor, was determined by a quantification process using HPLC-UV, the mixture was prepared by using the amount in ratio of the selected identified phenolic compounds in Pearl millet. 0.1mg/ml of the individual phenolic compound was prepared in acidified methanol in a sealable plastic container as single phenolic compounds. A total concentration of the major phenolic compound (ferulic acid) with each of the other minor identified phenolic compounds (0.2mg/ml) was also prepared in acidified methanol and kept in a sealable plastic container prior analysis as combined form.

2.4. Microorganisms and Culture Conditions

The bacterial strains employed were the Gram-positive *Staphylococcus aureus*, and Gram-negative; *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Salmonella typhi* obtained from Microbiology Department, Federal University of Technology Akure, Nigeria and was cultured aerobically at 37°C in nutrient agar medium. Before experimental use, cultures from solid medium was sub- cultivated in liquid media, incubated for 24hr and

was used as source of inoculums for each experiment. The individual and combined phenolics in pearl millet extract were dissolved in 10% DMSO and sterilised by filtration through 0.45µm millipore filter. The antimicrobial activities were examined by agar well diffusion method by [11].

2.5. Preliminary Phytochemical Screening of Pearl Millets

The aqueous-methanolic extract was screened for the presence of some secondary metabolite such as saponin, tannin, alkaloids, terpenoids, steroid flavonoids and cardiac glycosides as directed [16].

2.5.1. Test for Alkaloids

0.5g of crude powder was defatted with 5% ethyl ether for 15 minutes. The defatted sample was extracted for 20 minutes with 5ml of 1% hydrochloric acid (aqueous) in a steam bath. The solution was sieved through filter paper. Dragendorff´s reagent (1ml) was added to 1ml of filtrate. The mixture was observed for changes in the colour to black or formation of precipitate. The changes in colour indicate the presence of alkaloids.

2.5.2. Test for Saponin

The ability of saponin to produce fronting in aqueous solution was used as screening test for saponin. 2g of the powder pearl millets was dissolved in distilled water in a test-tube and the mixture was shaken. Frothing which persists on warming was taken as preliminary evidence for the presence of saponins.

2.5.3. Test for Flavonoids

The presence of flavonoid in the sample was determined by the method described [16], 5ml of dilute ammonia solution was added to a portion of the aqueous filtrate of the extract followed by the addition of concentrated H_2SO_4. A yellow coloration observed in the extract indicated the presence of flavonoids.

2.5.4. Test for Tannin

0.5g of the powder sample of pearl millets was boiled in 20ml of distilled water in a test tube and then filtered. 0.1% of ferric chloride was added. A blue-black or brownish green coloration was taken as evidence for the presence of tannin [17].

2.5.5. Test for Terpenoids (Salwowski Test)

0.5g of the extract was dissolved in 2ml of chloroform, after which 3ml of concentrated H_2SO_4 was carefully added to form a layer. A reddish brown coloration at the interface indicated the presence of terpenoids.

2.5.6. Test for Steroids

0.5g of the methanolic extract of the sample was added to 2ml of acetic anhydrides with 2ml of H_2SO_4. There is no colour generation indicating no presence of steroids.

2.6. Quantification of Phenolic Compounds Using HPLC

Chromatographic equipment consisted of a Hewlett-Packard (Avondale, PA) liquid chromatography model 1090 equipped with a diode array ultraviolet (UV) detector. A TSK-GEL Super-ODS (Supelco, Bellefonte, PA) column was used. The absorbance of the effluent was monitored at 254 and 238 nm. The mobile phase consisted of solvents A-C using three pumps equipped with the chromatograph. Solvent A was 0.1% trifluoroacetic acid in acetonitrile, solvent B, 0.1% trifluoroacetic acid in HPLC grade water, and solvent C 100% methanol. Flow rate was set at 1.0 mL/min, and column temperature was maintained at 37 °C throughout the test. The initial solvent condition was 100% solvent B. A linear gradient was used to increase solvent A from 0 to 10% within 7 min. This solvent composition was maintained at an isocratic flow for 3 min. Solvent A was then increased from 10% to 40% using a 20min linear gradient. This composition was then maintained for 2 min and returned to the initial condition in 3 min. Solvent C was used for column washings between and after runs. Sample sizes of 4µm for the intact phenolics and 12µm for hydrolysed phenolics were injected during HPLC analysis. The use of different sample sizes was due to the different phenolic concentrations in intact and hydrolysed samples. The concentrations of phenolic acids in pearl millet flour were calculated from standard curves calibrated using the 16 phenolic standards. The phenolic contents were expressed as milligrams per 100g pearl millet flour.

2.7. Antioxidant Assay

2.7.1. Determination of DPPH Antiradical Assay

The DPPH assay was done according to the method [18] with some modifications. The stock solution was prepared by dissolving 24 mg DPPH with 100mL methanol and then stored at -20°C until needed. The working solution was obtained by mixing 10mL stock solution with 45mL methanol to obtain an absorbance of 1.1 units at 515nm using the spectrophotometer. Phenolic extracts (300mL) were allowed to react with 2700mL of the DPPH solution for 6 hours in the dark. Then the absorbance was taken with respect to the reference at 515 nm.

2.7.2. Determination of ABTS Antiradical Assay

Antioxidant activity of the extracts was determined using the 2, 2'-azinobis-(3- ethylbenzothiazoline-6-sulfonic acid) ABTS antiradical assay [19]. The $ABTS^{\bullet+}$ (mother solution) was prepared by mixing equal volumes of 8 mM ABTS and 3 mM potassium persulphate ($K_2S_2O_8$) (both prepared using distilled water) in a volumetric flask, which was wrapped in foil and allowed to react for a minimum of 12 hours in a dark place. The working solution was prepared by mixing 5 ml of the mother solution with 145 ml phosphate buffer (pH 7.4). A range of trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-carboxylic acid) standard solutions (100–1000 µM) were prepared in acidified methanol. The working solution (2.9 ml) was added to the methanolic extracts (0.1 ml) The test tubes were allowed to stand for exactly 30 min. The absorbance of the reference and samples was measured at 734.

2.7.3. Determination of Lipid Oxidation Assay

Egg homogenate (0.5ml, 10% v/v) and 0.1ml of each extract were added to a test tube made up to 1ml with distilled water (Ruberto, 2000). 0.05 ml $FeSO_4$ (0.07M) was added to induce lipid peroxidation and incubated for 30min. Then 1.5ml of 20% acetic acid (pH adjusted to 3.5 with NaOH) and 1.5ml of 0.8% (w/v) TBA in 1.1% sodium dodecyl sulphate and 20% TCA were added and the resulting mixtures were vortexed and then heated at 95°C for 60min. After cooling, 5.0 ml of butan-1-ol was added to each tube and centrifuge at 300 rpm for 10min. The absorbance of the organic layer was measure at 532nm. The percentage inhibition of lipid peroxide formation by the extract was calculated. The results were expressed as percentage inhibition (Absorbance of the control- Absorbance of sample/ Absorbance of Control x 100).

2.8. Antimicrobial Assay

The agar diffusion assay was used to investigate the antibacterial effect of phenolic compounds in pearl millet. The assay was carried out according to the method [20] with slight modification using Mueller Hinton agar. Twenty millilitre of the specified molten agar (45°C) was aseptically mixed with 1000µl of a bacterial suspension (108 cfu/ml) and poured into sterile petri dish. For the preparation of the lnnocula, colonies of bacteria were suspended in Mueller Hinton broth. The bacterial suspensions were adjusted turbimetrically to McFarland solution. The total colony-forming unit of bacterial suspension was estimated by serial dilution followed by plate count method. An aliquot (50ml) of the extract were placed into 6-9mm wells borrowed using corle borer and the plates were incubated for 24hr at 37°C - the test were carried out in triplicate. The antibacterial activity was measured as clear zone of diameter (mm) formed due to inhibition of the growth of the microflora with antibiotic zone scale in mm and the experiment was carried out in triplicate. Solvent control (methanol) was included in every experiment as negative control. COT- Cotrimazole (25µg); CXC- Cloxacillin (5µg); ERY- Erythromycin (5µg); GEN – Gentamycin (10µg); AUG- Augmentin (30µg); STR-Streptomycin (10µg); TET- Tetracycline (10µg); CHL-Chloramphenicol (10µg); OFL- Ofloxacin (5µg); NAL-Nalidixic Acid (30µg); NIT- Nitrofurantoin (200µg); AMX-Amoxicillin (30µg); TET- Tetracycline (25µg); were used as positive controls for gram-positive and gram negative bacteria respectively. The phenolic acid obtained by HPLC analyses were also assessed for their antibacterial property individual and combined form.

2.9. Statistical Analysis

Graph pad prism version 7 was used to analyze the data obtained and these were expressed as mean ± standard error of mean follow by Tukey's test was used to analyze and compare the results at a 95% confidence level.

3. Results

3.1. Preliminary Phytochemical Screening

The phytochemical screening of pearl millet showed that alkaloids, terpenoids (triterpenoids), flavonoids, and tannins were present. (Table 1).

Table 1. Phytochemicals Screening of pearl millets (*pennisentum glacum*).

ALK	FLA	STE	TER	SAP	TAN	CAR-GLY
+	+	-	+	+	+	-

ALK=Alkaloids; FLA=Flavonoids, STE=Steroids, TER= Terpenoids, SAP=Saponins, TAN=Tannins, CAR-GLY= Cardiac Glycosides.

Table 2. Quantitative estimates of some phenolic compounds in Pearl millet (*pennisentum glacum*) with (HPLC-UV).

Compounds	Retention time (min)	(Amount mg/100g)
Cinnamic Acid	9.253	336.66977
Gentisic Acid	10.085	89.09372
Protocatechuic Acid	10.549	10.31365
Vanillic Acid	11.598	17.89320
O—Coumaric Acid	11.733	56.30077
p-hydroxybenzoic Acid	12.489	21.99586
Caffeic Acid	13.997	20.52505
Syringic Acid	14.805	17.12190
Ferulic Acid	16.777	647.06488
Apigenin	18.969	15.50342
Luteolin	21.809	32.35630

Total Phenolics = 1264.8384.

3.2. Antioxidant Capacity

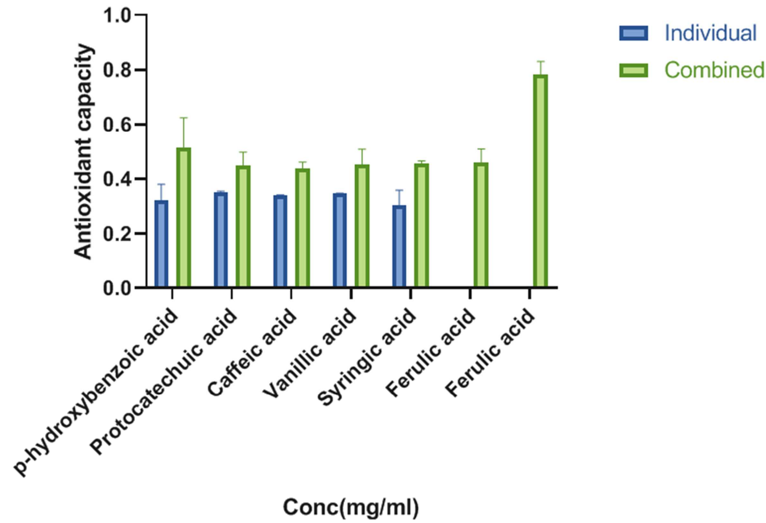

Figure 1. Effects of addition of the selected phenolics (100 mg/l) to ferulic acid (100 mg/l) on ABTS capacity. Mixture antioxidant capacity denoted the sum of antioxidant capacities of individual phenolics, which was presented in Table 2.

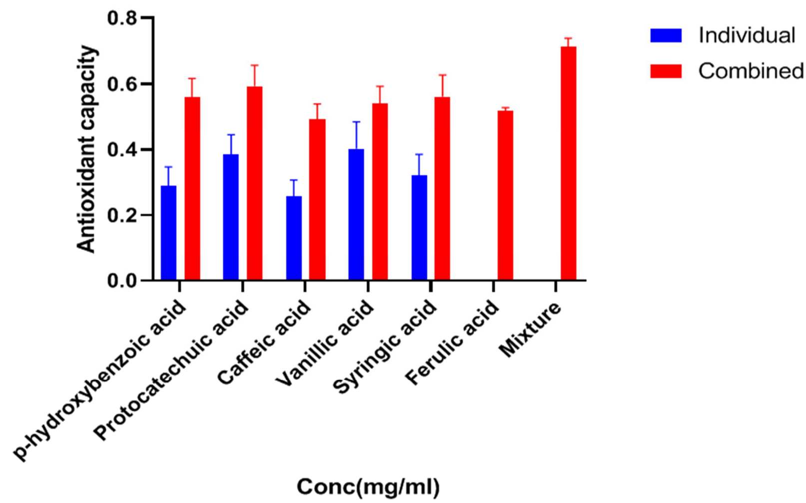

Figure 2. Effects of addition of the selected phenolics (100 mg/l) to ferulic acid (100 mg/l) on DPPH Scavenging Ability. Mixture antioxidant capacity denoted the sum of antioxidant capacities of individual phenolics, which was presented in Table 2.

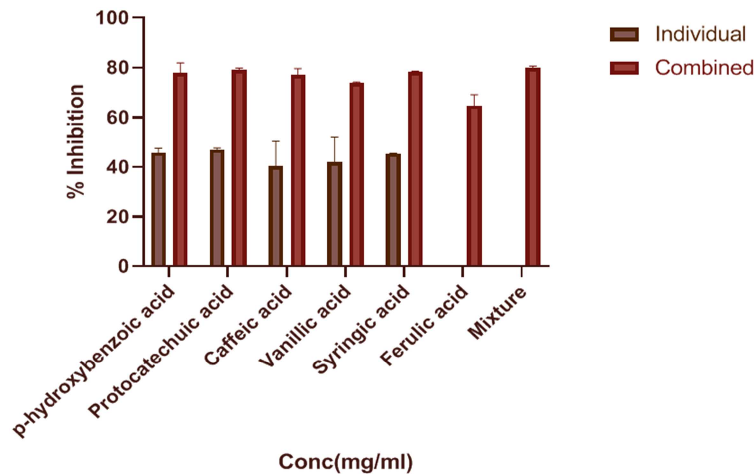

Figure 3. Effects of addition of the selected phenolics (100 mg/l) to ferulic acid (100 mg/l) on Lipid Peroxidation. Mixture antioxidant capacity denoted the sum of antioxidant capacities of individual phenolics, which was presented in Table 2.

Table 3. Mean Zones of inhibition of individual and combined phenolic compounds in pearl millet on bacterial isolates (mm).

Organisms	H	S	C	P	V	F
Escherichia coli	0.00±0.00^{a}	2.67±0.58^{b}	1.33±0.58^{b}	0.00±0.00	0.00±0.00	2.00±0.00^{bc}
Proteus mirabilis	0.00±0.00^{a}	0.67±0.58^{a}	0.00±0.00^{a}	0.00±0.00	0.00±0.00	1.33±0.58^{b}
Pseudomonas aeruginosa	0.00±0.00^{a}	1.00±0.00^{a}	1.67±0.58^{bc}	0.00±0.00	0.00±0.00	0.00±0.00^{a}
Staphylococcus aureus	1.67±0.58^{b}	0.67±0.58^{a}	2.33±0.58^{c}	0.00±0.00	0.00±0.00	3.67±0.58^{d}
Salmonella typhi	1.67±0.58^{b}	1.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00	0.00±0.00	2.67±0.58^{c}

Table 3. Continued.

Organisms	FH	FC	FP	FS	FV	Mix
Escherichia coli	1.67±0.58^{b}	2.00±0.00^{a}	0.00±0.00^{a}	3.33±0.57^{c}	3.33±0.58^{c}	9.00±1.00^{d}
Proteus mirabilis	0.00±0.00^{a}	1.67±0.58^{a}	0.00±0.00^{a}	1.00±0.00^{a}	0.00±0.00^{a}	5.67±0.58^{b}
Pseudomonas aeruginosa	2.33±0.58^{b}	2.00±0.00^{a}	1.33±0.58^{b}	2.67±0.58^{bc}	1.67±0.58^{b}	4.33±0.58^{a}
Staphylococcus aureus	3.67±0.58^{c}	5.67±0.58^{b}	2.00±0.00^{c}	2.33±0.58^{b}	2.33±0.58^{b}	7.67±0.58^{c}
Salmonella typhi	2.33±0.58^{b}	2.00±0.00^{a}	0.00±0.00^{a}	1.33±0.58^{a}	0.00±0.00^{a}	7.33±0.58^{c}

Conc (1000µg/disc), each.

$^{a-d}$ Means in the same column not sharing a common letter are significantly different ($P < 0.05$).

4. Discussion

Phytochemicals are natural bioactive compounds found in plant foods that work with nutrients and dietary fibre to protect against diseases by helping to slow down the ageing process and reduce risk of many diseases, including cancer, heart disease, stroke, high blood pressure, cataracts, urinary tract infections and osteoporosis. They also have complementary and overlapping mechanisms of action in the body including antioxidant effects, modulation of detoxification enzymes, stimulation of the immune system, modulation of hormone metabolism and antibacterial and antiviral effect [21]. The result showed the presence of alkaloids, terpenes, flavonoids, saponin and tannin, while cardiac glycosides, and steroids were absent in pearl millet as depicted in Table 1. Flavonoids found in millets could be used in management of various ailments like capillary and vascular weakness [22, 23]. Flavonoids have been reported to be useful in sexual stimulation [24]. It has been reported as a powerful water soluble free radical scavengers and prevention of oxidative cell damage, potent anticancer activity and inhibition of tumor growth [25]. It also contains hydroxyl functional group, which are responsible for antioxidant effect in some medicinal plants [26]. A study showed that flavonoids could inhibit the development of fluids that resulted in diarrhea by targeting the intestinal cystic fibrosis trans-membrane conductance regulator [27]. Flavonoids decrease capillary fragility and exert a cortisone-like effect on tissues [28]. The mechanism of action of flavonoids are said to be either through scavenging or chelating process [29, 30]. Studies have shown that tannins suppressed the production of the peptide responsible for hardening of arteries [31]. Saponins have potential in human health issues because they reduce serum cholesterol [32, 33]. Alkaloids have been used in the treatment of hypertension [34]. Animal studies have shown that dietary phytochemical antioxidants are capable of removing free radicals and thereby exhibiting potent antioxidant activities [35].

The HPLC result in Table 2 showed the phenolic compounds present in Pearl millet (*pennisentum glaucum*) which are categorised into two main groups namely flavonoids (quercetin, and kaempferol etc) and phenolic acids (cinnamic, ferulic, Caffeic, p-hydroxybenzoic, syringic, vanillic and protocatechuic acids. Ferulic acid was observed to be the most abundant phenolic acid in pearl millet, followed by cinnamic and p-hydroxybenzoic acid, Caffeic acid, vanillic acid, syringic acid, protocatechuic acid present in low amount. Phenolic constituents are very important in plants because of their scavenging ability due to presence of hydroxyl groups. A number of studies have focused on the biological activities of phenolic compound which are potential antioxidants and free radical scavengers. [36, 37].

The decolorization of ABTS^{+} cation radical is an unambiguous way to measure the antioxidant activity of phenolic compounds. Recently, [19] found positive correlations between the determination of phenolic antioxidant using the oxygen radical absorbance capacity (ORAC), ABTS and DPPH assays. Thus, monitoring the antioxidant activity of phenolic compounds by their ability to scavenge ABTS^{+} radical was demonstrated to give good prediction. Phenolics compounds present in pearl millet exhibited a strong radical scavenging ability as shown in Figure 1. It was observed that the mixture and combined phenolics in pearl millet have higher quenching ability than the individual identified phenolics considering their interaction, and this gives an account that phenolics interaction in pearl millet extract showed synergistic effects, and this is in agreement with the report [37].

1,1-diphenyl-2-picryl hydrazyl radical (DPPH) is a stable

free radical at room temperature and accepts electron or become a stable diamagnetic molecule [38] and has been used widely to evaluate the antioxidant activity of various natural products [39]. The decrease in the absorbance of DPPH radical is caused by antioxidants, which react with the radical. The model of scavenging the stable DPPH radical is a widely used method to evaluate antioxidant activities in a relatively short time compared to other methods. The addition of the extracts to the DPPH solution causes a rapid decrease in the absorbance at 515nm and the degree of discoloration indicates the scavenging capacity of the extract. The phenolics compounds present in pearl millet exhibited a strong ability to quench DPPH radical as depicted in Figure 2 and was observed that the mixture and combined phenolic in pearl millet have higher quenching ability The result showed that phenolics interaction in pearl millet extract exhibited synergistic effects. [37]. The DPPH radical scavenging activities of the extract have been attributed to the ability of the extract in pairing to the odd electron of DPPH radical [40].

Lipid peroxidation mediated by free radicals is considered to be the major mechanism of cell membrane destruction and cell damage. The damage has been implicated in the pathophysiology of various human diseases such as atherosclerosis, diabetes and cancer. The initiation of peroxidation sequence in membrane or polyunsaturated fatty acids is due to the abstraction of a hydrogen atom from the double bond in the fatty acids [41]. Malonyladehyde (MDA) is the major product of lipid peroxidation process. Incubation of egg yolk homogenates in the presence of $FeSO_4$ causes a significant increase in lipid peroxidation. The ability of the phenolic compounds presents in pearl millet to inhibit lipid peroxidation was tested using the method [42], as shown in Figure 3. The result also revealed that the mixture and the combined phenolic compound in pearl millet demonstrated highest inhibition of lipid oxidation as compared with the individual phenolic. Phenolic compounds have been reported to be activating lipid free or prevent the decomposition of hydrogen peroxide into free radicals [43]

The increasing occurrences of pathogenic resistant bacteria especially *S. aureus* to a wide range of antimicrobial agents particularly in hospital, including all kinds of *β*-lactams has made therapy more difficult. The increasing resistance to antibiotic represents the main factor justifying the need to find and/or develop new antimicrobial agents. Thus, many studies have been focused on antimicrobial agents and properties of plant-derived active principles [44]. Although strategies have been proposed in an attempt to control the spread of pathogenic bacteria, the search for new ways to treat infections stimulates the investigation for natural compounds as an alternative treatment of these infections [45]. In Table 3, the agar diffusion bioassay relating to antimicrobial activity of pearl millet phenolic compounds showed a very high levels of activity against the tested organism both grams positive and gram-negative bacteria. The antibacterial screening of the individual identified phenolic compounds, (p-hydroxybenzoic, protocatechuic, vanillic, syringic, caffeic, and ferulic) are presented revealed antimicrobial effects as follows; p-hydroxybenzoic acids have inhibitory eeffect against *S. aureus and S. typhi* (1.67mm) with no activities against *E. coli*, *P. mirabilis and P. aeuruginosa*. Syringic acid possessed activity against all the tested organism: *E. coli*, (2.67mm), *P. mirabilis* (0.67mm), *P. aeuruginosa* (1.00mm), *S. aureus* (0.67mm), and *S. typhi* (1.00mm), while caffeic acid have inhibitory action against *E. coli* (1.33mm), *P. aueruginosa* (1.67mm), *S. aureus* (2.33mm), and Ferulic acid inhibitory action against *E. coli* (2.00mm), *P. mirabilis* (1.33mm), *S. aureus* (3.67mm), *S. typhi* (2.67mm). The result showed a synergistic antimicrobial effect on the tested organisms, measured as zones of inhibition (mm) for combined phenolic compound: Ferulic/hydroxybenzoicacids: E. coli (1.67mm), *P. aueruginosa* (2.33mm), *S. aureus* (3.67mm), S. *typhi* (2.33mm), Ferulic/Caffeic: *E. coli* (2.00mm), *P. mirabilis* (1.67mm), *P. aueruginosa* (2.00mm), *S. aureus* (5.67mm), *S. typhi* (2.00mm), Ferulic/Protocatechuic: *P. aeruginosa* (1.33mm), *S. aureus* (2.00mm), Ferulic/Syringic, *E. coli* (3.33mm), *P. mirabilis* (1.00mm), *P. aeruginosa* (2.67mm), *S. aureus* (2.33mm), *S. typhi* (1.33mm), while Ferulic/Vanillic with inhibitory effect on: *E. coli* (3.33mm), *P. aeruginosa* (1.67mm), *S. aureus* (2.33mm). This is in agreement with Salawu *et al.*, (2011). Meanwhile, fferulic, caffeic, syringic and hydroxybenzoic showed a strong antimicrobial inhibitory effect (0.67-3.6mm) against some tested microorganisms (*E. coli*, *P. mirabilis*, *P. aeruginosa*, *S. aureus and S. typhi*). However, protocatechuic and vanillic acid did not show any antimicrobial effects against any of the organism. Interestingly, the Mixture phenolics showed better inhibitory effects (1.0-5.67mm) against the entire organism (*E. coli*, *P. mirabilis*, *P. aeruginosa*, *S. aureus and S. typhi*), no antimicrobial activities were observed for the combination of ferulic with hydroxybenzoic, protocatechuic and vanillic acids against *P. mirabilis* and *S. typhi*). These results indicate that ferulic, hydroxybenzoic, caffeic, syringic, protocatechuic and vanillic acids as contained in pearl millet extract offer certain synergistic effects in the mixed form. This by implication is that the pearl millet phenolic extracts could be harnessed as a potential antimicrobial plant. This result is in agreement with the reports [46].

5. Conclusion

This study showed that pearl millet phenolics extract have

antioxidant activities with a high level of radical scavenging action. The antioxidant and antimicrobial activities of the combination of the major phenolic compound (ferulic acid) with some selected minor compounds showed a synergistic interaction. In general, it could be inferred that the phenolics in pearl millet will be a useful natural product with a number of biological activities that could be channelled as a preventive measure towards the development of a number of diseases associated with free radicals. It is therefore recommended that some of the phenolic compounds in Pearl millet could be used as food additives in order to have a much better utilization of this phytoconstituents in health care and prevention.

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Availability of Data and Materials

The data sets analysed in this current study are available from the corresponding author on request.

Consent for Publication

Not applicable.

Declaration of Conflicting Interests

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