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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 on a regular basis 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, marula and black mustard). 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 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 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 and75.44for all concentrations (125, 250, 500, 1000 µg/ml). At a concentration of 1000 µg/ml, black mustard extract scavenged 46.65% of DPPH radicals, whereas that of 500, 250 and 125µg/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 and khella showed the most potent activity against *S.aureus* and *C. albicans.* Aqueous and chloroform extracts were failed to inhibit growth for the three strains. The MIC concentrations ranged from 0.37mg/ml to 0.75 mg/ml for both bacteria and fungi.

Keywords: *Medicinal plants, phytochemical constituents. Antimicrobial, Antioxidant*

INTRODUCTION:

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

The unique geographical position of the 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 72 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 the Sudan, as in many developing countries; [4] reported that the 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 with regard to the use of medicinal plants as drug alternatives. Therefore, conducting multi-disciplinary research is a must for 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 national health care system [6].

Although aromatic and medicinal plants in Sudan are widely distributed and exploited, they are 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 (*Ammivisnaga* 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 in 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 odor. 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, indurations 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].

Sclerocaryabirrea, known as 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 in the domestication process in the African farming system [11], for its usage as 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 as anti-bacterial [12], anti-proliferative [13] and anti-diabetes medicine [14].

The surveyed information revealed that limited studies were carried out on chemical composition and bioactivities of medicinal plants growing in the Sudan. Thus, the aim of this research was 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.

METHODOLOGIES

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.

Extracts preparation

The extraction of seed oil was done using 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 laboratory blender (Waring Commercial, HGBTWTG4, USA) and then extracted with soxhlet using water, chloroform, and ethanol solvent as described by Doughari et al., [15]. The extracts were subjected to antioxidant and antimicrobial analyses.

GC-MS method for analysis of FAME

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 initial temperature was 600C and increased to 3000C at rate of 100C/min. Carrier gas was Helium at flow rate of 50ml/min and the sample was injected in split mode. Identification of compounds was based on comparison of the relative retention time and mass spectral data and NIST mass spectral library of the GC-MS.

Antioxidant Activity Analysis

The antioxidant analysis was run at the faculty of Pharmacy, University of Gezira, Sudan. About 50 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 dryness. 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 Mensor et al., [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. The percentage of radical scavenging activity (RSA %) was calculated as follows:

$$RSA\% = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} X100$$

Antimicrobial Activity Assay

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 was *Candida albicans* (ATCC 10231).

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 Mcfrl and standard turbidity solution (0.05 ml of 1.175% barium chloride dihydrate ($BaCl2•2H2O$), with 9.95 ml of 1% sulfuric acid (H_2SO_4). This produced a suspension containing 1.5X108 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 vancomysin 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 370C for 24 hours and 25 °C for 48 hours for the bacteria and fungi, respectively. The zone of inhibition (if present) was measured [18].

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 other five tubes to create a concentration sequence from 10-1 to 10-5. 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 were determined by sub-culturing the test tubes dilutions, those used in 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 solid medium was taken as the MBC and MFC [19].

RESULTS AND DISCUSSIONS

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 founds were Oleic acid (1.86%) and Palmatic acid (1.34%). The major aromatic components found in the marula extract werePhenol,2,2'-methylenebis6-(1,1-dimethylethyl)-4-methyl (29.96%),Phenol,2,4-bis1,1-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%).

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 µg/ml, black mustard extract scavenged 46.65% of DPPH radicals, whereas that of 500, 250 and 125µg/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 µg/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. Daniela Russo et al. [20] reported that the aqueous bark extract had the highest antioxidant activity compared to leaf and root extracts obtained from different solvents. Generally, 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 µg/ml	Plant extracts		
	Black Mustard	Khella	Marula
1000 µg/ml	46.65%	75.44%	98.67%
500 µg/ml	28.31%	45.12%	97.06%
250 µg/ml	20.68%	21.15%	95.76%
125 µg/ml	19.38%	21.85%	80.84%

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 inhibition zone was 14 mm for *S. aureus* and *C. albicans*. In the ethanolic extract of khella, the mean diameter of 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 were failed to inhibit growth of *E. coli, S. aureus* and *C. albicans*. The different solvent extracts of black mustard produced no observable inhibitory effect. Majority of 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 revealinhibition zone versus all strains.

In table 3, the ethanolic Khella extract has MIC of 0.37 µg/ml for *S. aureus* and for *C. albicans*. Ethanolicmarula 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, ethanolickhella extract has MBC/MFC of 0.75 mg/ml for *S. aureus*, but for *C. albicans* has MFC of 1.5 mg/ml.

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

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

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			
	EthanolicKhella extract		EthanolicMarula extract	
	MIC	MBC/MFC	MIC	MBC/MFC
S. aureus	0.37	0.75	0.75	1.5
C. albicans	0.37	1.5	0.37	1.5

MIC = minimum inhibitory concentration; MBC = minimum bactericidal concentration; MFC = minimum fungicidal concentration

CONCLUSION

The results indicate that the extracts of the three investigated medicinal plants contain compounds with antioxidant. 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.

REFERENCES

1. Seebaluck-Sandoram, R., Lall, N., Fibrich, B., Blom van Staden, A., Saleem, H., Mahomoodally, M.F., (2019). Antimicrobial antioxidant and cytotoxic evaluation of two underutilised food plants: Averrhoabilimbi L. (Oxalidaceae) and Phyllanthusacidus L. Skeels (Phyllanthaceae). Biocatalysis Agric. Biotechnol. 18, 100998.
2. Eltohami, M.S. (1997). Medicinal, Culinary and Aromatic plants in Sudan. FAO corporate document repository originated by forestry department. Proceeding of the International Expert Meeting, Egypt.
3. El-Amin, H. M. (1990). Trees and shrubs of the Sudan. Ithaca Press Exeter, England, FAO.
4. Hala Ahmed Abdalla (2009). Quality of medicinal plants traditionally used in Sudan as affected by ionizing radiation treatments. PhD Thesis, Department of Botany and Agricultural Biotechnology, Faculty of Agriculture, University of Khartoum.
5. Al-Shtayeh, M.S., Abu Ghdeib, S.I. (1999). Antifungal activity of plant extract against dermatophytes. Mycoses, 42(11-12):665-72.
6. Rukangira, E (2001). The African Herbal Industry: Constraints and Challenges. A paper presented at "The natural Products and Cosmeceutcals 2001 conference" And published in "ErboristeriaDomani", August 2001.
7. Bisset, N.G. (1994). Herbal drugs and phytopharmaceuticals. Boca Raton, FL, CRC Press.
8. Farnsworth, N.R., ed. NAPRALERT database. Chicago, IL, University of Illinois at Chicago, (2001) production (an online database available directly through the University of Illinois at Chicago.
9. Gerald, E.T., Williams, C. (1989). Oil Crops of the World. 2nd ed. Oklahoma: Royal Botanic Gardens Inc. pp 341, 355–356
10. Obi, R. k., Nwanebu, F.C., Ndubuisi,U.U., Orji ,N.M.(2009). Antibacterial qualities and phytochemical screening of the oils of Curcubitapepo and Brassica nigra. Journal of medicinal plant research.3(5)5.
11. Russo, D., Kenny, O., Thomas, J.S., Milella, L., Mohammad, B.H., Diop, M.S., Dilip, K.R., Brunton, N.P. (2013). Profiling of phytochemicals in Tissue from sclerocaryabirrea by HPLC-MS and their link with Antioxidant Activity.11:3-8.
12. Eloff J.N. (2001). Antibacterial activity of Marula (Sclerocaryabirrea(A.rich.) Hochst. subsp. Caffra(Sond.) Kokwaro) (Anacardiaceae) bark and leaves J. Ethnopharmacol. 76(3): 305-308.
13. Tanih, N.F., Ndip, R.N. (2013). The acetone extract of sclerocaryabirrea (Anacardiaceae) possesses antiproliferative and apoptotic potential against human breast cancer cell lines (MCF-7). Sci World J 2013: 1-7.
14. Ojewole, J.A. (2004). Evaluation of the analgesic, anti-inflammatory and antidiabetic properties of Sclerocaryabirrea (A. Rich.) Hochst. Stembark aqueous extract in mice and rats. Phytother Res 18: 601-608.

15. Doughari, J.H, Pukuma, M.S and De, N. (2007). Antibacterial effects of Balanitesaegyptica and Moringaoleiferaon Salmonella typhi. *African journal of Biotechnology*. 6(19)2212-2215.
16. Mensor, L.I., Menezes, F.S., Leitao, G.G., REIS, A.S., Dos Santos, t., Coube, C.S. and Leitao, S.G. (2001). Screening of Brazilian plants extracts for antioxidants activity by the use of DPPH free radical method. Phytother. Res., 15, 127-130.
17. Andrensek, S., Simvnovska, B. and Vovk, I. (2004). Antimicrobial and Antioxidative enrichment of Oak (Quercusrobur), *International Journal of Food Microbiology*: 92; 181- 187.
18. Cheesbrough, M. (2006). District laboratory practice in tropical countries Part 2. Cambridge University Press.USA, 47-57.
19. Suffredini, I.A., Paciencia, M.L., Nepomuceno, D.C., Younes, R.N., Varella, A.D. (2006).Antibacterial and Cytotoxic Activity of Brazilian Plant Extracts Clusiaceae. Mem. Inst. Oswaldo Cruz, 101, 287-290.
1. Daniela Russo, Owen Kenny, Thomas J. Smyth, et al., (2013). Profiling of Phytochemicals in Tissues from Sclerocaryabirrea by HPLC-MS and Their Link with Antioxidant Activity. ISRN Chromatography, vol. 2013, Article ID 283462, 11 pages. https://doi.org/10.1155/2013/283462