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A study on the chemical constituents, antibacterial and antioxidant activities of *Vangueria madagascariensis* (Fruit)

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Abstract

Vangueria madagascariensis belongs to the family (Rubiaceae) is used as a herbal medicine against diabetes, gastrointestinal problems, malaria, pain, parasitic worms, and skin diseases. The aims of this study are to evaluate the phytochemical screening, antibacterial and antioxidant activities, Phytochemical screening was carried out by using three solvent (ethanol, ethyl acetate and n-hexane), two gram positive and two gram negative bacteria were tested and antioxidant properties were determined by measuring abilities to scavenge DPPH. The result of phytochemical cited that: there were present of (Cardiac glycosides, alkaloids, reducing compounds, saponins Tannin, Phenols, steroids and triterpens, flavonoids and coumarins. Antibacterial tests indicated that the ethanol, ethyl acetate and n-hexane extracts inhibited the growth of all microorganisms and most extracts showed same degree of antibacterial activity.

Keywords: *Vangueria madagascariensis*, Rubiaceae, South Kordofan, Phytochemical, Alkaloids.

Introduction

Vangueria madagascariensis is a multistemmed deciduous shrub or tree growing up to 15 meters in height. The species is native to Tanzania, Democratic Republic of Congo, South Sudan, Angola, Cameroon, Ghana, Benin, Central African Republic, Ethiopia, Eritrea, Madagascar, Mauritius, Mozambique, Nigeria, South Africa, Sudan, Swaziland, Togo, Uganda, Seychelles, and Kenya ^[1]. The species used as a herbal medicine against diabetes, gastrointestinal problems, malaria, pain, parasitic worms, and skin diseases and it is regarded as a popular fruit tree, medicinal plant, and it has positive effects on human health and well-being ^[2, 3, 4]. Which are beyond the provisions of basic nutritional requirements, there is no universally accepted definition of functional food and nutraceuticals, but ^[5, 6, 7] argued that functional food and nutraceuticals are natural foods that beneficially affect one or several body functions apart from nutritional effects, influencing both the health and well-being of the consumer. The value of pharmaceutical drugs derived from plants, other natural health products, nutraceuticals, and functional foods are being promoted throughout the world as an alternative strategy for disease risk reduction and reduction in health care costs ^[6] *V. madagascariensis* is an important food resource especially during droughts and in times of food shortages ^[8].

Materials and methods

Plant material, collection and identification

Plant collection

Vangueria madagascariensis dry fruits were collected from locality of Algoze (Dubibat-Alfingar mountain area) South Kordofan State- Sudan. In February 2020.

Preparation of the crude extracts

A weight of 150 gram of the coarsely powdered–dried sample was successively extracted with n-hexane, by using orbital mixer (shaker) for 48 hours, the n-hexane extract was evaporated by transferring it from conical flask to plate ; the residual powdered fruits was air dried and extracted again with ethyl acetate for 48 hours the extract was evaporated by transferring it in to conical flask to plate, Finally the air dried residual powdered fruits extract again with 70% ethanol for 48 hours and extract evaporated. Any solvent has different amount of extract from each other. Each evaporated extract was transferred from plates to bottles and stored in refrigerator.

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Phytochemical screening

Phytochemical screening was performed using standard procedures as described by [9, 10]. With some modification.

Test for reducing compounds (Fehling's test)

The aqueous ethanol extract (0.5 g in 5 ml of water) was added to boiling Fehling's solution (A and B) in a test tube. The red coloration indicates the presence of reducing sugars.

Test for anthraquinones

About 0.5 g of the extract was boiled with 10 ml of sulphuric acid (H₂SO₄) and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added. A pink coloration indicates the presence of anthraquinones.

Test for terpenoids (Salkowski test)

To 0.5 g each of the extract was added 2 ml of chloroform. Concentrated (H₂SO₄) (3 ml) was carefully added to form a layer. A reddish-brown coloration of the interface indicates the presence of terpenoids.

Test for flavonoids

Three methods were used to test flavonoids:

First, dilute ammonia (5 ml) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1 ml) was added. A yellow coloration that disappears on standing indicates the presence of flavonoids. Secondly, a few drops of 1% aluminum solution were added to a portion of the filtrate. A yellow coloration indicates the presence of flavonoids. Thirdly, a portion of the extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow coloration indicates the presence of flavonoids.

Test for saponins

To 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for tannins

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

Test for alkaloids

About 0.5 g of extract was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken

gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Dragendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish-brown precipitate (with Dragendorff's reagent) was regarded as positive for the presence of alkaloids.

Biological activities

Antibacterial test

The procedures used for the antimicrobial screening in the present study were described previously as [11, 12]. The disc diffusion method was used as a preliminary test to find out if plant extracts were active. Clear inhibition zones around discs indicated the presence of antibacterial activity.

Antioxidant activity

DPPH (radical scavenging assay)

DPPH radical scavenging was determined according to the methods of [13]. In 96-wells plate the test samples were allowed to react with 2,2-Di (4-tert-octylphenyl)-1-picrylhydrazyl stable free radical (DPPH) for half an hour at 37 °C. The concentration of DPPH was kept at (300µM) the test sample was dissolved in DMSO while DPPH was prepared in ethanol after incubation; decrease in absorbance was measured at 517nm using multiplate reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group. All tests and analysis were run in triplicate.

IV. Results

Phytochemical screening of *Vangueria madagascariensis* fruits.

Table 1: Results of Phytochemical screening

Phytochemical	Results
Alkaloids	+
Cardiac glycosides	+
Reducing compounds	+
Phenols	+
Saponin	+
Tannins	+
Flavonoids	+
Steroids and Triterpens	+
Coumarins	+

Phytochemical screening showed the presence of Cardiac glycosides, alkaloids, reducing compounds, saponins Tannin, Phenols, steroids and triterpens flavonoids and coumarins, these results are agreement with [14].

Antibacterial activities of *Vangueria madagascariensis* fruits.

Table 2: Antibacterial activity of *Vangueria madagascariensis* fruits

Plant extraction	Standard bacterial strains				
	Concentration in mg/ml	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>
Ethanol	100	17	18	18	17
	50	16	17	17	17
	25	16	16	16	16
	12.5	14	15	15	15
Ethyl acetate	100	20	21	15	17
	50	19	20	14	16

	25	18	18	13	16
	12.5	17	17	12	15
n-hexane	100	21	17	14	15
	50	20	16	13	14
	25	19	15	12	13
	12.5	18	14	12	12

Concentration of extracts (100, 50, 25, 12.5mg/ml). Zone of inhibition in (mm), - no inhibition, <9mm inactive, 9-12mm partially active, 13-18mm active, >18mm very active.

The n-hexane and ethyl acetate extracts showed high activity at all concentrations (100,50,25,12.5) against *Escherichia coli* (20,19,18,17), partially active (15,14,13,12) against *Staphylococcus aureus* in ethyl acetate and n-hexane extracts; the low activity against *Bacillus subtilis* (14,13,12,12), respectively as well as *staphylococcus aureus* (20,17,14,12), and low activity against *Bacillus subtilis* (15,14,13,12) in n-hexane extract, ethanol extract cited activity against all bacteria in all concentrations (100,50,25, 12.5), as well as *Bacillus subtilis* in ethyl acetate extract (17,16,16,15).

Antioxidant activity

Table 3: Antioxidant activity of *Vangueria madagascariensis* fruit extracts on free radical scavenging activity on DPPH (RSA%±SD).

No	Extracts	RSA% ± SD (DPPH)
1	Ethyl acetate	60± 0.04
2	Ethanol	65 ± 0.09
3	n-hexane	80 ± 0.08
Standard	Propyl Gallate	91 ± 0.01

The antioxidant activity of different extracts of *Vangueria madagascariensis* fruits

Have been evaluated using important parameters (RSA%±SD) free radical scavenging activity on DPPH the most potent activity on the DPPH scavenging activity was observed by n-hexane extract (80±0.08); while the ethyl acetate exhibited less activity on DPPH as compared with other extracts.

Discussion

Phytochemical screening conducted on the plant extracts (Table 3) revealed the presence of compounds which are known to exhibit biological as well as physiological activities [15]. These are alkaloids, flavonoids, polyphenols, saponins, sterols and triterpens. Triterpenoids are also known to possess antimicrobial, antifungal, anti-parasitic, antiviral, antiallergenic, antispasmodic, anti-hyperglycemic, anti-inflammatory and immunomodulatory properties [16]. Flavonoids were tested positive in *Vangueria madagascariensis* fruits extract possess significant antioxidant activities and their effects on human nutrition and health are considerable with known properties which include free radical scavenging, inhibition of hydrolytic oxidative enzymes and anti-inflammatory action [17, 18]. Antioxidant properties, especially radical scavenging activities, are very important due to the deleterious role of free radicals in foods and biological systems [19] (Hussein. *et al*; 2014). DPPH-molecule that contains a stable free radical has been widely used to evaluate the radical scavenging ability of antioxidants. As indicated in table (3) the result of DPPH scavenging activity assay in this study indicates that n-hexane extract (80±0.08) represented the maximum activity which is comparable to propyl gallate as standard this could suggest moderately polar anti-oxidant compounds.

Conclusion

These findings showed that *Vangueria madagascariensis* fruits can be regarded as promising resources for anti-oxidant drugs, this study serves customs in developing countries in addition to contributing further depths to the growing literature on plant materials recognized as a reservoir of important to antibacterial and anti-oxidant compounds and provided scientific support for the antibacterial activity of extracts of the *Vangueria madagascariensis* fruits.

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