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Lethality and Antioxidant Activity of some Sudanese Medicinal Plants' Fixed Oils

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MFB, YIH and HMA designed the study and wrote the protocol. Author MFB wrote the manuscript. Author YIH designed biochemical analysis and author EF performed them. Author HMA authenticated the plant species. Authors MFA and YIH managed the analyses of the study. Author HEK supervised the study at his laboratory and revised the manuscript. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

The objective of the undergoing work is investigating antitumor therapy potential and antioxidant capacity of Sudanese medicinal plants *Helianthus annuus* L. (Asteraceae), *Ricinus communis* L. (Euphorbiaceae), *Nigella sativa* L. (Ranunculaceae), *Sesamum indicum* L. (Pedaliaceae) and *Balanites aegyptiaca* (L.) Del (Zygophyllaceae) by studying the plants seeds fixed oils for their cytotoxicity and antioxidant activity. This study was conducted at the Sudan National Research Center, Medicinal & Aromatic Plants Research Center, Khartoum, Sudan, in June-August, 2013. The five fixed oils cytotoxicity was studied using Brine Shrimp Lethality and antioxidant activity by DPPH and Iron Chelating Assays. Obtained results showed that *Ricinus communis* fixed oil has the highest toxicity with LC_{50} 1.7014, followed by *Nigella sativa* with LC_{50} 606.2, when analyzed by Finney Probit Analysis. Antioxidant activity results of the five fixed oils measured using DPPH showed that *Nigella sativa* fixed oil showed high antioxidant potential (85%), while *Ricinus communis* and *Helianthus annuus* fixed oils showed moderate antioxidant activity (52%) and (51%) respectively. All of the fixed oils showed no

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activity when using Iron Chelating Assay. Results justify the plants use in folkloric medicine although dosages should be monitored for its safe use. Studies directed towards identification of bioactive compounds are recommended.

Keywords: Medicinal plants; fixed oils; cytotoxicity; antioxidants.

1. INTRODUCTION

About 80–90% of the populations in African countries are dependent on traditional medicine for their primary health care. In Sudan, traditional medicine plays an important role in health care, since access to hospitals and other medicinal facilities is limited and a high percentage of the population are nomads. Sustainability of the use of medicinal plants is an actual and important issue. The demand for medicinal plants is increasing in Africa as the population grows and pressure on medicinal plant resources will become greater than ever. Interest in plant derived medicines has also increased in the West, among the pharmaceutical companies [1].

Medicinal plants contain various phytochemicals that are used for treatment of various diseases. Antioxidants present in the plants play an essential role in protecting the cells and tissues against damage caused by reactive oxygen species [2].

Bioactive compounds are often toxic to *Artemia salina* shrimp larvae. The eggs of the brine shrimp are readily available as fish food in pet shops. When placed in artificial seawater the eggs hatch within 48 hours, providing large numbers of larvae. This is a rapid and inexpensive general bioassay which has been developed for screening, fractionation and monitoring of physiologically active natural products [3].

Balanites aegyptiaca, known as ‘desert date’ is a spiny shrub or tree up to l0 m tall, widely distributed in dry land areas of Africa and South Asia. It is traditionally used in treatment of various ailments such as jaundice, intestinal worm infection, wounds and malaria. It contains protein, lipid, carbohydrate, alkaloid, saponin, flavonoid, and organic acid [4].

The Seeds of *Helianthus annuus* have medicinal importance as diuretic, expectorant and also used for cough, throat and lung infections. Healing properties of seed oil of *Helianthus annus* are used in folk medicine for the treatment of bronchitis, diarrhea, scorpion stings and snakebite [2].

Among natural products, the seeds and oil of *Nigella sativa* have attracted the interest of medical scientists. It is an annual herbaceous plant with black seeds. The black seeds of *Nigella Sativa* have been used as a spice and a food preservative in different countries. Emerging studies have demonstrated that black seed oil (BSO) and its different components, such as thymoquinone, manifest anti-inflammatory, anti-tumour, immune stimulatory and healing properties. In addition, oral administration of BSO can decrease the disease scores in patients with allergic rhinitis, bronchial asthma and atopic eczema [5].

Sesame, *Sesamum indicum* is one of the world’s important oil crops. Its primary marketable products are the whole seeds, seed oil and meal. Also, they have desirable physiological effects including antioxidant activity, blood pressure and serum lipid lowering potential as proven in experimental animals and humans. Sesame oil is mildly laxative, emollient and demulcent. The seeds and fresh leaves are also used as a poultice. The oil has wide

medical and pharmaceutical application. Sesamin has been found to protect the liver from oxidative damage. The oil has been used for healing wounds for thousands of years. It is naturally anti-bacterial for common skin pathogens such as Staphylococcus and Streptococcus as well as common skin fungi such as athlete's foot fungus. It is anti-viral and anti-inflammatory [6].

The castor oil plant *Ricinus communis*, also known as Palma(e) Christi or wonder tree is a perennial crop of the spurge family. *Ricinus communis* probably originates from Africa and was used in ancient Egypt and by the Romans and Greeks. Nowadays the plant grows wild in many tropical and subtropical regions and is found as an ornamental plant virtually all around the world. Historically, the plant, the seeds and in particular the oil have been used for a variety of medical purposes, for example, as a laxative or for treatment of infection and inflammation [7]. Roots are used in urinary trouble; Leaves juice with lime is used to suppress newly formed boils [8].

In the present study fixed oils of the five Sudanese medicinal plants *Helianthus annuus*, *Ricinus communis*, *Nigella sativa*, *Sesamum indicum* and *Balanites aegyptiaca* were studied for their lethality and antioxidant activity. The study aimed at verifying the traditional use of these oils as well as investigating the potential of these oils as curative agents for several diseases.

2. METHODOLOGY

2.1 Tested Plants

Helianthus annuus cypsela, *Ricinus communis* regma, *Balanites aegyptiaca* drupe, *Nigella sativa* seeds and *Sesamum indicum* seeds were bought from Omdurman crop local market, Khartoum, Sudan, in July, 2013. Seeds from fruits were taken at the laboratory. All seeds were identified, authenticated and classified by Dr. Haidar Abd Algadir in the herbarium of The Medicinal & Aromatic Plants Research Institute (MAPRI), Khartoum, Sudan. Samples were deposited at the museum at the herbarium with serial numbers as follows; *Helianthus annuus* (HELAN 713), *Ricinus communis* (RICOM 713), *Balanites aegyptiaca* (BALA 713), *Nigella sativa* (NIGSAT 713) and *Sesamum indicum* (SESIN 713).

2.2 Extraction of Fixed Oils [9]

Specific weight of each sample seeds was coarsely powdered using mortar and pestle and extracted with Petroleum ether (500 ml for each sample) using soxhlet extractor apparatus for about eight hours. Extract was then filtered through filter paper and the solvent was evaporated under reduced pressure using rotary evaporator apparatus. The yield percentage was calculated as follows: Weight of extract obtained/weight of plant sample *100, *Helianthus annuus* (44.1%), *Ricinus communis* (41.2%), *Nigella sativa* (32.5%), *Sesamum indicum* (42.3%) and *Balanites aegyptiaca* (38.4%).

2.3 Brine Shrimp Lethality Test [10]

Artemia salina (shrimp eggs) was placed in sea water and eggs hatched within 48 hrs, providing a large number of larvae (nauplii). The tested fixed oil (20 mg) was dissolved in 40 mg DMSO and made up to 2ml with seawater. From this solution 5, 50 and 500µl were transferred to vials (triplicate for each concentration), forming concentrations of 10, 100 and

1000 µ/ml respectively. Volume was made to 5 ml with seawater. 10 larvae were placed in each vial using a Pasteur pipette. Potassium Dichromate (0.54 mg/ml) was used as positive control. Vials were left for 24 hrs and numbers of survived larvae were counted. Data were analyzed by Finney Probit Analysis computer program to determine Ld50 values with 95% confidence intervals. LC_{50} values below 249 µg/ml were considered as highly toxic, 250–499 µg/ml as moderately toxic and 500–1000 µg/ml as lightly toxic. Values above 1000 µg/ml were regarded as non-toxic [3].

2.4 Antioxidant Activity

2.4.1 DPPH radical scavenging assay

The DPPH radical scavenging was determined according to the modified method of Shimada et al. [11]. In 96-wells plate, the test samples were allowed to react with 2.2 di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37ºC. The concentration of DPPH was kept as 300µM. The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517 nm using multiple reader spectrophotometers (Thermo Fisher Scientific 1500). Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group. All tests and analysis were run in triplicates.

2.4.2 Iron chelating assay

The iron chelating ability was determined according to the modified method of Dinis et al. [12]. The Fe^{+2} were monitored by measuring the formation of ferrous ion-ferrozine complex. The experiment was carried out in a 96 microtiter plate. The plant extracts were mixed with $FeSO_4$. The reaction was initiated by adding 5mM ferrozine. The mixture was shaken and left at room temperature for 10min. The absorbance was measured at 562nm. EDTA was used as standard and DMSO as control. All tests and analysis were run in triplicates.

3. RESULTS AND DISCUSSION

The results in Table 1 revealed that *Nigella sativa* (NS) seed fixed oil had a high antioxidant activity (85%) as well as a potential toxicity on Brine shrimp (LC_{50} 604.2). Previous studies on NS have identified many enriched bioactive molecules, both in fixed oil as well as essential oil. It has been demonstrated that much of the biological activity of NS is due to thymoquinone, which is the main component of the essential oil and fixed oil. The extracts of NS have anti-inflammatory and antimicrobial properties (against several germs) as well as antiox-idant properties due to its activity of free-radical elimination [13].

N. sativa and the oil obtained from it are reservoirs of different bioactive compounds, and they have been shown to reduce tissue injury by reducing oxidative stress through their antioxidant activity of eliminating radicals. Moreover, it has been observed that NS oil promotes the activity of antioxidant enzymes like GSH-PX and SOD and it reduces the lipid peroxidation of the biological membranes thanks to their antioxidant properties and elimination of ROS [13].

Our findings emphasize the useful implications of *Nigella sativa* fixed oil in the treatment of many diseases. Future research into bioactive compounds must be implemented to address the problem of dosage.

In this study the fixed oil of *Ricinus communis* showed a remarkably high lethality on Brine Shrimp (LC_{50} 1.7) as well as a moderate antioxidant activity by DPPH radical scavenging assay (51%) Table 1 our results agree to some extent with other studies which demonstrated that the oil contains high levels of the unusual fatty acid, ricinoleic acid that is valued for its unique chemical properties. Furthermore, with respect to medical applications, the ability of the A-subunit to induce cell death has been exploited for the development of immunotoxins [7]. Also the roots of *Ricinus communis* were shown to be an effective antimicrobial agent [14]. The observed pharmacological activity may be due to the presence of phytochemicals like flavonoids, alkaloids and tannins present in the plant extract with various biological activities [15].

Due to the high toxicity obtained by its fixed oil, the present study suggests the potential antimicrobial and anticarcinogenic properties of *Ricinus communis L*., indicating the possibilities of its potential use in the formulation of natural remedies for the topical treatment of infections. However, recommendations for further studies are needed to evaluate active compounds and probable medicinal benefits in chemotherapy among humans.

This study confirmed the antioxidant activity of fixed oil of *Helianthus annuus* seed as reported by previous workers [16,17]. The fixed oil showed moderate antioxidant activity (52%) by DPPH radical scavenging assay and low lethality (LC_{50} 1570) Table 1. The methanolic seed extracts of *Helianthus annuus* had shown very significant DPPH (1, 1-diphenyl-2-picryl-hydrazyl) radical scavenging activity compared to standard antioxidant Ascorbic acid [2]. The results concluded that the seed extracts of *Helianthus annuus* are a potential source of antioxidants of natural origin and have strong potential for use as natural antimicrobials in several applications requiring these properties.

Toxicity of *Sesamum indicum* seed fixed oil was light, with LC_{50} 855.9 in the current study and its antioxidant activity was moderate by DPPH radical scavenging assay (32%) Table 1 This herb oil is used widely in Sudan for many therapeutic purposes against several diseases. Our results thus support the folkloric use of *Sesamum indicum* oil.

Supporting the therapeutic uses of *Sesamum indicum* also a previous study [18] showed that Sesamin and sesamolin, two unique phytoconstituents isolated from *Sesamum indicum* seeds, possess excellent cholesterol-lowering effect in humans and prevents high blood pressure. They serve as a good source of copper, manganese and calcium which are effective in reducing pain, in osteoporosis and in reduction of swelling in rheumatoid arthritis. Antioxidant action was also observed with seeds [18]. Results have thus demonstrated considerable antioxidant activity of sesame products tested especially black sesame hulls. Moreover, Sesame as a valued oil seed appears to have numerous industrial applications. It is therefore important to fully develop Industrial processing and utilization of sesame to meet the current demands. In addition to other uses of the oil, like cooking as well as for medicinal purposes such as the treatment of ulcers and burns, the oil extract could equally be used in making soap and skin moisturizers [19].

In spite of the fact that the aqueous extract of the fruit mesocarp from *Balanites aegyptiaca* is used in Sudanese folk medicine in the treatment of jaundice [20], the present study of the plant's fixed oil revealed a weak lethality and antioxidant activity of its fixed oil Table 1

Furthermore, it has been experimentally proven that *B. aegyptiaca* posses antioxidant, antimicrobial, anticancer, diuretic, hypocholesterolemic, wound-healing, antiviral, antidiabetic, hepatoprotective, mosquito larvicidal, anti-inflammatory and analgesic, antivenin, cardioprotective, antioxidant activity and antinociceptive properties [21]. So, further studies need to be carried out to explore the bioactive compounds of *B. aegyptiaca* for its potential in curing and treating diseases.

Table 1. Lethality and antioxidant activity of five sudanese plants' fixed oils

Plant sample	LC_{50}	%RSA ±SD (DPPH)	Iron chelating assay
Balanites aegyptiaca	99099.890	17±0.03	Not active
Helianthus annuus	1570.1680	52±0.24	Not active
Ricinus communis	1.7014	51±0.12	Not active
Nigella sativa	604.209	85±0.13	Not active
Sesamum indicum	855.9596	34±0.11	Not active

4. CONCLUSION

Our Findings revealed the high antioxidant potential of *Nigella sativa* fixed oil. This may explain its role in altering the oxidative stress and its usefulness in the treatment and management of many diseases. Due to the high toxicity obtained by *Ricinus communis L*. fixed oil, the present study suggests it may have greater potential as an antimicrobial and anticarcinogenic agent. Results justify the plants' use in folkloric medicine although dosages should be monitored for safety. Studies directed towards identification of bioactive compounds are recommended.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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