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Evaluation of antimicrobial and antioxidant properties of leaves of *Emex spinosa* and fruits of *Citrillus colocynthis* from Saudi Arabia

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The crude methanol extract of *Citrullus colocynthis* fruit and *Emex spinosa* leaves were examined for antimicrobial and antioxidant potentialities. The phytochemical analysis revealed presence of some bioactive principles, such as alkaloids, flavonoids and anthraquinones for *E. spinosa* and saponin, flavonoids, terpenoids and alkaloids for *C. colocynthis*. The antimicrobial activities were determined against seven bacterial strains (*Proteus vulgaris* NCTC 8196, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumonia* ATCC 53651, *Salmonella typhi* NCTC 0650, *Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* NCTC 8236) and one fungal strain (*Candida albicans* ATCC 7596). *E. spinosa* leaf methanol extract was most active against fungus, while *C. colocynthis* fruit methanol extract was most active against bacteria, particularly *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853. The antioxidant properties of extracts were investigated *in vitro* using 1,1-diphenyl, 2-picryl hydrazyl (DPPH) radical scavenging assay and *in vivo* in rats using serological and enzymatic tests. Both plant extracts showed considerable antioxidant activities. The promising findings of this investigation could be used as a novel natural antimicrobial and antioxidant agents.

Key words: *Emex spinosa*, *Citrillus colocynthis*, antimicrobial, antioxidant activity.

INTRODUCTION

The medicinal properties of plants were an issue of human interest since times immemorial. Since modern medicine has increasingly benefitted from exploring compounds present in traditional medicine, researches should focus on medicinal plants which are rich sources of natural remedies (Abdallah and El-Ghazali, 2013). Saudi Arabia which is located in the Arabian Peninsula has an arid hot desert climate and rainfall is mostly scarce. Based on these harsh conditions, the studies on the pharmaceutical properties of the flora of Saudi Arabia have been neglected for a long time (El-Ghazali et al., 2010). However, arid climate may activate the production of secondary phytochemical compounds in high concentrations which are presumably more capable of fighting its natural enemies such as insects, herbivores and diseases. *Citrullus colocynthis* (L.) Schrader from family Cucurbitaceae

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Abbreviations: DPPH, 1,1-Diphenyl, 2-picryl hydrazyl; **ALT,** alanine aminotransferase; **AST,** aspartate aminotransferase; ALP, alkaline phosphate; LDH lactate dehydrogenase; **γ-GT,** γ-glutamyl transpeptidase.

and *Emex spinosa* (L.) Campd. from family Polygonaceae are among the plants of folk medicinal applications growing in the deserts of Arabian Peninsula. The fruits of *C. colocynthis* have many folk medicinal applications, its juice with sugar are prescribed for dropsy (Anonymous, 1970), against tumors of gastrointestinal tract, joint pains, anti-leukemia and as anti-cancerous drug (Rodge and Biradar, 2012). The leaves of *E. spinosa* are edible by the local inhabitants and Bedouin in eastern Saudi Arabia as well as its carrot-like tap root (Mandaville, 1990). It is used to relief dyspepsia, appetite stimulant, diuretic and also used for gastrointestinal disorders (Watt and Breyer-Brandwjk, 1962). The present investigation describes the antimicrobial and antioxidant properties of two medicinal plants, *C. colocynthis* and *E. spinosa* growing in the arid desert regions in Saudi Arabia.

MATERIALS AND METHODS

Plant collection

E. spinosa (leaves) and *C. colocynthis* (fruits) were collected manually from Qassim district, Saudi Arabia. The botanical identification was confirmed by Gamal E. El-Ghazali (Taxonomist). The fresh plant samples were washed and dried in shade for up to 15 days for leaves and 30 days for fruits. Then, they were crushed into fine powders using crushing machine and kept in dark well tight bottles for further investigations.

Plant extraction

Extraction was performed as described by Samie et al. (2005) with minor modifications. 50 g of each ground plant material was soaked in 500 ml of methanol for up to 72 h with frequent shaking. Then, samples were filtered twice using Whattman No.1 filter paper and evaporated to dry (semi-solid residues) under reduced pressure at 40°C. The semi-solid residues were left in Incubator at 40°C until totally dried (about two days). Dry extracts were reconstituted with methanol 70% (50 and 100 mg/ml) and kept in refrigerator in a dark well tight bottles.

Tested microorganisms

Seven reference bacterial strains representing the gram negatives (*Proteus vulgaris* NCTC 8196, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumonia* ATCC 53651 and *Salmonella typhi* NCTC 0650) and gram positives (*Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* NCTC 8236,), and one reference fungal strain (*Candida albicans* ATCC 7596) were used in this study.

Phytochemical screening

Some phytochemical compounds claimed to have many bioactive properties were investigated; those compounds were tannins, saponins, flavonoids, terpenoids, phenolic compounds, alkaloids and anthraquinones (Edeoga et al., 2005; Krishnaiah et al., 2009; Abdallah et al., 2009).

Determination of antimicrobial activity

The antimicrobial activity of the methanol extract of leaves of *E. spinosa* and fruits of *C. colocynthis* were investigated using agar-well diffusion method as mentioned by Güven et al. (2006), with minor modifications. 15 ml of molten Mueller-Hinton for bacterial isolates or potato dextrose agar (PDA) for fungal isolates (Oxoid Ltd, UK) was poured in a sterile Petri-dish and left until it solidied. Fresh cell suspensions were prepared and adjusted to 0.5 McFarland's standard. Then, 100 µl was spread onto the surface of the plates of Mueller-Hinton agar or potatoes dextrose agar. 6 mm wells were punched into the agar with a sterile cork borer. The methanol extracts of leaves of *E.spinosa* and fruits of *C. colocynthis* were dissolved in 70% methanol to a final concentration of 50 and 100 mg/ml. 80 µl (8000 µg/well) from each concentration was loaded into the wells and incubated for 24 h at 37°C for bacterial strains and 72 h at 28°C for fungal strain. Reference antibiotics, chloramphenicol (5 mg/ml, 50 µg/wells) and clotrimazole (5 mg/ml, 100 µg/wells) were used as antibacterial and antifungal agents, respectively. Tests were repeated two times and the mean inhibition zone was recorded.

1,1-Diphenyl, 2-picryl hydrazyl (DPPH) radical scavenging activity

Radical scavenging activity of tested extracts was evaluated using DPPH as a reagent as reported by Kirby and Schmidt (1997) with minor modifications. 1 ml of a 4 % (w/v) solution of DPPH radical in methanol was mixed with 500 µl of sample solutions in ethanol at different concentrations. Then, the mixture was incubated for 20 min in the dark at room temperature. The scavenging capacity was determined spectrophotometrically by monitoring the decrease in absorbance at 517 nm against a blank using a spectrophotometer (Bio-Rad Smart SpecTM plus). Lower absorbance of the reaction mixture indicated a higher free radical scavenging activity. Ascorbic acid was used as positive control. The percent DPPH scavenging effect was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = (A_{control} - A_{sample} / A_{control}) \times 100$$

Where, $A_{control}$ is the absorbance of the control reaction containing all reagents except the tested compound and A_{sample} is the absorbance of the tested compound.

The extract concentrations providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against the extract concentration. Tests were repeated three times.

In vivo antioxidant properties

This test was performed as described by Parthasarathy et al. (2006) with some modifications. Male albino *Wistar* rats (weight of 200-220 g) were used in this study. The animals were purchased from the Central Pharmacy of Tunisia (SIPHAT, Tunisia). They were housed at standard conditions and fed with a commercial balanced diet (SICO, Sfax, Tunisia). The drinking water was offered *ad libitum*. Our Institutional animal Care Committee approved the protocols for the animal study; the animals were cared for in accordance with the institutional ethical guidelines.

After two weeks of acclimatization, the rats were allocated randomly to four experimental groups of eight animals each with free access to food and water. Based on the preliminary experiments, the hepato protective dose of the methanolic extract of leaves of *E. spinosa* and fruits of *C. colocynthis* were decided. In multiple dose pretreatment experiment, methanolic extract was administered at 250 mg/kg bw by intraperitoneal injection. Group I (control rats) received the vehicle (olive oil, 1 mL/kg orally) at day 8; Group II received CCl_4 in olive oil (1 mL/kg, i.p) at day 8; Group III received the methanol extract of tested plant (250 mg/kg BW) daily by i.p injection for 8 days followed by a single dose of CCl4 in olive oil at a dose of 1 ml/kg using an intragastric tube twenty-four hours after

Table 1. The phytochemical analysis of leaves of *Emex spinosa* and fruits of *Citrillus colocynthis*.

Plant	Tannin	Saponin	Flavonoid	Terpenoid	Phenolic compound	Alkaloid	Anthraquinone
Emex spinosa	–	–	+	–	–	+	+
Citrillus colocynthis	±	+	+	+	–	+	–
Negative control (D.W.)	–	–	–	–	–	–	–

+, Presence; – , absence; ±, weak positive reaction; D.W., distilled water.

Table 2. Antimicrobial activities of leaves of *Emex spinosa* and fruits of *Citrillus colocynthis*.

Test *	Concentration (mg/ml)	Mean zone of inhibition (mm) of microorganisms (Mean ± SEM)**							
		Pr	Ec	Bc	Sal	Kp	Ps	Sa	Cand
Emex spinosa	50	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	12.0 ± 1.0
	100	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	14.5 ± 0.5
Citrillus colocynthis	50	8.5 ± 0.5	19.5 ± 1.5	12.5 ± 0.5	13.0 ± 1.0	8.25 ± 0.25	17.0 ± 0.0	10.75 ± 0.75	6.0 ± 0.0
	100	9.0 ± 1.0	22.5 ± 0.5	16.5 ± 1.5	17.0 ± 0.0	10.0 ± 1.0	22.0 ± 2.0	15.0 ± 0.0	9.0 ± 0.5
Chloramphenicol	5	26.0 ± 1.0	13.7 ± 1.05	21.0 ± 1.0	13.0 ± 1.0	22.0 ± 0.2	24.0 ± 1.0	16.5 ± 1.5	–
Clotrimazole	10	–	–	–	–	–	–	–	13.0 ± 1.0

*Plants tested are as methanol extracts at 100 mg/ml; chloramphenicol as antibacterial at 5 mg/ml and clotrimazole as antifungal at 10 g/ml. **Mean ± standard error of means (SEM), mm=millimeter; Pr, *Proteus vulgaris* NCTC 8196; Ec, *Escherichia coli* ATCC 25922; Bc, *Bacillus cereus* NCTC 8236; Sal, *Salmonella typhi* NCTC 0650; Kp,*Klebsiella pneumonia* ATCC 53651; Ps, *Pseudomonas aeruginosa* ATCC 27853; Sa, *Staphylococcus aureus* ATCC 25923; C and, *Candida albicans* ATCC 7596.

the last dosing; Group IV received methanol extract (250 mg/kg BW) daily by i.p injection for 8 days.

The animals were killed on day 9 by cervical decapitation. Blood samples were collected, allowed to clot at room temperature and serum separated by centrifuging at 4000 r.p.m. for 15 min for various biochemical parameters. The liver and the kidney were quickly excised, minced with ice cold saline, blotted on filter paper and homogenized (Ultra Turrax T25, Germany) (1:2, w/v) in 50 mmol/l phosphate buffer (pH 7.4). The supernatant and serum were frozen at -30°C in aliquots until analysis.

Blood samples were collected, allowed to clot at room temperature and serum separated by centrifuging at 4000 rpm for 15 min for various biochemical parameters. The supernatant and serum were frozen at -30°C in aliquots until analysis. The levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphate (ALP), lactate dehydrogenase (LDH), γ-Glutamyl transpeptidase (γ-GT), urea and creatinine were measured using Chemistry Automatic analyzer 911, with the appropriate reagents purchased from Hitachi LTD., Japan.

RESULTS AND DISCUSSION

The phytochemical testing on the studied plant extracts revealed presence of many compounds of biological activities as presented in Table 1. Leaves of *E. spinosa* showed presence of alkaloids, flavonoids and anthraquinones. Similar study confirms the current results (Rizk, 1986). Fruits of *C. colocynthis* revealed presence of saponin, flavonoids, terpenoids and alkaloids. This is in partial agreement with the results of Rodge and Biradar (2012) who found that *C. colocynthis* growing in India consists of tannins, saponins, flavanoids, terpenoids, alkaloids, steroids and cardic glycoloids. The current study did not detect tannins. Variation of season of harvesting or different geographical localities may have effect on the production of some phytochemical compounds. Such phytochemical compounds are interesting, as they are claimed to have many biological activities on animals and human including antioxidant, anti-inflammatory and anti-cholinesterase effects (Loizzo et al., 2007), in addition to antimicrobial properties (Cowan, 1999).

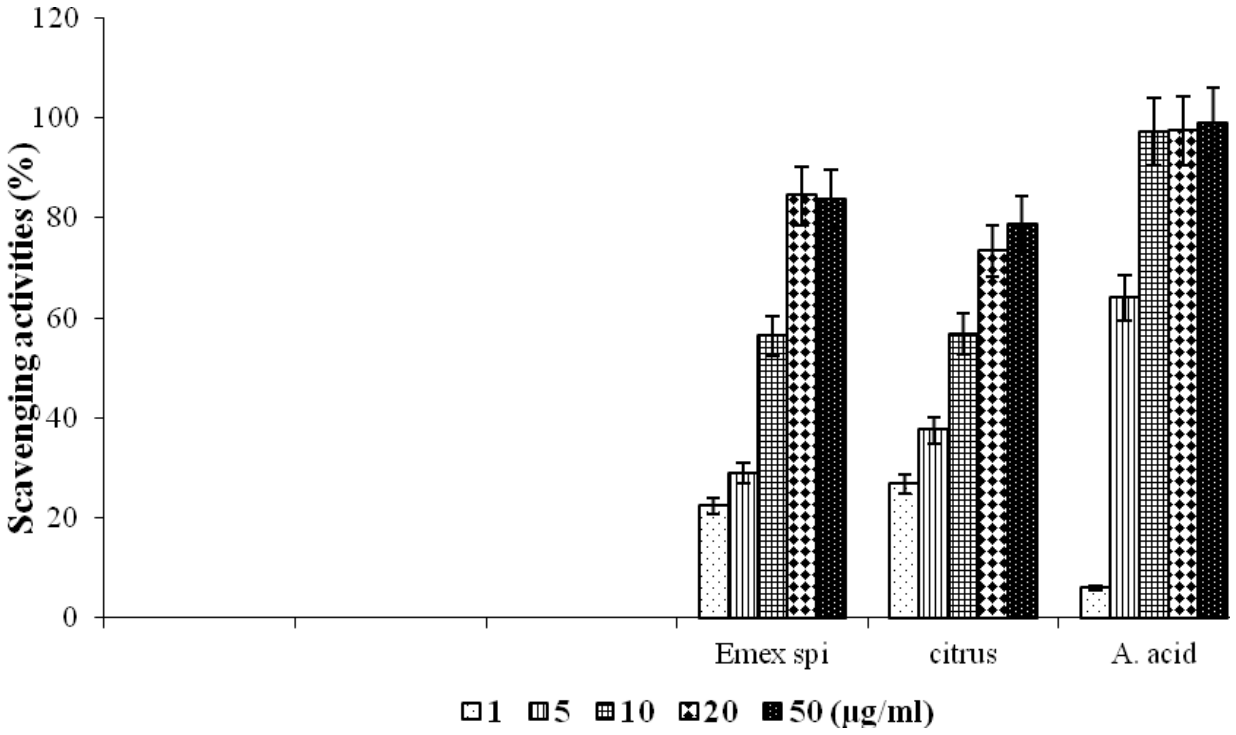


Figure 1. The DPPH free radical-scavenging activity of leaves of *Emex spinosa* and fruits of *Citrillus colocynthis* at different concentrations. A. acid, Ascorbic acid; Emex spi, *Emex spinosa,* Citrus, *Citrillus colocynthis.* Each value represents the mean ± SD of three experiments.

Table 2 shows the antimicrobial testing results of methanol extracts. It is clear that leaves of *E. spinosa* did not show any antibacterial activity against tested bacteria, while it revealed a considerable antifungal activity against *C. albicans* ATCC 7596 particularly at concentration 100 mg/ml, which was 14.5 ± 0.5 mm, higher than clotrimazole (10 mg/ml) (13.0 ± 1.0 mm). This interesting result requires more investigation on the chemical constituents of leaves of *E. spinosa* in order to categorize the active antifungal compound(s). However, different result was published by Abd El-kader et al. (2006) who reported that ethyl acetate extract of areal parts of *E. spinosa* from Egypt showed weak antifungal and variable antibacterial activity. Fruits of *C. colocynthis* showed weak antifungal activity and significant antibacterial activity at 100 mg/ml, the highest susceptible bacterium was *E. coli* ATCC 25922 (22.5 ± 0.5 mm), followed by *P. aeruginosa* ATCC 27853 (22.0 ± 2.0 mm), *S. typhi* NCTC 0650 (17.0 ± 0.0 mm), *B. cereus* NCTC 8236 (16.5 ± 1.5 mm), *S. aureus* ATCC 25923 (15.0 ± 0.0 mm), *K. pneumonia* ATCC 53651 (10.0 ± 1.0 mm) and the least susceptible bacterium was *P. vulgaris* NCTC 8196 (9.0 ± 1.0) compared to the antibiotic chloramphenicol 5 mg/ml (Table 2). Similar study was conducted by Rodge and Biradar (2012) who mentioned that the methanol, ethanol and water fruit extracts of *C. colocynthis* showed signi-ficant antibacterial and antifungal activities.

Figure 1 presents the *in vitro* antioxidant activities of leaves of *E. spinosa* and fruits of *C. colocynthis*. As shown in the figure, the tested plant extracts and the standard for the *in vitro* antioxidant activity using DPPH method revealed significant antioxidant activity. The DPPH radical is used for assessment of the free radical-scavenging activity in plant extracts (Brand-Williams et al., 1995). These antioxidants generally include flavonoids, tannins, phenolic compounds, coumarins, anthocyans and essential oils (Ennajar et al., 2009; Bettaieb et al., 2010).

Table 3 shows the effects of CCl_4, the methanol extracts of examined plants and their combination (EACs /CCl_4) on hepatic markers in serum of control and experimental rats. The positive effects of these extracts on serum parameters are clearly seen. Table 4 reveals the effects of CCl_4, the methanolic extract of tested plants and their combination on the activities of enzymatic antioxidants in liver of control and experimental rats, which possesses significant protective effect against hepatotoxicity induced by CCl_4. Moreover, the phytochemical analysis of the studied plants revealed presence of some compounds known to have hepatoprotective activities such as alkaloids, flavonoids and phenolic compounds (Ranawat et al., 2010).

Conclusion

The medicinal plants mentioned in folk medicine are rich

Table 3. Effects of CCl_4, the methanolic extract *of* studied plants and their combination (Emex/CCl4 and Citrillus/CCl4) on hepatic markers in serum of control and experimental rats

Enzyme (U/L)	Experimental group			
	C	CCl4	Emex/CCl4	Citrillus/CCl4
AST	61.7 ± 2.5	125 ± 2.5	55 ± 2.4	65.15 ± 3.4
ALT	16.5 ± 1.25	34.5 ± 4.5	20.1 ± 4.4	18.7 ± 2.4
ALP	32.2 ± 0.75	44.5 ± 2.3	33.3 ± 1.5	31.2 ± 1.9
LDH	450 ± 45	520 ± 45	525 ± 46	523 ± 47
γGT	1.25 ± 0.3	2.23 ± 0.15	1.5 ± 0.25	1.84 ± 0.20

*AST, Aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; LDH, lacatate dehydrogenase; γGT, gamma glutamyl transferase. Values are mean ± SEM for eight rats in each group; CCl4, rat group treated with carbon tetrachloride; Emex/CCl4, rat group treated with a combination of Emex spinosamethanol extract and carbon tetrachloride,Citrillus/CCl4, rat group treated with a combination of Citrillus methanol extract and carbon tetrachloride.

Table 4. Effects of CCl_4, the methanol extracts and their combination on the activities of enzymatic antioxidants in liver of control and experimental rats.

Treatment	SOD (Units/mg protein)	CAT (µmol H_2O_2/mg protein)	GPx (µmol GSH/min/mg protein)
C	9.1 ± 1.15	267.5 ± 8.4	5.2 ± 0.25
CCl_4	6.65 ± 0.25	96 ± 1.33	3.3 ± 0. 45
Emex/CCl_4	7.96 ± 1.25	243.25 ± 14.5	4.4 ± 0.3
Citrus/CCl_4	8.82 ± 1.4	244 ± 3.15	4.3 ± 0.828

Emex, *Emex spinosa;* Citrus, *Citrillus colocynthis*. C, control group; CCL4, rat group treated with carbon tetrachloride; Emex/CCl4, rat group treated with a combination of Emex spinosamethanol extract and carbon tetrachloride,Citrus/CCl4, rat group treated with a combination of Citrillus colocynthis methanol extract and carbon tetrachloride. Values are mean ± SEM for eight rats in each group.

sources for new therapeutics. Leaves of *E. spinosa* revealed significant antifungal activities and fruits of *C. colocynthis* showed significant antibacterial activities. Both plants are rich sources for antioxidant compounds. These promising plants require more interest and deep research in order to explore their medicinal properties.

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