

EVALUATION OF PHYTOCHEMICAL VARIATION, ANTIOXIDANT AND ANTIMICROBIAL POTENTIAL OF THREE SUDANESE TRADITIONAL MEDICINAL PLANTS

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ABSTRACT: Herbal medicine has a long history of being for treating various ailments ranging in severity. Traditional medicine is widely used regularly by Sudanese. This research aims to determine the phytochemical constituents and evaluate the antioxidant and antimicrobial activities of the crude extracts from three Sudanese traditional medicinal plants khella (*Ammi visnaga* L.), marula (*Sclerocarya birrea*) and black mustard (*Brassica nigra*). The phytochemicals of the oils were determined by Gas Chromatography - Mass Spectrometry (GC-MS). The GC-MS analysis showed that the predominant constituents in the black mustard seed oil were 2,4-Decadienal (31.72%), 2-Pyrrolidinone 1-methyl (19.41%), phenol (9.43%), Limonene (6.8%), Benzoic acid (2.12%), 2-(1-phenylethyl)- (1.78%), and Eugenol (0.96%). The identified fatty acids were Oleic acid (1.86%) and Palmatic acid (1.34%). The major aromatic components found in the marula bark extract were Phenol,2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl (29.96%), Phenol, 2,4-bis(1,1-dimethylethyl) (1.94%). The major fatty acid present was Linoleic acid (18.51%). The most abundant components found in the khella seed oil were α -Farnesene (11.8%), Ammidin (5.44%), Methoxsalen (3.62%), Stearic acid (2.81%), Palmitic acid (2.81%), Decanoic acid, propyl ester (1.10%) and Oleic acid (0.81%). To assess the antioxidant activity of the three aqueous extracts, the radical scavenging activity (RSA %) for khella seed extract varied between 21.15 and 75.44 for all concentrations (125, 250, 500, 1000 μ g/ml). At a concentration of 1000 μ g/ml, black mustard seed extract scavenged 46.65% of DPPH radicals, whereas that of 500, 250 and 125 μ g/ml caused 28.31%, 20.68%, and 19.38% RSA, respectively. The aqueous extract of marula bark had the highest RSA% for all concentrations (98.67% 97.06%, 95.76%, and 80.84%, respectively) compared to the aqueous seed extract for khella and mustard. Antimicrobial activities of different solvents extract of the three medicinal plants studied against three selected microorganisms (*E. coli*, *S. aureus* and *C. albicans*). Ethanolic extract of marula bark and khella seed showed the most potent activity against *S. aureus* and *C. albicans*. Aqueous and chloroform extracts failed to inhibit growth for the three strains. The MIC concentrations ranged from 0.37 mg/ml to 0.75 mg/ml for both bacteria and fungi.

KEY WORDS: *Medicinal plants, Phytochemical constituents, Antimicrobial, Antioxidant.*

1. INTRODUCTION

Worldwide, plants have played a vital role in human civilization as constituents of food, cosmetics, clothing, dyes, fibres, flavours, fragrances, medicines, pesticides, fertilizers and herbicides, etc. [1].

The unique geographical position of Sudan is reflected in its diverse climate and its varied natural resources. It ranges from completely arid to tropical zones with a wide range of bioclimatic regions [2]. The Sudan flora consists of: flowering plants; about 3137 species from 170 families and 1280 genera [3]; identified 278 species, 210 genera and seventy-two families as medicinal, culinary and aromatic plants (MCA) [3]. Medicinal plants have played an important role in the treatment of diseases, especially in rural areas in Sudan, as in many developing countries; [4] reported that Sudan has been exporting medicinal plants in dried form to different African, Asian, European, North and South American countries since 1952.

Medicinal and aromatic plants are important pillars of healthcare and traditional medicinal systems of the world [5]. Today, about 80% of the world population residing in the third world countries still rely almost entirely on plant products for their primary health care. The main problem facing the use of herbal medicines is the proof required that the active ingredients contained in medicinal plants are useful, safe and effective. This is a highly important requirement to get the approval of health authorities, and to assure the medical staff and the public concerning the use of medicinal plants as drug alternatives. Therefore, conducting multi-disciplinary research is a must for the formulation of efficacy and safety standards for herbal healthcare products. This will help to install a mechanism to screen all the herbal healthcare products for consumer protection and to keep the accountability on public health [6]. A very large number of scientific studies were conducted on herbal medicines, and this reflected the importance of such studies of medicinal plants for their scientific utilization and uses. The scientific study of herbal medicines is a must, because it reflects the importance of the scientific use of these medicinal plants in diseases treatment and control. There is a world-wide recognition of the vital importance of these medicinal plants in health care, their importance in the eradication of diseases, and their expanding role in health care services. In addition, it has always been the directions of the WHO to the national health authorities in developing countries to pay attention to these medicinal plants and give them due care and role in the national health care system [6].

Although aromatic and medicinal plants in Sudan are widely distributed and exploited, they still do not find serious attention from the government, scientists and researchers. This could be attributed to the following reasons:

1. Availability and cost of improved seeds.
2. Absence of modern technologies.
3. Traditional production methods.
4. Most plants are wild.
5. Insufficiently trained human staff.
6. Difficulty of screening material resources for small producers.

Khella (*Ammi visnaga* L.) belongs to the family Umbelliferae and has been commonly used for colic and gastrointestinal cramps, kidney stones and painful menstruation [7]. Also, it is used in the treatment of mild angina. Further, Khella is used as a supportive treatment for respiratory conditions such as asthma, bronchitis, cough and whooping cough [8].

Mustard (*Brassica nigra*) is of the family *Brassicaceae*. It has a pungent taste and a rich nutty odour. In addition to its importance as a food flavoring agent, Mustard seeds also have important medicinal uses such as in the treatment of rheumatism and joint pains, induration of the liver and spleen, throat tumors and as a laxative [9]. Phytochemical screening of mustard oil reveals the presence of tannins, flavonoids and alkaloids, together with antibacterial activity [10].

Sclerocarya birrea, known as the Marula tree, has gained attention in numerous chemical, biological and environmental investigations since 1906 and has been identified as one of five fruit tree species that should be integrated with the domestication process in the African farming system [11], for its usage as a source of food and medicine and its potential to increase income in rural communities. The stem bark, roots and leaves of *S. birrea* have been used for decades as a traditional anti-bacterial [12], anti-proliferative [13] and anti-diabetes medicine [14].

The surveyed information revealed that limited studies were carried out on the chemical composition and bioactivities of medicinal plants growing in Sudan. Thus, this research aimed to determine the chemical constituents and evaluate the antioxidant and antimicrobial activities of the crude extracts from three Sudanese traditional medicinal plants, which will be a baseline for future search to identify the pharmaceuticals.

2. MATERIALS AND METHODS

2.1. Plant materials

The seeds of Khella and Mustard and bark of Marula were collected from local traditional herbalists' shops (Atareen) at Khartoum and authenticated by National Centre for Research, Khartoum, Sudan.

2.2. Extracts preparation

The extraction of seed oil was done using a soxhlet apparatus (solvent extraction) and mechanical pressing at the laboratory of the National Oilseed Processing Research Institute (NOPRI), University of Gezira, Sudan. Marula bark was ground using a laboratory blender (Waring Commercial, HGBTWG4, USA) and then extracted with soxhlet using water, chloroform, and ethanol solvent as described by [15]. The extracts were subjected to antioxidant and antimicrobial analyses.

2.3. GC-MS method for analysis of FAME

The fatty acid composition was determined at the International Institute for Halal Research and Training (INHART), the International Islamic University Malaysia. The GC-MS Instrument (QP 22010 Ultra), equipped with a capillary column (0.25 diameter; film thickness 0.25 μm) was used. The carrier gas used was helium (99.99%). The GC oven's initial temperature was 600C and increased to 3000C at a rate of 100C/min. The carrier gas was helium at flow a rate of 50ml/min and the sample was injected in split mode. Identification of compounds was based on a comparison of the relative retention time and mass spectral data and the NIST mass spectral library of the GC-MS.

2.4. Antioxidant Activity Analysis

The antioxidant analysis was run at the faculty of Pharmacy, University of Gezira, Sudan. About fifty grams of each coarsely powdered plant material were macerated in 500 ml of ethanol (70%) for 72 hours with occasional shaking, then filtered using the Buchner filtration unit. The combined ethanolic extracts were evaporated using the

rotary evaporator unit to dry. The free radical-scavenging activity of the extracts was evaluated by 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) according to the method reported by [16]. Sample stock solutions (1000ppm) were diluted to final concentrations of 1000, 500, 250, and 125 µg/ml in methanol. One ml of a 0.3 mM 2, 2 DPPH in methanol solution was added to a 2.5 ml solution of the different concentrations of the extracts and allowed to react at room temperature for 30 minutes. The absorbance was measured at 550 nm against an aliquot blank. Methanol was used as a control. All determinations were performed in triplicate. The percentage of radical scavenging activity (RSA %) was calculated as follows:

$$\text{RSA\%} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100 \quad (1)$$

2.5. Antimicrobial Activity Assay

2.5.1. Microorganisms

The following standard strains of microorganism, obtained and authenticated by the Faculty of Medical Laboratory Sciences (FMLS), University of Gezira, Sudan, were used: Gram-negative *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 23235), and the fungal pathogenic yeast strain used in this research project were *Candida albicans* (ATCC 10231).

2.5.2. Antimicrobial susceptibility testing

Both antibacterial and antifungal activities of the extracts were evaluated by the well diffusion method [17]. Each control for suspension was prepared by emulsifying 3-5 colonies of the strain in 5 ml of sterile physiological saline, then was compared with the Mcfrland standard turbidity solution (0.05 ml of 1.175% barium chloride dihydrate ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$), with 9.95 ml of 1% sulfuric acid (H_2SO_4). This produced a suspension containing 1.5×10^8 CFU/ml [18] for strains of bacteria and fungi. Using a sterile cotton swab, each bacterial suspension was sub-cultured on a plate of Mueller-Hinton agar. At one side, a well of 6 mm in diameter was made on the surface of the agar media and then the extract was added in it. Each treatment was replicated three times. At the other side, a disc of vancomycin and ceftriaxone antibiotic were placed as control for *S. aureus* and *E. coli* respectively, and a disc of amphotercine B for *C. albicans*. Then, the plates were incubated at 37°C for 24 hours and 25 °C for 48 hours for the bacteria and fungi, respectively. The zone of inhibition (if present) was measured [18].

2.5.3. Assessment of Minimum Inhibitory Concentration and Minimum Bactericidal/Fungicidal Concentration

The minimum inhibitory concentration (MIC) was measured to the plant extracts for which the organisms show sensitivity. For MIC, seven test tubes were prepared and filled with 1.0 ml sterilized Mueller Hinton agar. The first tubes received an additional 100 µl of the plant extract, then the mixture was serially diluted through the other five tubes to create a concentration sequence from 10^{-1} to 10^{-5} . An amount of 100 µl of the strain suspension was added to all tubes except the 7th tube. So, tube 6 served as antibiotic/extract control containing culture media and extract (negative control), and tube 7 as growth control containing culture media and the bacterial suspension (positive control). All test tubes were incubated for 24 h at 37°C. The resulting turbidity was

observed; and the MIC was determined to be at the tube where growth was no longer visible. On other hand, the MBC/MFC was determined by sub-culturing the test tubes dilutions, those used in the MIC test, on to a fresh solid medium plate and then were incubated further for 24 h at 37°C. After that, all plates were checked for visible growth. The highest dilution that yielded no bacterial/fungal growth on a solid medium was taken as the MBC and MFC [19].

3. RESULTS AND DISCUSSION

3.1. GC-MS Phytochemical Determination

The GC-MS revealed that the most abundant aromatic compounds found in the black mustard seed oil were 2,4-Decadienal (31.72%), 2-Pyrrolidinone 1-methyl (19.41), phenol (9.43%), Limonene (6.8%), Benzoic acid (2.12%), 2-(1-phenylethyl)- (1.78%), and Eugenol (0.96%). The predominant fatty acids found were Oleic acid (1.86%) and Palmitic acid (1.34%). The major aromatic components found in the marula extract were Phenol, 2,2'-methylenebis(6-(1,1-dimethylethyl)-4-methyl (29.96%), Phenol, 2,4-bis(1,1-dimethylethyl) (1.94%). The major fatty acid was Linoleic acid (18.51%). The most abundant components found in khella seed oil were α -Farnesene (11.8%), Ammidin (5.44%), Methoxsalen (3.62%), Stearic acid (2.81%), Palmitic acid (2.81%), Decanoic acid, propyl ester (1.10%) and Oleic acid (0.81%).

3.2. Antioxidant scavenging activity

The percentage of antioxidant activity of the three aqueous extracts, obtained from seeds of mustard and khella, and bark of marula, was assessed by DPPH free radical assay (Table 1). At a concentration of 1000 $\mu\text{g/ml}$, black mustard extract scavenged 46.65% of DPPH radicals, whereas that of 500, 250 and 125 $\mu\text{g/ml}$ caused 28.31%, 20.68%, and 19.38% RSA, respectively. The RSA% for khella seed extract was 75.44, 45.12, 21.15, and 21.85% for the concentration of 1000, 500, 250, and 125, respectively. At 1000 $\mu\text{g/ml}$ the seed extract of khella showed higher RSA% than the mustard seed extract. The aqueous extract of marula bark had the highest RSA% for all concentrations compared to the aqueous seed extract for khella and mustard (Table-1). High RSA% in Marula bark could be ascribed to the high level of phenolic compound. [20] reported that the aqueous bark extract had the highest antioxidant activity compared to leaf and root extracts obtained from different solvents. The total antioxidant capacity of all the extracts increased with an increase in extract concentration.

Table 1: Antioxidant activity results of the three samples extract

Concentration $\mu\text{g/ml}$	Plant extracts		
	Black Mustard	Khella	Marula
1000 $\mu\text{g/ml}$	46.65%	75.44%	98.67%
500 $\mu\text{g/ml}$	28.31%	45.12%	97.06%
250 $\mu\text{g/ml}$	20.68%	21.15%	95.76%
125 $\mu\text{g/ml}$	19.38%	21.85%	80.84%
$\pm\text{SD}$	0.12	0.25	0.08

*The result is a mean of three replicates \pm SD (standard deviation)

3.3. Measurement of Antimicrobial activity using different solvents

As can be seen in table 2, the microorganisms exhibited different susceptibility responses against those extracts. Ethanolic extract of marula and khella showed the most potent activity against *S. aureus* and *C. albicans*. In the ethanolic extract of marula, the diameter of the inhibition zone was 14 mm for *S. aureus* and *C. albicans*. In the ethanolic extract of khella, the mean diameter of the inhibition zone was recorded as 12 mm and 14 mm for *S. aureus* and *C. albicans*, respectively. In the aqueous and chloroform extract, both marula and khella extracts failed to inhibit the growth of *E. coli*, *S. aureus* and *C. albicans*. The different solvent extracts of black mustard produced no observable inhibitory effect. Most traditional healers use water to prepare their extracts because water is not harmful and is the only solvent available. However, non-polar bioactive compounds cannot be extracted when using water for extraction. This could justify that the aqueous extract did not reveal an inhibition zone versus all strains.

In table 3, the ethanolic Khella extract has an MIC of 0.37 µg/ml for *S. aureus* and *C. albicans*. Ethanolic marula extract has MIC of 0.75 µg/ml for *S. aureus* but 0.37 µg/ml for *C. albicans*. The MBC and MFC were the lowest concentration that kills the test organism. In table 3, ethanolic khella extract has an MBC/MFC of 0.75 mg/ml for *S. aureus*, but *C. albicans* has an MFC of 1.5 mg/ml.

Table 2: Susceptibility pattern of different plant extracts against microbial strains

Solvents	Susceptibility pattern								
	Marula Extract			Khella Extract			Mustard Extract		
	W	Ch.	Eth	W	Ch.	Eth	W	Ch.	Eth
Microbe									
<i>E. coli</i>	R	R	R	R	R	R	R	R	R
<i>S. aureus</i>	R	R	S (ZID, 14 mm)	R	R	S (ZID, 12 mm)	R	R	R
<i>C. albicans</i>	R	R	S (ZID, 14 mm)	R	R	S (ZID, 14 mm)	R	R	R

*Where: W: Water Extract, CH: Chloroform Extract, Eth: Ethanol Extract.
R: Resistance; S: sensitive; ZID: Zone of Inhibition Diameter

Table 3: MIC and MBC values for marula and khella extracts against *S. aureus* and *C. albicans*

Microbial species	MIC and MBC/MFC (µg/mL) of plant extracts			
	Ethanolic Khella extract		Ethanolic Marula extract	
	MIC	MBC/MFC	MIC	MBC/MFC
<i>S. aureus</i>	0.37	0.75	0.75	1.5
<i>C. albicans</i>	0.37	1.5	0.37	1.5
±SD	0.0	0.53	0.26	0.0

*The result is a mean of three replicates ± SD (standard deviation)

*Where: MIC = minimum inhibitory concentration; MBC = minimum bactericidal concentration; MFC = minimum fungicidal concentration

4. CONCLUSION

The results indicate that the extracts of the three investigated medicinal plants contain compounds with antioxidants. As a consequence of the high antioxidant activities and phenolic compounds, aqueous extract from marula bark could be a very effective antioxidant. The *E. coli*, *S. aureus*, and *C. albicans* were found to be resistant to chloroform and water extracts of the three investigated plants. *S. aureus*, and *C. albicans* showed considerable susceptibility to ethanolic extract of marula and khella.

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