Original Article

Chemical Composition, Antioxidant and Antimicrobial Properties of *Vangueria madagascariensis* J.F.Gmelin (Kirkir) Fruit

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ABSTRACT

Background: *Vangueria madagascariensis* fruits (Kirkir) produces compounds own various nutritional and medicinal properties. **Objectives:** The study purpose was to investigate the chemical composition and antioxidant properties of kirkir fruits (*Vangueria madagascariensis*). **Materials and Methods:** Proximate chemical composition, minerals and antioxidant properties of kirkir fruits were consucted via determination of total flavonoids, Ferric reducing antioxidant power (FRAP) and DPPH free radicals scavenging Assay. The Antimicrobial activity of plant extracts was also investigated. **Results:** The results of chemical properties indicated that kirkir fruit contained: moisture 6.96%, protein 7.40%, carbohydrates 59.85%, fats 2.00%, dietary fibers 17.50% and ash 3.10%. It also contained micro nutritional elements such as ascorbic acid (145.00 mg/100 g) and beta-carotene (102 IU/100 g). The fruits were proved to be rich in phosphorus and potassium as 11.55 and 30.96 mg/g, respectively. The antioxidant activity of methanolic extract was determined by DPPH and FRAP. The radical scavenging effect was observed with IC_{50} = 36 μg/ml and 341 mM TE/g, respectively. The study showed that methanolic extract of kirkir fruit was proved to be effective as an antioxidant containing polyphenols 52.00 mg GAE/100 g and flavonoids 7.25 mg RE/100 g. **Conclusion:** Kirkir fruit has showed its high nutritive value (macro nutritional elements, important micro nutritional elements such as ascorbic acid and beta-carotene and minerals of tested fruits. The extract of kirkir fruit was demonstrated to be effective as a an antimicrobial and antioxidant containing polyphenols and flavonoids. The study recommends isolation of active ingredients and development of novel drugs from the *Vangueria madagascariensis* plant.

Key words: *Vangueria madagascariensis*, Minerals, Polyphenols, Ascorbic acid, Radical scavenging effect, Methanolic extract.

INTRODUCTION

Vangueria is tripe of blooming plant in the Rubiaceae family which contains around 600 species in 25 genera. *Vangueria madagascariensis* a perpetual plant found in both amazingly hot living spaces, for example, the dry desert-like regions of the horn of Africa like Sudan, the downpour backwoods of tropical Africa and the southernmost piece of Madagascar.[1,2] It belongs to the family Rubiaceae, it is a multipurpose wild fruit tree in western Sudan. A few species of genus *Vangueria* are broadly used in customary drug in different nations, example, in Tanzania, it is generally utilized for the redyment or potentially the executives of malaria, wounds, menstrual, and uterine issues. It is also utilized for the treatment of smallpox and bruises, herpes labialis, and in the administration of diabetes.[3-5]

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Figure 1: *Vangueria madagascariensis* dry fruits and seeds.

Each fruit of *V. madagascariensis* has 4 to 5 seeds, and the seed kernel (Figure 1) contains significant measure of oil which is higher than that of conventional oilseeds such as groundnut, cottonseed and sunflower.[5] Some of the phytochemical components such as flavonoids identified from *V. madagascariensis* are known to have antiallergic, antiinflammatory, antimicrobial, antiproliferative, antioxidant, enzyme inhibition, and oestrogenic activities, synergism with antibiotics, and concealment of bacterial virulence.[6-9]

It has been reported that the antioxidant activities of flavonoids include extinguishing free radical elements, metal chelation, concealment of enzymes involved in free radical scavenging, and stimulationof enzymes that activate antioxidant activities.[6] Research has additionally uncovered that food assets described by significant levels of flavonoids and related phenolic components may diminish the danger of cardiovascular sicknesses.[10] This study aimed to determine the physicochemical properties of *Vangueria madagascariensis* J.F. Gmelin. Kirkir fruit and to determine its antioxidant activity using DPPH and FRAP methods.

MATERIALS AND METHODS

Raw materials

Vangueria madagascariensis (Kirkir) ripe fruits which had been originated and grown naturally in Zalengi, western Sudan, were purchased from Abougahal market in Elobaied city, Western Sudan, during seasons (2016-2017) and (2017-2018). The fruits were collected in clean plastic bags.

Preparation of samples

Samples were prepared as described by.[11-13] The dirt, unripe fruits and extraneous matter were removed. Then fruit diameters was measured. In addition, the whole fruits, edible part, peel and a top loading balance weighed stones of fruits (model: D0001 – H R 120, A and D Company, Limited E C). The fruits were washed well with tap water to remove soil and dust particles, left in the shade to dry, and collected. Dry fruits were peeled, crushed to remove the seeds. Finely the edible part was collected and grinded (fine powder) by electric grinder(model-LM240-Groupe-SEB-53104, Mayenne-France), and kept tightly in a dark glass container at room temperature for further analysis.

Physico-chemical analysis

The physical properties of kirkir fruits were determined using standard methods.[14] These variable included: the dimension (for 100 fruits) included length (cm), width (cm) and thickness (cm), weight of 30 fruits (g), percentage of seeds and peels.

The total extractable matter was estimated.[15] Calculated the content of extractable matter as following:

$$\text{Total extractable matter (\%)} = \frac{\text{Volume of extract} \times \text{dried weight}}{25 \times \text{weight sample}} \times 100$$

Proximate composition estimation

The contents of moisture, ash, crude fibre, crude fat, dietary fibre of kirkir ripe fruit were determined by method described.[14] Carbohydrate contents were calculated as percentage by difference. The following formula is depicted in equation as shown below.

Carbohydrate % = 100 – (% Moisture+ % Protein + % Crude fibre +% Crude fat +% Ash content).[14]

Caloric values

The caloric values of the different samples were calculated by summing the values obtained through multiplying the contents of fats, protein and carbohydrates by the coefficients recorded bellow.[16]

Fat factor	= 8.37 K cal/g
Protein factor	= 3.87
Carbohydrate factor	= 4.12
1 K cal	= 4.184 KJ

Estimation of minerals content

Mineral contents (calcium, sodium, iron, zinc, and potassium) of kirkir ripe fruit were determined by the use of Unicam 919 Atomic Absorption Spectrophotometer U.K following.[14] official method. Test portions were

dried and then ashed at 4500°C under a gradual increase (about 500°C/hr) in temperature. The ash was dissolved in 20 ml of 1N HCl and heated for 5 min at 70°C. The solute was then transferred quantitatively to a 100 ml volumetric flask and made up to volume with distilled water. The absorbance of sample and standard solutions were determined. The standard curve plot of absorbance against the known concentration of standard solutions was used to determine the concentration of minerals in samples and expressed as shown in equation as shown in:

$$\text{Mineral content mg/100 g} = R \times \text{Extract vol. (L)} \times D.F \times S \text{ (Kg)}$$

Where:

R is mineral concentration in ppm (mg/Kg) as calculated using linear regression equation, D.F is Dilution Factor and S is sample weight (Kg).

Estimation of ascorbic acid

Vitamin C for kirkir ripe fruit was determined by 2,6 Dichlorophenol indophenols (DCIP) sodium salt method.[17] Under this method, titration was performed in the presence of phosphoric acid/acetic acid solution, to maintain proper acidity (pH 1-3) for titration, and to inhibit oxidation of the acid whereby 5g of powdered edible part fruit sample were taken into 250 ml erlenmeyer flask. 50 ml of orthophosphoric acid were added to extract, to lower pH as well as to deproteinize the sample. The extracted samples were then filtered and titrated against standardized dichlorophenol indophenols until pink color which is the end point of the reduction process was observed. The volume of Dichlorophenol indophenols used was recorded and vitamin C content in samples was calculated according to the following equation:

$$\text{Mg of ascorbic acid} = (X-B) \times (F/E) \times (V/Y)$$

Where:

X is titre value, B is blank, F is mg of ascorbic acid equivalent to 1.0 ml indophenols, E is number of ml assayed, V is initial assay solution volume and Y is volume of sample aliquot titrated.

Polyphenols (Tannins content)

Quantitative determination of tannins was carried out by using the modified vanillin-HCl colorimetric method as described.[18] Tannins (polyphenols) after complexing with vanillin-HCl reagent give distinct colour proportional to their concentration.Thecolour intensity is then measured spectrophptometerically**(**model: Shimadzu – AA - 6800**)** at wavelength of 500 nm whereby the concentration of tannin is calculated.

A weight of 0.2 g sample was placed in a test tube. Then, 10 ml of 1 % vanillin/ HCl/ methanol reagent were added. The test tube was capped and continuously shaken for 20 min and centrifuged (Fisher, USA) at 2500 rpm for 5 min. The absorbency of the clear supernatant (1.0 ml) was then measured spectrophptometerically (Milton Roy, USA) at a wavelength of 500 nm after incubation for 20 min at 30°C. After that, the concentration of condensed tannins was determined and expressed as catechin equivalent (CE) from catechin standard curve prepared under the same conditions. For zero setting of the spectrophotometer, 1.0 ml-redistilled water was mixed with 5 ml (4 %) HCl / methanol and 5 ml vanillin in a test tube as a reference standard and the absorbency of the reference solution was adjusted to zero at 500 nm.

Calculation

$$\text{Catechin equivalent (CE) \%} = C \times V \times 100/ W$$

Where:

C = Concentration corresponding to the optical density

V = Volume of extract (ml)

W = Sample weight (g).

Total pectin

Total pectin was estimated.[17] Twenty-five g of sample were mixed with 300 mL of 0.05% of the sodium salt of Ethylene Diamine Tetracetic Acid (EDTA), treated with 1.0 N NaOH to reach a pH of 11.5, allowed to sequester for 30 min at room temperature and the pH was adjusted to 5.0 with 1.0 N acetic acid. To this mixture, 0.1 g pectinase was added and stirred for about an h, diluted to 500 ml with distilled water, filtered through Whatman no. 1 and the first few mL of the filtrate were discarded. Two mL of the filtrate were diluted to 50 ml, from which 2 mL were taken for colorimetric determination of total pectin.

Sugars determination

Sugars in kirkir fruits powder were estimated by Lane and Eynon's method.[19] In this method, reducing sugars and total sugars were estimated using Fehling solution, then non-reducing suagrs contentwas calculated as follows:

$$\text{Non-reducing Sugars (\%)} = [\text{Total Sugar (\%)} - \text{Reducing Sugar (\%)}] \times 0.95.$$

Antioxidant Properties of *Vangueria madagascariensis* (Kirkir) Fruit

Total phenolic content

Total phenolic content was determined by,[20] method.

Total flavonoid content

Total flavonoid content was determined,[20] where 0.5 mL aliquot of the extract solution was mixed with 2.0 ml distilled water and subsequently with $NaNO_2$ solution (5%, 0.15ml). After 6 min, $AlCl_3$ solution (10%, 0.15 mL) was added and allowed to stand for 6 min, thereafter, NaoH solution (4%, 2.0 mL) was added to the mixture. Immediately, distilled water was added to bring the final volume to 5.0 mL. Then the mixture was properly mixed and allowed to stand for 15 min. The intensity of pink colourwas measured at 510 nm. The results were expressed as mg (+) – catechin equivalents (CEs) per g extract.

(1-1) Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay

The assay was carried out.[21] Stock solutions of crude extracts and the positive control, ascorbic acid (400 μg/ml), were prepared in methanol at appropriate concentrations and added to DPPH (200 μl at100 μm prepared in methanol) in a 96 microlitre plate.The plate was then incubated for 30 min at 37°C. Absorbance of each solution was measured at 517 nm.The extract and standard were analyzed in triplicate at different concentrations and the IC_{50} values were determined as follows:

$$\%\ \text{Inhibition} = \frac{\text{absorbance blank sample} - \text{absorbance extract}}{\text{absorbance blank sample}} \times 100$$

Ferric reducing antioxidant power (FRAP) Assay

The FRAP assay was determined.[1] In this assay, the antioxidant activity is determined based on the ability to reduce ferric (III) to ferrous (II) iron. To perform this test, stock solutions which included acetate buffer (300 mm, pH 3.6), tripyridyltriazine (TPTZ) (10mM) solution in HCl (40 (mM) mmol/L), and $FeCl_3 \cdot 6H_2O$ solution (20(mM)mmol/L) were used. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution, and 2.5 mL $FeCl_3 \cdot 6H_2O$ solution and then equilibrating at 37°C for 15 min before using. Plant extracts (0.15 mL) at known concentrations were allowed to react with FRAP solution (2.85 mL) for 30 min in the dark. Analysis of extracts and positive control trolox (200 mMmmol/L) were done in triplicate. Readings of the Persian blue complex were then taken at 593 nm. Results were expressed in mM Trolox equivalent (TE)/g.

Antimicrobial activity of plant extracts

Dimethyl sulfoxide (DMSO) (3%w/v) was used to prepare the stock of water and ethanolic extract solutions to evaluate their activities against the standard pathogenic bacteria and fungi that used in this study. 200, 400 and 600 mg/ml concentration of water and ethanolic extracts were used according to the,[22] method. Ketoconazole antifungal and Gentamycin antibiotic were used as control.

Statistical analysis

Replicates of each sample were analyzed using Statistical Analysis System (SAS). The analysis of variance (ANOVA) and least significant difference (LSD at 5%) were utilized to assess significant differences between means of samples.

RESULTS AND DISCUSSION

Physical properties of Vangueria madgascariensis kirkir fruit

The physical properties of kirkir fruits were carried out to determine the length, width and thickness, colour, weight of fruit, kernel and seed as percentage. Table 1 shows the dimensions of kirkir fruit for 100 fruits, from this Table the range was between 8.6 to 2.19 cm for length, this values were higher than the range of 2.03 to 1.05 cm for length that recorded,[23] for the same fruit, 2.00 to 3.13 cm for width also this values were higher than the range of 2.22 to 0.92 cm for width, these values 3.60 to 2.23 cm for thickness wwere higher than the range of 2.23 to 0.90 cm for thickness,[24] and 12.30 to 5.20 g for weight, these values were high than the range of 9.46 to 4.09 g for weight that obtained,[23] for the same fruit.

The correlation coefficient between weight and length was week, and between weight and width was moderate, while between weight with thickness was strong. Table 1 shows the weight of 30 fruits, there were higher significant differences ($P \geq 0.05$) in the weights. Large size11.399 g was reported as the higher weight, and small size 6.657g showed as the lower weight. The means of those weights were higher than the values 9.46g, 8.00g that obtained,[23,25] respectively.

Table 1 also shows the percentage of edible part (flesh) counted from the whole weight of the fruit, the results reported 3.8% for the large size fruit and 3.1% for the medium size fruit while only 2.0% for the small size fruit and that, these significant ($P \leq 0.05$) differences in

Table 1: Physical properties of different size of kirkir fruits.*					
Parameters	Fruit size			$LSD_{0.05}$	SE±
	Small	Medium	Large		
Length (cm)	5.00	5.52	8.67	0.06013	0.01632
Width (cm)	4.50	5.75	6.22	0.1287	0.03510
Thickness (cm)	5.08	5.18	6.10	0.1060	0.03015
Weight (g)	6.675	8.860	11.399	0.8987	0.4380
Edible part (flesh) (%)	2.0480	3.1150	3.8030	0.5885	0.2868
Seeds (%)	3.2530	4.0880	5.4060	0.5068	0.2470
Peels (%)	1.3660	1.6670	2.1460	0.3321	0.1619
Number of seeds	2.0	4.0	5.0	0.6560	0.2261

*Results are expressed as mean of three replicates.

Table 2: Proximate composition of kirkir fruits (on dry weight basis) and energy value.*	
Component	Percentage (%) + SE
Moisture	6.95 ±0.01
Crude protein	7.40±0.02
Fat	2.00±0.08
Fibre	17.50±0.08
Carbohydrate	59.85±0.05
Ash	3.10±0.05
Energy value (Kcal)	291.9±0.05

*Results are expressed as mean ± standard deviation of three replicates.

percentages of flesh were due to the differences in fruit weight. Table 1 also shows the percentage values for the fruit peels which reported 2.14%, 1.66% and 1.36% for large, medium and small size, respectively. There were no significant differences ($P \leq 0.05$) between the fruits size. The large size fruits had seed % of 5.41%, and medium size fruits had a seed % of 4.08% while the small size fruits had a 3.35%, there were significant differences ($P<0.05$) between the all sizes.

The number of seeds in small size fruit were only two seeds, and in medium size fruit were four while in large size were 5 seeds. The Figure of fruit was pale with bright-yellow-brown color in skin and edible part.

Proximate composition of kirkir fruit

Table 2 shows the percentage of moisture content obtained was 6.96%, this value was slightly more than that reported Maroy (2018),[26] who reported a value of 6.4%, and less than that reported by Abdelrahaman *et al.* (2014),[23] which was reported 7.92. Protein content of kirkir fruit was found to be 7.40%. This value was in agreement with that reprted by Abdelrahaman *et al.* (2014)[24] which was 7.74%, higher than than the value of 1.4% recorded by Ramalingum and Mahamoodally (2014)[1] and less than that reported by Mustafa,[27] who reported a higher value (22.2%). The changes of proteins however, indicate variations in metabolic activity during different development stages.[24] The fat content value for kirkir fruit was found to be 2.0%, this value was higher than 0.1% which was recorded by[1] and similar to 2.35% that reported by Abdelrahaman et al. (2014).[23]

Table 2 also shows that the percentage of crude fibre content of kirkir fruit was 17.50%. which was similar to 18.89% that reported by Abdelrahaman *et al.* (2014),[23] and higher than 4.7% which, was recorded by Ramalingum and Mahamoodally (2014).[1] This difference may be due to varieties. The ash content was found to be 3.10%. This value was similar to 3.63 obtained by Abdelrahaman (2014),[23] and for the same fruit, whereas lower than 4.90% recorded by Abdelmuti (2002),[25] for the same fruit. The carbohydrate content of kirkir fruit as 59.85%.This result was higher than 28% that obtained by Ramalingum and Mahamoodlly (2014),[1] and close to 67.42% that was recorded by Abdelrahaman (2014).[23] The energy value of kirkir as 291.9 kcal, this results is close to that reported by Abdelrahaman (2014)[24] and higher than 119.03 kcal for the same fruit reported by Ramalingum and Mahamoodally (2014).[1]

Physico-chemical properties of kirkir fruit

Table 3 presents the physico-chemical properties of kirkir fruit, from the table, the total extractable matter of kirkir fruit was reported as 38.60g/100 g edible part, this result was close to 40.10 g recorded by Abdelrahaman.[23] The pH value of kirkir fruit was 3.25, this result was similar to 3.60 which was recorded by Abdelrahaman *et al.*[24]

The total phenols content (aqueous) of kirkir fruit was 1.95 mg/g as GAE, this result was found to be similar to 2.22 mg/g which was reported by Abdelrahaman *et al.*[23] for the same fruit, whereas the (TPC-methanolic extract) of kirkir fruit was 52 mg/g as GAE, this value was lower than the range 112.5 to 170.4 that was recorded by Mustafa,[27] and it was in close agreement with the range

Table 3: Chemical properties of kirkir fruit (on dry weight basis.*

Component	Percentage (%)
Total extractable matter (%)	38.60±0.03
pH value (%)	3.25±0.05
Total sugar (%)	28.50±0.03
Reducing sugar (%)	16.00±0.08
Non-reducing sugar (%)	12.50±0.03
Titratable acidity* (%)	0.50±0.01
Vitamin c (mg/100 g)	145.30±0.03
β-carotene (IU/100 g vitamin A)	102.00±0.05
Polyphenols (Tannins) (%)	1.95±0.02
Pectin (%)	0.21±0.01
Colour (0.D at 420 nm)	1.525±0.002

*Results are expressed as mean ± standard deviation of three replicates.

(22.30–95.73 mg/g) for fourteen wild edible fruits from Burkina Faso recorded by Meda *et al.*[28] and lower than 61.22 mg/g for the same fruit reported by Ramalingum and Mahamoodally.[1] Phenolic compounds are auxiliary metabolites, broadly circulated in plants. They are significant parts of numerous fruits and vegetables not only for their major influence on sensory qualities of the fruit (color, flavor, and taste), yet additionally for their antioxidant, anticarcinogenic, antimicrobial, antiallergic, antimutagenic, and mitigating properties.

Table 3 shows the total sugar of kirkir fruit was 28.50%, reducing sugars were 16.00% and non-reducing sugars were 12.50%. While the titratable acidity of kirkir was 0.50, this finding agreed with the study carried out by Abdelrahaman *et al.*[23] The ascorbic acid content obtained was 145 mg/100 g. This value was higher than 4.7 mg/100 g that was reported by Ramalingum and Mahamoodally,[1] and close to 169.69 mg/100 g, that was reported by.[23] The β-carotene content of kirkir fruit was (102.00I U/100 g), this result was close to 115.61 obtained by Abdelrahaman *et al.*[24]

Pectin is notable to be a significant component of the essentia cell wall and intracellular substance of higher plants. As a pervasive component of leafy foods, pectin is a characteristic component of the human diet and is considered as a constituent of dietary fibre due to be resistant in the human stomach and small intestine.[29,30] Pectins have recently been appeared to have diverse biological activities, which may have a role in the beneficial effects of fruit and vegetable diets. In particular, pectins have been found to possess ROS scavenging activity which is known to rely upon the auxiliary features of pectin.[31,32]

Table 4: Mineral composition of kirkir fruit (mg/g).*

Mineral	Value (mg/g)
Calcium (Ca)	0.503±0.001
Iron (Fe)	0.067±0.001
Phosphour (P)	11.550±0.02
Sodium (Na)	3.20±0.02
Magnesium (Mg)	3.910±0.03
Manganese (Mn)	0.196±0.001
Potassium (K)	30.690±0.04

*Results are expressed as mean ± standard deviation of three replicates.

Table 4 shows that the minerals content of kirkir fruit were Ca 0.67 mg/g, Mn 0.196 mg/g, K 30.690 mg/g, Fe0.503, P11.550 and Magnesium 3.910.Abdelrahman,[23] reported Ca 0.075, Fe 0.522, P 12.880, Na 3.727, Mg 4.369, Mn 0.028 and K 32.820 for the same fruit, Whereas Ramalingum and Mahamoodally,[23] reported Ca 25.0 mg/g, Fe 1.1.mg/g, Mn 39.0 mg/g, P 366.0, K 521.0 and Na 28.0 for the same fruit. While Muhammad *et al.*[33] found Ca 0.37, Mg 0.966, Na 2.03, K 1.21, P 2.23, Fe 0.57, Mn 0.02 mg/g for Gardenia aqualla (*Gaudendutse*) fruit. Mustafa *et al.*[27] found that kirkir fruits had good quantities of protein and carbohydrtaes as well as appreciable amounts of macromierlas; K, Na, Mg and Ca.

Total flavonoids (TFC)

The total flavonoids content was found as 7.95 mg RE/g of the methanolic extract presented as mg of rutin equivalent/g of extract (Table 5), this result agreed with the findings of Ramalingum and Mahmoodally (2014).[23] and lower than the range of 23-298.8 mg RE/g for the same plant (different parts) that recorded by Mustafa (2017).[34]

Antioxidant Activities

DPPH free radicals scavenging Assay

Results presented in Table 5 also shows the (DPPH) free radicals scavenging activity of the methanolic extract of kirkir fruit was 36 µg/ml, this result was lower than 48.46 µg/ml for the same fruit that recorded by Ramalingum and Mahmoodally (2014),[23] and within the range of 7.81- 62.5 µg/ml for the same plant (different parts), that recorded by Mustafa *et al.*[27]

A free radical is characterizedas any atom or molecule having unpaired electrons.[35] In living systems, free radicals are producedas a feature of the body's typical metabolic procedure.[36] Antioxidants fight free radicals and protect us from various diseases.

Ferric reducing antioxidant power (FRAP)

Results presented in Table 5 shows that the ferric reducing antioxidant power (FRAP) of the methanolic

Table 5: Total phenolic content, Total flavonoid content, DPPH and FRAP of kirkir fruit extract.		
Sample	**Tests**	**Result (a mount)**
Methanolic extract of ripe fruit of kirkir (pericarp)	Total phenolic content (TPC)	52 (mg GAE/g of ripe fruit)
	Total flavonoid content (TFC)	7.95 (mg RE/g of ripe fruit)
	DPPH	36 (IC_{50}) (µg/mL)
	FRAP	341 (mM) mmol/Ltorolx equivalent (TE)/g ripe fruit

Table 6: Antimicrobial activity (mean zone of inhibition in mm) of kirkir fruit methanolic extracts.		
Tested micro-organisms	**Sample**	**Control**
Fungi	Kirkir fruit methoanlic Extract	Ketoconazole
Aspergillus fumigatus (RCMB 002008)	20	17
Aspergillus niger (RCMB 002005)	10	15
Candida albicans RCMB 005003 (1) ATCC 10231	13	20
Gram Positive Bacteria		Gentamycin
Staphylococcus aureus (RCMB010010)	11	24
Bacillus subtilis RCMB 015 (1) NRRL B-543	08	26
Staphylococcus epidermidis RCMB 009 (2)	13	28
Gram Negatvie Bacteria		Gentamycin
Escherichia coli (RCMB 010052) ATCC 25955	13	30
Salmonella typhimurium RCMB 006 (1) ATCC 140281``1`12	09	17
Proteus vulgaris RCMB 004 (1) ATCC 13315	10	25

Mean zone of inhibition in mm beyond well diameter (6 mm) produced on a range of pathogenic microorganisms. Results are depicted in the following Table above: The test was done using the diffusion agar technique, Well diameter:6.0 mm (100 µl was tested), RCMB: Regional Center for Mycology and Biotechnology.

extract of kirkir fruit was reported in this study as 341 mM (TE)/g, this value was lower than 357.08 mM (TE)/g which was recorded by Ramalingum and Mahmoodly.[23]

Antimicrobial activity of plant extracts

Results presented in Table 6 shows the reaction of the methanolic extract used of 20 mg/ml against a number of fungi and bacterial species. This extract showed higher inhibitory zone (20 mm) against *Aspergillus fumigatus* (RCMB 002008) compared to Ketoconazole antifungal with an inhibitory zone of 17 mm. In conrast Ketoconazole showed higher efficacy with inhibition zones of 15 mm and 20 mm, respectively against *Aspergillus niger* (RCMB 002005) and *Candida albicans (*RCMB 005003 (1) ATCC 10231) compared to methanolic extract of kirkir fruit which showed inhibition zones of 10 mm and 13 mm, respectively.

On the other hand, gentamycin showed stronger reaction against the tested gram (+) bacteria *Staphylococcus aureus* (RCMB010010), *Bacillus subtilis* RCMB 015 (1) NRRL B-543 and *Staphylococcus epidermidis* RCMB 009 (2) with inhibition zones of 24, 26 and 28 mm, respectively, compared to methanolic extract of kirkir fruit which showed inhibition zones of 11, 8 mm and 13 mm,respectively (Table 6).

Gentamycin showed stronger reaction against the tested gram (-) bacteria *Escherichia coli* (RCMB 010052) ATCC 25955, *Salmonella typhimurium* RCMB 006 (1) ATCC 14028 and *Proteus vulgaris* RCMB 004 (1) ATCC 13315) with inhibition zones of 30, 17 and 25 mm, respectively, compared to methanolic extract of kirkir fruit which showed inhibition zones of 13, 9 and 10 mm, respectively. Results conducted revealed that methanolic extract of kirkir fruit have antifungal and antibacterial activity against the tested microorganisms.

CONCLUSION

In conclusion, the chemical properties of kirkir fruit has showed its high nutritive value (macro nutritional elements, important micro nutritional elements such as ascorbic acid and beta-carotene and minerals of tested fruits. It was proved to be rich in phosphorus and potassium. The study showed that methanolic extract of kirkir fruit was demonstrated to be effective as a an antioxidant containing polyphenols and flavonoids, very effective as a an antifungal and antibacterial. In addaition, kirkir has a DPPH radical scavenging with great power. In order to utilize these phenolic compounds as antioxidants, it is advisable to substitute methanol solvent with other solvents with low toxicity. Further studies are needed to isolate and purify active

ingredients and development of novel drugs from the *Vangueria madagascariensis* plant.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

TFC: Total flavonoid content; **DPPH:** Diphenyl-2-picrylhydrazyl; **FRAP:** Ferric reducing antioxidant power; **SAS:** Statistical Analysis System (SAS); **ANOVA:** Analysis of Variance.

Author contributions

A. E. Sulieman, A. A. Mariod,– developed the concept and designed the experiment; H.Abdelgair – collected data and performed analyses; A. E. Sulieman, A. A. Mariod, N. Alshammari –A. Abdel Muhsin, Z. Salih analysed the data and wrote the paper.

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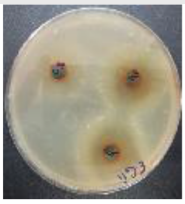
PICTORIAL ABSTRACT

Kirkir fruits

Determination of minerals



Inhibition of E.coli by kirkir

Mineral composition of kirkir fruit (mg/g).*

Mineral	Value (mg/g)
Calcium (Ca)	0.503±0.001
Iron (Fe)	0.067±0.001
Phosphour (P)	11.550±0.02
Sodium (Na)	3.20±0.02
Magnesium (Mg)	3.910±0.03
Manganese (Mn)	0.196±0.001
Potassium (K)	30.690±0.04

Total phenolic content, Total flavonoid content, DPPH and FRAP of kirkir fruit extract.

Sample	Tests	Result (a mount)
Methanolic extract of ripe frust of kirkir (pericarp)	Total phenolic content (TPC)	52 (mg GAE/g of ripe frui)
	Total flavonoid content (TFC)	7.95 (mg RE/g of ripe fruit)
	DPPH	36 (IC_{50}) (µg/mL)
	FRAP	341 (mM) mmol/Lterols equivalent (TE)/g ripe fruit

SUMMARY

Vangueria madagascariensis fruits (Kirkir) is reported for its various nutritional and medicinal properties. Chemical methods as well as well antimicrobial activity of the plant fruit were carried out. The results of chemical properties indicated that kirkir fruit contained: moisture 6.96%, protein 7.40%, carbohydrates 59.85%, fats 2.00%, dietary fibers 17.50% and ash 3.10%. It also contained micro nutritional elements such as ascorbic acid (145.00 mg/100 g) and beta-carotene (102 IU/100 g). The fruits were proved to be rich in phosphorus and potassium as 11.55 and 30.96 mg/g, respectively. The antioxidant activity of methanolic extract was determined by DPPH and FRAP. The radical scavenging effect was observed with IC_{50}= 36 µg/ml and 341 mM TE/g, respectively. The study showed that methanolic extract of kirkir fruit was proved to be effective as an antioxidant containing polyphenols 52.00 mg GAE/100 g and flavonoids 7.25 mg RE/100 g. The study recommends isolation of active ingredients and development of novel drugs from the *Vangueria madagascariensis* plant.

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