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Research Article

Antimicrobial Activity of the Seed Extracts of *Carthamus tinctorius*, *Moringa oleifera* and *Jatropha curcas* on Pathogenic Bacteria

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Abstract

Background and Objective: In the last decade, the resistance in pathogenic bacteria has been increasing. The objective of this study was to evaluate the antibacterial activity of water, ethanol and the hexane seed extracts of *Carthamus tinctorius* (*C. tinctorius*), *Moringa oleifera (M .oleifera)* and *Jatropha curcas* (*J. curcas*). **Methodology:** The agar disc diffusion method was used *in vitro* to determine the zone of inhibition (ZI) against four different species of pathogenic bacteria [*Escherichia coli (E. coli)*, *Staphylococcus aureus* (*S. aureus*), *Bacillus cereus* (*B. cereus)* and *Salmonella* spp (*S. spp*)]. Three different concentrations of the extracts were subjected to these organisms. **Results:** Results of the present study showed that the ethanolic extracts (50%) of Jatropha, Carthamus and Moringa seeds observed a significantly ($p<0.05$) higher zone of inhibition (5.9 ± 0.1, 1.8 ± 0.02 and 1.03 ± 0.00 mm, respectively) against *E. coli*, compared with other extracts. However, the hexane extract (30%) of Carthamus revealed the lowest zone of inhibition on *S.* spp (0.5 ± 0.00 mm). **Conclusion:** These findings indicate that *J. curcas*, *C. tinctorius* and *M .oleifera* has the potential to inhibit and control the growth of pathogenic bacteria.

Key words: *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella* spp, seed extracts, *Carthamus tinctorius*, *Moringa oleifera*, *Jatropha curcas*

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Plants derivatives have constituted a huge contribution to human health as herbal medicine for their therapeutic properties since early times[1]. The prevalent usage of drugs has led to the development of pathogen resistance, which encourages research of new drugs for the treatment of diseases[2,3]. Some bio active compounds present in these plants are responsible for their medicinal value, food industries, cosmetics and, more recently in agriculture, for pest control[4]. Their concentrations may vary in different plants, which result in unique medicinal properties for a specific plant. Antimicrobial agents are components that kill microorganisms or suppress the growth of the microorganisms[5]. They are well recognized for their ability to cure bacterial diseases. Antimicrobial agents break up microbial processes or structures that differ from those of the host[6].

Carthamus tinctorius L. *©. tinctorius*) (safflower) belongs to the genus Carthamus, family Compositae. There are approximately 13 species in the genus Carthamus and *C. tinctorius* is the sole existing species in Sudan[7]. *C. tinctorius* is almost distributed all over the world. Thus, there is no uncertainty that *Carthamus tinctorius* is the one source for medicinal material in the pharmacopeia in many countries, especially the People's Republic of China. The biochemical compounds in safflower are reported to be flavonoids, triterpene alcohols, polysaccharides and lignans.

Moringa oleifera L. (*M. oleifera*) belongs to the genus of family Moringaceae. The family is made up of 12 species. *M. oleifera* is the most cultivated species of the genus Moringa and it is widespread over the tropical region of Africa and Asia[8]. The value of the seeds and the different parts of the plant have long been recognized in folklore medicine. The chemical constituents in Moringa are reported to be flavonoids, phenolics, ascorbic acid and carotenoids. Many studies have identified certain compounds as flavonoid derivatives (e.g., kaempferol, rhamnetin, isoquercitrin and kaempferitrin), alkaloid compounds (e.g., pterygospermin, moringin and moringinine) and 4-(L-rhamnosyloxy) benzyl isothiocyanate, which may act as antibacterial agents[9].In addition, Poumaye *et al*.[10] reported that the active agents of the *Moringa oleifera* seeds are cationic peptides that have molecular weights between 6 and 16 kDa. Recently, *M. oleifera* seeds have been widely used for the treatment of many different diseases[11].

The *Jatropha curcas* L. (*J. curcas*) plant belongs to the family Euphorbiaceae. In many subtropical and semiarid regions, traditionally, *J. curcas* is used for its medicinal properties and its seeds contain semi dry oil, which has been found to be useful for medicinal purposes[12]. The seeds contain substances, such as curcin and phorbol esters, which are responsible for the antimicrobial activity against pathogenic bacteria, such as *S. aureus*[13]. The *J. curcas* seed has played a major role in the treatment of various diseases, including bacterial and fungal infections. The extracts of many Jatropha species, including *J. curcas*, have shown to display potent antimicrobial activities in different assays[14]. The aim of this study was to evaluate the antibacterial activity of different seed extracts of *C. tinctorius*, *M. oleifera* and *J. curcas* against pathogenic bacteria.

MATERIALS AND METHODS

Sample collection: Healthy seeds of *C. tinctorius*, *M. oleifera* and *J. curcas* were collected from the college of Agricultural and Natural Resources, University of Bakht Al-Ruda (Ed Dueim, White Nile State, Sudan).

Preparation of seed extracts: Each plant seed sample was washed and air dried at ambient temperature (25°C). The sample was then ground into a powder using a mortar and pestle and kept in dark, airtight bottles for the extraction process.

All the extractions were conducted by following the methods of Okorondu *et al*.[8] with some modification. Approximately 10, 30 and 50 g of the powdered Carthamus, Moringa and Jatropha seeds were percolated into 100 mL water and kept for 24 h. The percolates were filtered with Whitman No. 1 filter paper. The same quantities of the powdered seed material were again percolated with ethanol and hexane, following the same method, in order to obtain three extracts per sample, namely, water, ethanolic and hexane extracts.

Preparation of inoculum: Four pathogenic bacterial isolates of *E. coli*, *S. aureus*, *B. cereus* and *S. spp* were obtained from the Department of Plant Biotechnology, University of Khartoum, Sudan. They were isolated and purified on nutrient agar plates, characterized and identified[8].

Zone of inhibition (ZI): Antibacterial activity analyses of the extracts were conducted using the agardisc diffusion method with somemodification[15]. Approximately 0.5 mL of the previously prepared inoculum of the dilute cultures was aseptically inoculated onto the surface of sterile Petridishes containing sterile solid nutrient agar. Discs (6 mm diameter) impregnated with the seed extracts (water, ethanol and

hexane) were aseptically placed onto the agar surface and thereafter incubated at 37°C for 24 h. The inhibition zone was observed and then recorded in millimeters using a transparent meter ruler. The tests were conducted in triplicate.

Statistical analysis: The experiments were performed in a completely randomized design. Analysis of variance (one-way ANOVA) was performed and means were compared by the least significant difference (LSD) test at a significance level of $p<0.05$, using the Data Processing System (Zhejiang University, Hangzhou, China) and Graph Pad Prism 5 (California San Diego, USA).

RESULTS AND DISCUSSION

In the present scenario of emergent multi drug resistance in human pathogenic infections, it has become highly necessary to search for novel antimicrobial substances from other sources, such as plants[16,17]. This exploration will hopefully lead to the development of a phytomedicine to act against microbes[18,19]. Figure 1a-c shows the antibacterial activity of different extracts of *C. tinctorius* seeds against pathogenic bacteria at different concentrations. The antibacterial activity test results for influences of Carthamus extracts on pathogenic bacteria are characterized by the inhibition zone around the discs. Figure 1b shows the highest zone of inhibition (ZI) of ethanolic extracts (50%) for Carthamus (1.03±0.0) was demonstrated significantly against *E. coli*. Both *E. coli* and *S. aureus* showed the same zone of inhibition (ZI) (1.0±0.0) against hexane extracts (50%) (Fig. 1c). Interestingly, the ethanol and hexane solvent extracts (50 and 30%) showed antimicrobial activity against selected pathogenic bacteria, which was not found in water except at a concentration of 50%, which inhibited *E. coli* and *S. aureus* (Fig. 1a). A higher concentration of the extract could be expected to increase the antimicrobial activity, as increasing the concentration of an extract should increase the diameter of the inhibitory zone formed due to greater abundance of active components in the extract[20]. The low antimicrobial activity demonstrated in the present study is associated with the type of solvent used.

In Fig. 2b, the ethanolic extract (50%) of Moringa was revealed as demonstrating the highest antibacterial activity against *E coli* (5.9±0.10 mm). In the same manner, the hexane extract inhibited *E. coli* at the same concentration (1.9±0.05 mm) (Fig. 2c).The lowest zone of inhibition (ZI) was found with the water extract (50%) against *S. aureus* (0.57±0.004 mm) (Fig. 2 a).These results were consistent with

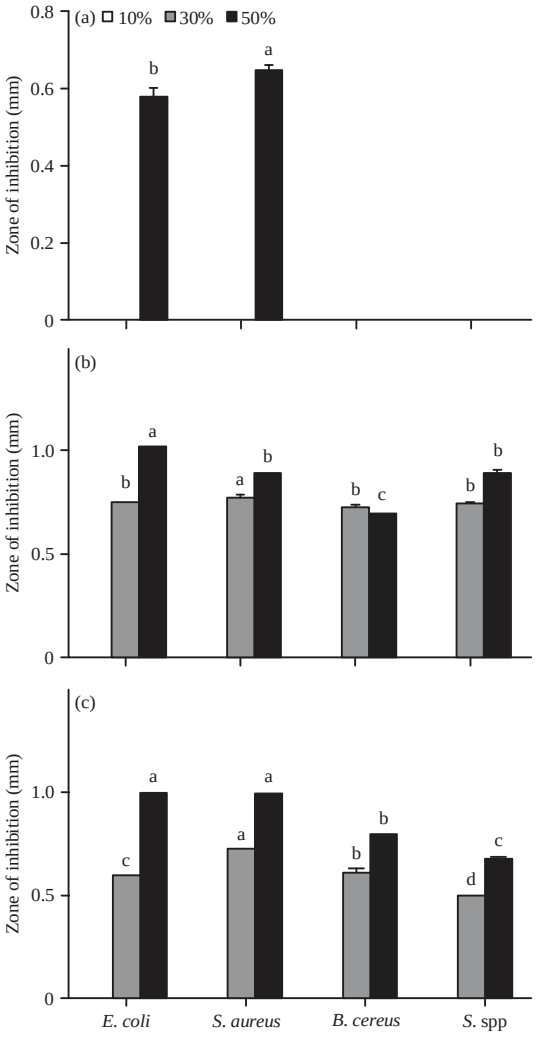

Fig. 1(a-c): Antibacterial activity of different extracts of *C. tinctorius* seeds against pathogenic bacteria at different concentrations: (a) water extract, (b) ethanol extract and © Hexane extract.

Different letters indicate significant differences between different groups ($p<0.05$) among the mean values

those reported by other researchers[8], who found that Moringa leaf extracts were effective against *S. aureus* and *E. coli*. In addition, Talreja[21] reported that the *Moringa oleifera* flower has antimicrobial activity against gram-positive and gram negative bacteria.

Figure 3 shows that the zone of inhibition for the hexane and ethanol extracts of *J. curcas* was significantly higher (1.8±0.02 and 1.3±0.02 mm) against *E. coli* at 50 and 30% concentration, respectively. Dada *et al.*[22] reported that ethanolic extract of Jatropha leaves consist of polyphenol compounds, such as flavonoids, tannin and saponins, which have antibacterial activity against *Staphylococcus aureus*

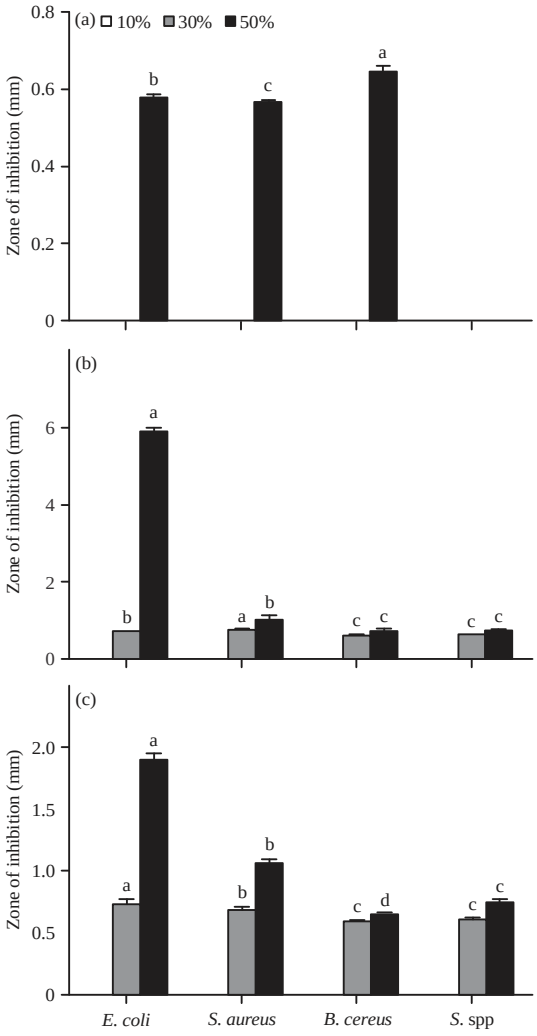

Fig. 2(a-c): Antibacterial activity of different extracts of *M. oleifera* seeds against pathogenic bacteria at different concentrations: (a) Water extract, (b) Ethanol extract and © Hexane extract.

Different letters indicate significant differences between different groups (p<0.05) among the mean values

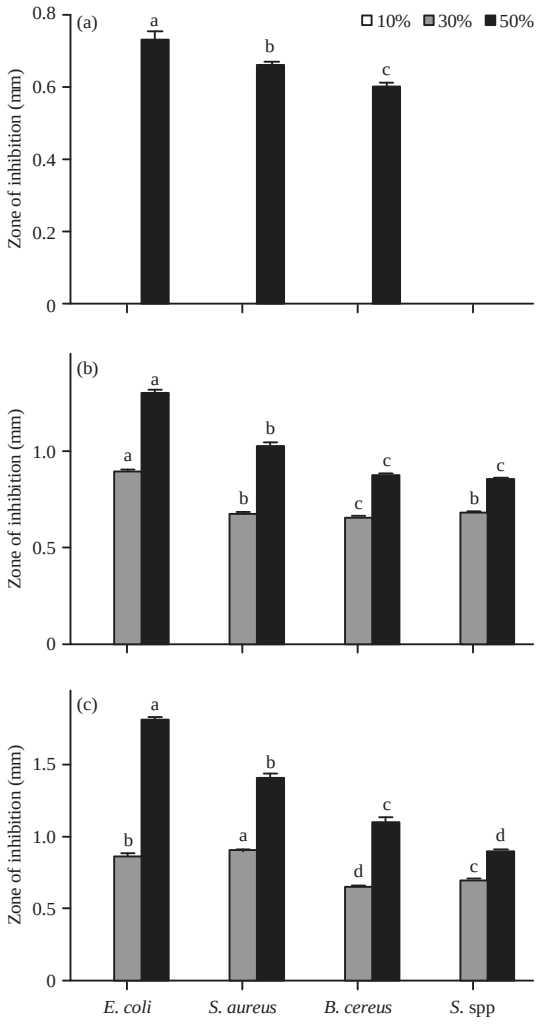

Fig. 3(a-c): Antibacterial activity of different extracts of *J. curcas* seeds against pathogenic bacteria at different concentrations: (a) Water extract, (b) Ethanol extract and © Hexane extract.

Different letters indicate significant differences between different groups (p<0.05) among the mean values

and coliform bacteria. However, the lowest zone of inhibition (0.60±0.012 mm) was shown with the water extract against *B. cereus* at a 50% concentration. Generally, the different seed extracts revealed a concentration-dependent inhibition effect on the bacteria tested.

Based on the results obtained, both ethanol and hexane extracts showed different rates of inhibitory effect on both gram-positive and gram-negative bacteria. It is hoped that these results will lead to the identification of compounds that could help to establish antibacterial drugs from natural products. Further purification of components can be carried out and the components may be subjected to animal studies.

CONCLUSION

This study has shown that different seed extracts *©. tinctorius*, *M. oleifera* and *J. curcas*) have a potential antibacterial agent against the pathogenic bacteria tested herein. The ethanolic extract (50%) of the three seed plants had the largest effects against pathogenic bacteria compared to other extracts. These three seed plant extracts could be studied further as future alternatives to control contamination in diets and diseases associated with common pathogenic bacteria.

SIGNIFICANCE STATEMENT

This study discovers the possible antibacterial effects of seed extracts from *Carthamus tinctorius*, *Moringa oleifera* and *Jatropha curcas* against pathogenic bacteria, which can be beneficial for drug industries, food industries and cosmetics. This study may also help researchers to discover novel drugs from a natural antibacterial source to control some bacterial infections or use as a food additive to control pathogenic bacteria.

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