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Phytochemical screening, assessment of biological activity and nutritional value of Sudanese *Hibiscus sabdariffa* L. seeds

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Abstract

Medicinal plants are widely used in traditional cultures all over the world and they are becoming increasingly popular in modern society as natural alternatives. This research aimed to conduct phytochemical screening; assess of antimicrobial and antioxidant activities; evaluate toxicity and detect the nutritional value of *Hibiscus sabdariffa* L. seeds. The Phytochemical screening was carried out using standard procedures. The samples were extracted using hexane and absolute ethanol respectively. The extracts were evaluated for their effectiveness against microorganisms using the disc diffusion method and the antioxidant potential was measured by the DPPH assay. The proximate analysis of seed powder and mineral composition was determined using standard procedures. Toxicity was evaluated by the brine shrimp lethality test and the LD_{50} was calculated. Phytochemical screening revealed the presence of alkaloids, saponins and flavonoids, while tannins and anthraquinones were not detected. Generally, the hexane extract showed higher antibacterial activity than the ethanolic extract against 4 bacteria. Both extracts exhibited antifungal activity against *Candida albicans* ranging from 12-17 mm. The hexane extract showed higher antioxidant activity than the ethanolic extract (60 % and 47 % respectively). Both extracts were non-toxic against brine shrimps, suggesting that they are safe for medicinal use. The results of the proximate analysis showed that the seeds contain high amounts of fat (34.19 %) and protein (33.25 %). The seeds of *H. sabdariffa* contained considerable amounts of some important elements, while lead was not detected. The findings of this study revealed that *H. sabdariffa* seeds are a rich source of secondary metabolites and nutritional value and could be used for pharmaceutical preparation and drug development.

Keywords

Hibiscus sabdariffa; antimicrobial; antioxidant; nutrition; toxicity

Introduction

Historically, medicinal plants have been essential sources of pharmacological lead compounds (1). Traditional societies worldwide extensively employ medicinal herbs and they are also becoming increasingly popular in modern civilization as organic substitutes for synthetic chemicals (2).

Most of the tribal populations of Sudan use alternative medicine for treatment. They have simple and effective remedies to treat common ailments, with plants being the main source of their traditional remedies (3).

Hibiscus sabdariffa, commonly known as Roselle, belongs to the family Malvaceae and is a popular herb used for its medicinal properties. It offers several health benefits and is considered a nutraceutical. Used in traditional medicine as a source of many therapeutic agents, it grows well in tropical countries. In Sudan, it serves as a major crop export, especially in the western areas (4). Recent pharmacological studies have shown that *H. sabdariffa* possesses an antibacterial effect (5). It is rich in antioxidants, such as anthocyanins, which have been found to have potential protective effects against chronic conditions such as heart disease and cancer. *H. sabdariffa* also contains a variety of compounds such as polysaccharides, organic acids and flavonoids, which have anti-inflammatory, anti-diabetic and anti-hypertensive properties (6).

It is also known for its diuretic and laxative properties, which can help promote bowel regularity and aid in weight loss. Additionally, it has been found to have a cooling effect on the body and can assist in reducing fever and inflammation. The seeds are composed of starch, cellulose, carbohydrates, campesterol, ß-sitosterol, ergosterol, pelargonic acid, palmitic acid, oleic acid, myristic acid, methanol, malvalic acid, linoleic acid, sterulic acid, caprylic acid, isoamyl alcohol, ethanol, 3-methyl-1-butanol, fiber and minerals (7).

Populations in developing countries may only have access to local traditional medicines as their main source of primary healthcare. The Sudanese people frequently use medicinal herbs, which can help combat the issue of antibiotic resistance, one of the largest challenges to current world health and development. Access to high-quality antibiotics is still a significant problem in Sudan. *H. sabdariffa* seed is low cost compared to antibiotics. The present study aims to evaluate the antimicrobial activity, detect the presence of secondary metabolites, determine the antioxidant activity, nutritional value and evaluate the toxicity of the seeds.

Materials and Methods

Collection of Plant Material, Preparation and Extraction

The *Hibiscus sabdariffa* seeds were collected directly from the field in Kordofan state and authenticated at the Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), Khartoum, Sudan. The *H. sabdariffa* seeds were cleaned and air-dried at room temperature (30 °C) and ground to a coarse powder. 300 g of the powder were extracted using hexane and absolute ethanol by a Soxhlet extractor for 8 h respectively. The extracts were dried under reduced pressure and the % yields were calculated as follow:

Yield (%) = Volume of extract obtained (g)/ weight of raw material (g) × 100 (Eqn. 1)

Phytochemical Screening

Phytochemical examinations were carried out on the powder of *H. sabdariffa* seeds. The chemical constituents, include alkaloids, flavonoids, tannins, saponins and anthraquinones, were detected according to Treas and Evans standard methods (8).

Antimicrobial Assay

The antimicrobial activity of hexane and ethanolic extracts was evaluated by the disc diffusion method (9). Four isolates and standard bacterial strains, including 2 Gram-positive (*Bacillus subtilis* NCTC 8236 and *Staphylococcus aureus* ATCC 25923) and 2 Gram-negative (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) bacteria as well as the standard fungal species *Candida albicans* ATCC 7596, were used in the study. Ciprofloxacin (5 mcg) and gentamicin (10 mcg) standard antibiotics were also included. The sensitivity test was conducted using the Kirby-Bauer disc diffusion method on Muller-Hinton agar. Initially, the discs were soaked in various concentrations of the extracted material for 2 h and then dried in an oven at 40 °C for 10 min. Next, a sterile cotton swab was dipped into the suspension, rotated multiple times and firmly pressed against the inner wall of the tube above the level of saline to eliminate excess inoculum. Subsequently, the bacterial suspension was evenly spread across the entire surface of the plate by stroking the swab back and 3-4 times. This process was repeated in 3 different directions by rotating the plate 60 °C after each stroke. To remove any excess moisture, the plates were then left on the bench for 4-5 min. Subsequently, the dried discs, along with the antibiotics discs, were placed onto the surface of the medium previously inoculated with the tested organisms. Using sterile forceps, each disc was gently pressed onto the agar to ensure consistent contact. The plates were then incubated at 37 °C for 24 h. DMSO 30 % serves as negative control, while various antibiotics served as positive control. Subsequently, each plate was inspected for zones of inhibition and the average diameter of each zone was measured.

Determination of Nutritional Value

Proximate Analysis of Plant Samples

The powdered sample of the seeds was analyzed to determine the moisture, protein, fat, ash and fiber content using standard analytical procedures (10).

Mineral Analysis

The mminerals content in the seeds (iron, sodium, potassium, calcium, phosphorus and lead) were determined using an atomic absorption device (11).

Antioxidant Activity

DPPH Radical Scavenging Activity

The DPPH radical scavenging activity was assessed by following method with some modifications (12). In a 96-well plate, the test samples were allowed to react with a stable free radical called 2,2-Diphenyl-1-picrylhydrazyl (DPPH) for 30 min at 37 °C. The concentration of DPPH used was 300 µM. The test samples were dissolved in DMSO, while the DPPH solution was prepared in 80 % ethanol. After incubation, the reduction in absorbance was measured at 517 nm using a spectrophotometer and the %

of DPPH radical scavenging activity was calculated using the following formula:

Scavenging activity (%) = [(Absorbance of control - Absorbance of the sample)/Absorbance of control] × 100. (Eqn. 2)

Brine Shrimp Lethality Test

The cytotoxicity of hexane and ethanol seed extracts was evaluated using the method with some modifications (13). Shrimp eggs, specifically *Artemia saline*, were placed in natural seawater, resulting in the hatching of a significant number of larvae within 48 h. For the experiment, 20 mg of the 3plants' extracts were dissolved in 2 mL of solvent. From this solution, 5, 50 and 500 µL were taken and transferred to separate vials, creating concentrations of 10, 100 and 1000 µg/mL respectively. The solvent was allowed to evaporate overnight and the volume was adjusted to 5 mL with seawater. Each vial was then populated with 10 larvae using a Pasteur pipette and the samples were incubated at room temperature for 24 h. The numbers of dead and surviving larvae were recorded and the data was analyzed using the Finney Probit Analysis computer program to determine LD_{50} values.

Results and Discussion

Yield %

The hexane extract yield the highest % (16.57 %), followed by the ethanolic extract (2.13 %).

Qualitative Preliminary Phytochemical Screening

The qualitative phytochemical analysis was initially performed using different chemical reagents to detect the presence of secondary metabolites in the seed. The results are presented in Table 1.

The presence of alkaloids, saponins and flavonoids has been reported (5). However, the presence of tannins was not clear compared to the findings that the seeds contained an appreciable amount of tannin (14). Previous studies reported that the seeds contain alkaloids, which are known for their medicinal properties such as local anesthetics, stimulants, psychedelics, analgesics, antibacterial, anticancer drugs, antihypertensive agents, antiasthma therapeutics and antimalarial (15). *Hibiscus* is a good source of flavonoids, which are plant compounds known for their antioxidant and anti-inflammatory properties. Studies have shown that consuming *Hibiscus* regularly may help reduce high blood pressure, lower LDL cholesterol levels and improve liver health (16). Saponins are also present in *Hibiscus*; these compounds are considered rich foods that provide preferential chemical preventive measures, reducing the risk of human cancer (16).

Table 1. Phytochemical Screening of *Hibiscus sabdariffa* seed.

Phytochemicals	Specific test	Result
Alkaloids	Mayer	+ve
	Wagner	+ve
	Dragendorffs	+ve
Saponins	Froth test	+ve
Flavonoids	Lead acetate	+ve
Tannins	Ferric chloride	-ve
	Gelatin	-ve
Anthraquinones	Borntrager test	-ve

Key: (+) Presence; (-) Not detected

Antimicrobial Activity

Different extracts showed variable activity against the tested bacteria, as shown in Tables 2 and 3. Generally, the hexane extract exhibited higher antibacterial activity than the ethanolic extract. Specifically, at a concentration of 100 mg/mL, the hexane extract displayed the highest activity against *Pseudomonas aeruginosa* (18 mm), *Bacillus subtilis* (17 mm), *Escherichia coli* (16 mm) and *Staphylococcus aureus* (15 mm), while the ethanolic extract showed lower activity against *P. aeruginosa* (13 mm) followed by *S. aureus* (13 mm), *E. coli* (13 mm) and *B. subtilis* (12 mm). Additionally, all extracts demonstrated significant antifungal activity against *Candida albicans*, with inhibition zones ranging from 12 to 17 mm. Standard antibiotics such as Ciprofloxain and gentamicin exhibited activities ranging from 15 to 25 %.

The seed hexane and ethanol extracts exhibited lower antimicrobial activities when tested against isolated bacteria. These results align with published data in the literature (5), indicating the likelihood of microbial resistance among isolated bacteria from humans.

Previous studies have demonstrated that the seed extracts of *H. sabdariffa* possess significant antimicrobial activity against a wide range of microorganisms. This activity is attributed to the presence of various phytochemicals such as flavonoids, alkaloids, phenolics and terpenoids (17).

The seed extract exhibited significant inhibitory activity against common foodborne pathogens such as *E. coli* and *Salmonella enterica* (18). Additionally, another study demonstrated its significant antimicrobial activity against bacterial strains responsible for dental caries. Moreover, the extract has been found to be effective against fungal pathogens like *C. albicans*, which cause skin and mucous membrane infections (19). Furthermore, *H. sabdariffa* seed extracts have potential applications in the food, pharmaceutical and cosmetic industries as a natural preservative and antimicrobial agent (20). The seed extract can be used as a natural alternative to synthetic preservatives, which have been associated with various health risks. Additionally, previous research has demonstrated the antifungal activity of *H. sabdariffa* seeds. A study investigated the antifungal activity of an ethanolic extract of *H. sabdariffa* seeds against *C. albicans*, a common fungal pathogen known to cause infections in the mouth, throat and genitals (21). The extract was found to significantly inhibit the growth of *C. albicans*, indicating its potential as a natural antifungal agent (21). Another study investigated the antifungal activity of *H. sabdariffa* seed oil against 3different fungal pathogens, including *Aspergillus niger*, *C. albicans* and *Fusarium solani* (17). The seed oil exhibited strong antifungal activity against some pathogens, suggesting its potential as a natural alternative to synthetic antifungal agents (17). Furthermore, these studies suggest that *H. sabdariffa* seeds may possess

Table 2. Antimicrobial activity of *Hibiscus sabdariffa* seed extracts and standard antibiotics against standard organisms

Extract	Concentration	Gram-ve negative bacteria		Gram –ve positive bacteria		Fungi
		E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*	*C. albicans*
Hexane	100 mg/mL	16 ± 1.40	18 ± 0.00	15 ± 2.80	16.5 ± 0.70	16.5 ± 0.70
	50 mg/mL	15.5 ± 2.01	15 ± 1.40	14 ± 1.40	16.5 ± 0.70	16.5 ± 0.70
	25 mg/mL	13 ± 0.00	15 ± 1.40	13 ± 0.00	16 ± 0.70	16 ± 2.10
	12.5 mg/mL	13 ± 0.70	13 ± 4.20	14 ± 1.40	15 ± 0.70	15 ± 0.70
Ethanol	100 mg/mL	13 ± 0.70	13 ± 0.00	13 ± 0.00	12 ± 0.00	12 ± 0.70
	50 mg/mL	12 ± 0.70	12 ± 0.70	12 ± 0.00	11 ± 0.00	11 ± 0.70
	25 mg/mL	12 ± 0.70	11 ± 0.00	11 ± 1.40	10 ± 0.00	10 ± 0.70
	12.5 mg/mL	11 ± 104	11 ± 0.00	11 ± 1.40	10 ± 0.70	9 ± 1.40
Ciprofloxacin	5 mcg	25	20	24	25	-
Gentamicin	10 mcg	17	15	25	18	-

Zone of inhibition (ZOI): < 9 mm, inactive; 9-12 mm, partially active; 13-18 mm, active; >18 mm, very active.

Table 3. Antimicrobial activity of *Hibiscus sabdariffa* seed extracts and standard antibiotics against isolate organisms

Extract	Concentration	Gram -ve bacteria		Gram -ve bacteria	
		E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*
Hexane	100 mg/mL	10 ± 0.70	11 ± 2.10	9 ± 2.80	13 ± 0.70
	50 mg/mL	8 ± 0.00	10 ± 2.80	12 ± 2.10	10 ± 2.10
	25 mg/mL	7 ± 0.00	9 ± 1.40	8 ± 2.80	11 ± 3.50
	12.5 mg/mL	6 ± 0.70	7 ± 0.70	8 ± 4.20	10 ± 3.50
Ethanol	100 mg/mL	11 ± 2.80	11 ± 0.70	12 ± 0.70	-
	50 mg/mL	9 ± 1.40	8 ± 0.70	9 ± 1.40	-
	25 mg/mL	9 ± 0.70	9 ± 2.10	7 ± 0.70	-
	12.5 mg/mL	8 ± 0.00	6 ± 0.70	7 ± 0.00	-
Ciprofloxacin	5 mcg	45	31	29	30
Amoxicillin	5 mcg	6	6	6	6
Ceftraixone	30 mcg	6	6	6	6

Zone of inhibition (ZOI): < 9 mm, inactive; 9-12 mm, partially active; 13-18 mm, active; >18 mm, very active.

antifungal activity and have the potential to be used as a natural antifungal agent.

Nutritional Value of *Hibiscus sabdariffa* Seed

Proximate Analysis

The results of the proximate analysis of the nutritional value of the seeds were presented in Fig. 1. The study aimed to determine the nutritive value of the seeds using standard procedures. The caloric value of the organic constituents was calculated from ash content, moisture content, fiber, crude protein, carbohydrates and fat. The total ash value was found to be 6.21 %, indicating the presence of normal complexes of inorganic and organic compounds (22). The moisture content was determined to be 1.89 %, suggesting minimal chances of microbial degradation of the drug during storage, as excess moisture can lead to the breakdown of important constituents through enzymatic activity (23, 24). The value of crude fiber in the seeds was measured at 22.65 %, while the amount of crude protein was found to be 33.25 %, indicating higher protein content. Additionally, the amount of carbohydrate was determined to be 1.81 %.

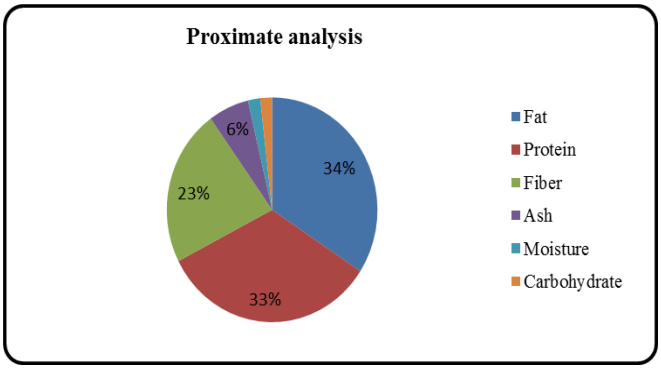

Fig. 1. Proximate analysis of *Hibiscus sabdariffa* seed.

Plants can serve as additive supplements, offering natural source of vitamins, minerals, antioxidants and other compounds beneficial to human health. Previous studies have indicated that plant seeds are rich sources of valuable nutrients. In comparison with published data, carbohydrates were found to be similar, but there were notable differences in crude fiber, ash, protein and moisture levels (22). These variations may be attributed to difference in climate zones and seasonal fluctuations. Furthermore, the nutritional value of plants can vary depending on the species, part of the plant and growing conditions.

Mineral Composition

The mineral contents of the seeds were determined using atomic absorption and the results are presented in Fig. 2. Among the mineral analysed, potassium exhibited the highest content at 150.20 ppm, followed by calcium (43.550 ppm), sodium (18.46 ppm), phosphorous (10.40

ppm) and iron (5.050 ppm). Lead was not detected, suggesting it may be present in trace, undetectable amounts.

According to previous studies, the seed is a rich source of various minerals. The mineral content in the recent study aligns with findings (25), who reported variable minerals compositions in the seeds, notably an increase in potassium (in some cases), sodium and calcium levels. Minerals play crucial roles in normal growth, muscles activity and skeletal development (such as calcium), cellular function and oxygen transport (iron), chemical reactions in the body, intestinal absorption, fluid balance and nerve transmission (sodium and potassium) (26). Deficiencies in these minerals can adversely affect the performance and health of both humans and livestock (27).

Antioxidant Activity

The most common method used to investigate the antioxidant activity of plant extracts is the DPPH radical scavenging assay, resulting in the formation of stable free radicals detected by common spectrophotometric techniques. The decrease in absorbance indicates the more efficient antioxidant activity of the extraction in terms of hydrogen atom donating capacity (28, 29). The antioxidant activity of the hexane and ethanolic extracts of the seeds were evaluated using the DPPH assay, with the highest result shown by the hexane extract (60 %) followed by the ethanol extract (47 %). Propyl gallate (91 %) was used as a pharmaceutical reference standard (Table 4). A previous study showed that the DPPH scavenging activity of seed oil possessed a moderate antioxidant potential of 50 ± 0.01 µg/mL compared to Propyl gallate of 89 ± 0.01 µg/mL (5). Several studies suggest that plants rich in antioxidants play a protective role in health and against diseases, lowering the risk of cancer, heart diseases, hypertension and stroke (19). According to previous studies, *H. sabdariffa* seeds have been reported to possess potent antioxidant activity due to their high content of phenolic compounds such as anthocyanins, flavonoids and phenolic acids. These compounds have been shown to scavenge free radicals and inhibit lipid peroxidation, which are major contributors to oxidative stress and various diseases (30). In a study, the antioxidant activity of *H. sabdariffa* seed extracts was investigated. The results demonstrated high levels of antioxidant activity, with the extracts capable of scavenging free radicals and inhibiting lipid peroxidation. The study attributed these observed activities to the presence of phenolic compounds (31). Another study confirmed the potent antioxidant activity of the methanol extract of *H. sabdariffa* seeds. The research revealed that the seeds are a rich source of phenolic compounds and exhibited high antioxidant activity (32). These findings suggest that *H. sabdariffa* seeds have the potential to be utilized as a natural source of antioxidants (33).

Brine Shrimp Lethality Assay

The toxicity of hexane and ethanolic extracts of the seed was evaluated using the brine shrimp lethality test (34, 35). All extracts demonstrated no toxicity to brine shrimps, with $LD_{50} \geq 1000$ µg/mL. The results are presented in Table 5.

Based on the literature review, *H. sabdariffa* (Roselle) is generally safe for consumption and no toxicity has been reported in humans. However, excessive use of the plant or its extracts may lead to adverse effects such as diarrhea, stomach cramps, nausea and vomiting (36). It is important to note that some studies have reported potential toxicity in animals at high doses of *H. sabdariffa*

Table 4. DPPH radical scavenging activity of *Hibiscus sabdariffa* seeds

Sample	% RSA ± SD (DPPH)
Hexane extract (5 mg/mL)	60 ± 0.04
Ethanol extract (5 mg/mL)	47 ± 0.02
Propyl gallate (Standard)	91 ± 0.01

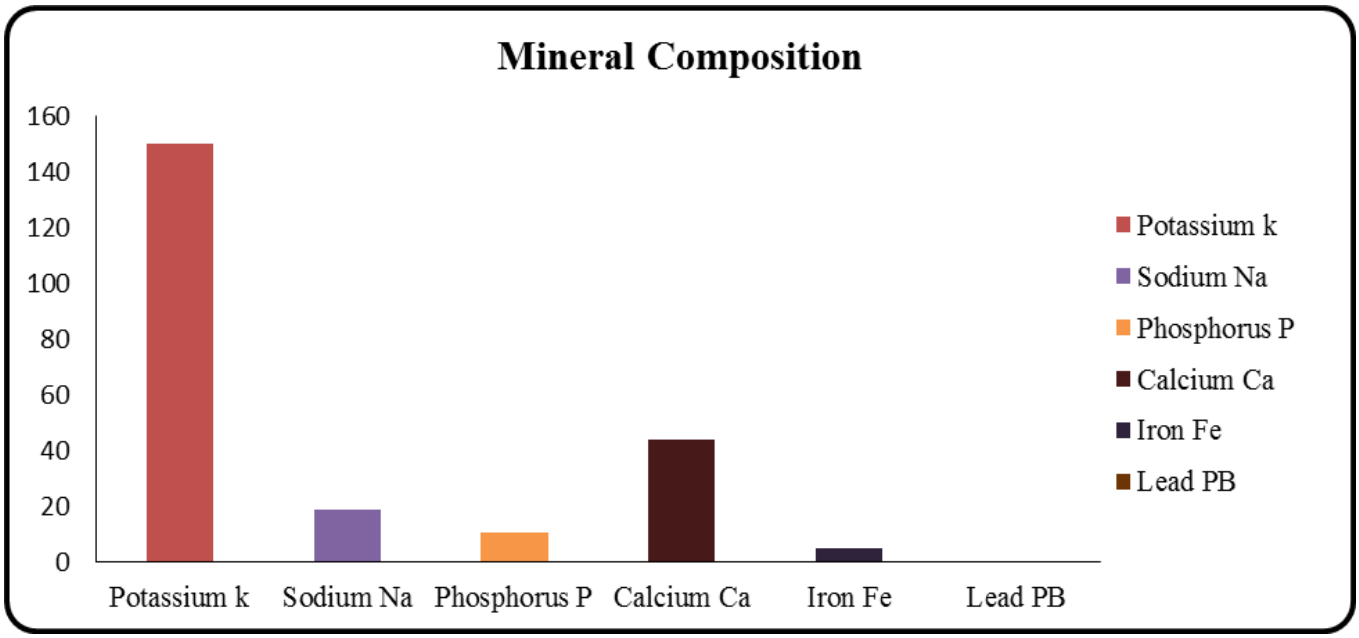

Fig. 2. Mineral analysis of *Hibiscus sabdariffa* seed.

Table 5. Toxicity of *Hibiscus sabdariffa* seeds extracts

Extract	LD_{50} (µg/mL)	Result
Hexane	6701.55988	Non-toxic
Ethanol	37898.11337	Non-toxic

Key:≥ 1000 µg/mL: Non-toxic; 500-1000 µg/mL: weak;100-500 µg/mL: moderate; 0-100 µg/mL: strong; ≤20 µg/mL: very active (34)

extracts. Therefore, pregnant and lactating women as well as individuals with pre-existing medical conditions are advised to consult a healthcare professional before consuming the plant or its products (37).

Conclusion

The findings of this study revealed that *Hibiscus sabdariffa* seed is a potent nutraceutical with a wide range of health benefits. It is rich in antioxidants and antimicrobial compounds, making it a valuable dietary supplement. The seed could be considered safe for consumption based on the study's results. These findings support some of the traditional uses of the seeds and could pave the way for the development of new active natural products. Further studies are recommended to characterize the active constituents responsible for the therapeutic activities of the seeds.

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Authors' contributions

IMM initiate the idea, performed sample collection, performed the laboratory work and wrote the original draft. AAA performed analysis, supervised the experimental process, responsible for conceptualization and investigation of the study. SMHA was responsible for conceptualization and investigation and revised the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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