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# Phytochemical screening, antimicrobial, antioxidant and cytotoxicity activities of bark's crude extracts of *Cordia sinensis*

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**ABSTRACT**

*Cordia sinensis* which is known locally as Andrab is a medicinal plant used traditionally in Sudan and in many other countries as an antioxidant, anti-glycation, anti-malarial and anti-inflammatory. This study was aimed to prepare various crude extracts of *C. sinensis* bark using different polarities of solvent; phytochemical screening, antimicrobial, antioxidant, and cytotoxicity of the bark crude extracts. Bark of *C. sinensis* was collected from Omdurman area, dried under shade, powdered and then extracted successively with petroleum ether, chloroform, ethyl acetate, and methanol by using shaker apparatus (at room temperature). The phytochemical examinations were carried out for all extracts. The preliminary phytochemical analysis of the bark crude extracts revealed the presence of various amount of flavonoids, tannins, cardiac glycosides, sterols/triterpense, alkaloids and showed no saponins and coumarins. Crude extracts at concentrations 25 and 50 mg/ml were applied against four standard bacteria (Gram positive: *Bacillus subtillis* and *Staphylococcus aureus*, Gram negative: Klebsiella and *Pseudomonas aeruginosa*) and two standard fungi (*Candida albicans*, *Aspergillus niger*) using the disc diffusion method assay. The methanolic extract at concentration 50 mg/ml was found to be more active against two types of bacteria (Klebsiella and *P. aeruginosa*) and showed an intermediate activity against the other two types of bacteria (*B. subtillis* and *S. aureus*) and also against the two types of fungi (*C. albicans* and *A. niger*). No activity was observed with the three solvents (chloroform, ethyl acetate and petroleum ether) (25 and 50 mg/ml) extracts and the methanolic (25 mg/ml) extract against all the microorganisms tested, except ethyl acetate extract at concentration 50 mg/ml showed intermediate inhibition zone (15 mm) against Klebsiella. All extracts were found to be potent against brine shrimps with moderate $LD_{50}$. The free radical scavenging activity of the crude extracts was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. The antioxidant activity of methanolic extract was the strongest (60%), followed in descending order by ethyl acetate (52%), chloroform (48%), and petroleum ether (32%) extracts. The results showed that higher concentrations are more active than lower concentrations in case of antimicrobial and cytotoxicity activities and also showed that methanolic extract was found to be having potent effect more than other extracts. The study recommends further study for identification of chemical compounds in each extract and specifies the active components that make antimicrobial, cytotoxicity and antioxidant activities of this plant.

**Keywords:** *Cordia sinensis,* bark extracts, phytochemical screening, cytotoxicity.

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## INTRODUCTION

The use of plants as medicines has a long history in the treatment of various diseases. The earliest known records for the use of plants as drugs are from Mesopotamia in 2600 B.C., and these still are a significant part of traditional medicine and herbal remedies (Koehn and Carter, 2005). According to the World Health Organization (WHO), a medicinal plants is defined as any plant, which one or more of its content contain substance that can be used for treatment of numerous diseases or as precursors for synthesis of

useful drugs (WHO, 1978). The use of traditional medicine is wide spread throughout the world. The term traditional medicine is interchangeably used with herbal medicine and natural medicine (Hazan and Atta, 2005). The evolution of these plant-based medicine systems, primarily based on plants within a local area, produced the well-known traditional medicine systems in several local systems within Africa and other worlds. Specifically, the medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human or animal body (Edeoga et al., 2005). The most important of these bioactive constituents which are mainly secondary metabolites like flavonoids, triterpenes, tannins and alkaloids possessing wide range of bioactivities were isolated from different plant parts of Cordia species (Thirupathi et al., 2007). The roots and bark of *Cordia sinensis* are used for stomach disorders in both children and adults. A decoction of boiled roots is used to treat malaria but can cause an abortion. Bark and roots are mixed to treat conjunctivitis in cattle (Orwa et al., 2009). Many plants possess antimicrobial activities are used for treatment of different diseases (Najafi et al., 2010). Since antiquity, man has used plants to treat common infectious diseases and even long before mankind discovered the existence of microbes; the idea that certain plants had healing potential was well accepted (R`ios and Recio, 2005). *C. sinensis* grows in the Middle East, Pakistan, India, Sri Lanka and in Africa from West Africa to Ethiopia, Somalia, Sudan, Egypt, south to Namibia and north-east South Africa. The species is found in dry riverine vegetation, usually with Salvadora persica, or in open bush land, usually from sea level to 1,400 m in alluvial, sandy, red loam and rocky soils (Maundu et al., 1999).

## MATERIALS AND METHODS

### Plant material

The bark of *C. sinesis* was collected from Khartoum state and authenticated by Yahya Suleiman at herbarium of Medicinal and Aromatic Plants Research Institute National Centre for Research. The plant material was shade-dried and coarsely powdered separately in hammer mill.

### Preparation of crude extracts

The powdered bark (350 g) was treated successively with different solvents, firstly defatted with petroleum ether for 24 h by cold method using shaker apparatus at room temperature, the residual of the powdered bark was dried and extracted again with chloroform for 24 h and then with ethyl acetate and finally with methanol using the same methods for all. Each extract was filtered and concentrated under reduced pressure to the dryness using rotary evaporator.

### Preliminary phytochemical screening of *Cordia sinensis* bark

The bark extracts were screened for the presence of saponins, tannins flavonoids, steroids, triterpenes, cumarins, quinones organic acids and alkaloids according to the methods described by Sofowora (1993).

### Antioxidant activity

The antioxidant activity of bark extracts of *C. sinensis* was measured *in vitro* using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay. The DPPH free radical scavenging was determined according to the modified method of Shimada et al. (1992). In 96-wells plate, the test samples were allowed to react with DPPH free radical for half an hour at 37°C. The concentration of DPPH was kept at 300 µM. The test samples were dissolved in dimethyl sulfoxide (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 as a control group. All tests and analysis were run in triplicates.

### Antimicrobial activities

#### *Preparation of nutrient agar*

28 g of the powdered nutrient agar, was weighed, dispersed in 1 L of distilled water and allowed to soak for 10 min, swirl to mix then sterilized by autoclaving for 15 min at 121°C, cooled to 47°C, mix well then poured in Petri dishes.

#### *Preparation of sabouraud dextrose agar*

62 g of the powdered sabouraud dextrose agar was weighed, dispersed in 1 L of distilled water and allowed to soak for 10 min, swirl to mix then sterilized by autoclaving for 15 min at 121°C, cooled to 47°C, mix well then poured in Petri dishes.

#### *Preparation of standard microorganisms suspensions*

The bacterial cultures were maintained on nutrient broth. 10 ml of nutrient broth was sterilized in autoclave; full loop of standard bacteria was inoculated in nutrient broth at 37°C in incubator for 18 to 24 h. After 24 h, when growth evidenced, the individual colonies were again sub-culture to nutrient agar slopes and inoculated at 37°C in incubator for 24 h. The overnight cultures were harvested and washed off with 10 ml normal saline. Then serial of sterile normal saline was added to 1 ml bacteria suspension that was taken with digital pipette and shaken gently to produce a suspension containing about $10^8$ to $10^9$ colony-forming unit per ml. The suspension was stored in the refrigerator at 4°C till used.

The fungal cultures were maintained on sabouraud dextrose agar broth and incubator for 7 days. The fungal growth was observed, the broth was sub-cultured for 24 h. When growth evidenced, the individual colonies were again sub-cultured to sabouraud dextrose agar slopes, inoculated at 25°C in incubator until the growth occurred, suspended into 100 ml of sterile normal saline and then the same procedure as for bacterial culture was followed.

### Antibacterial activity

Antimicrobial activity was carried out using disc-diffusion method as described by Bauer et al. (1966). 0.5 McFarland standards of standard organisms were prepared by the method of Koneman et al. (1992). 5 ml of peptone water was placed into a sterile test tube.

An incubation of each isolate was prepared from subculture of bacterial suspension. 4 to 5 colonies of the isolates were emulsified in sterile normal saline and the turbidity adjusted to $10^5$ to $10^8$ CFU/ml (corresponding to 0.5 McFarland standards).

Sterile discs (Oxoid) were soaked separately with 50 µl of each of the organic extract prepared in petroleum ether, chloroform, ethyl acetate and methanol solvents, at a concentration of 100 mg/ml and then dried. Petri plates were prepared with 20 ml of sterile nutrient agar for standard bacteria after the test cultures (100 µl of suspension containing $10^8$ CFU/ml bacteria) were swabbed on the top of the solidified media and allowed to dry for 10 min. These discs were placed on nutrient agar plates, previously swabbed with the target bacterial isolate at a concentration of $10^5$ to $10^8$ CFU/ml.

200 mg crude extract dissolved in 2ml of suitable solvent per disc. The sterile 6 mm disc impregnated with different concentrations of extracts. The loaded discs were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. Negative control was prepared using respective solvent. The plates were incubated for 24 h at 37°C. Zone of inhibition was recorded in millimetres and the experiment was repeated twice.

**Antifungal activity**

The same method as for bacteria was adopted. Instead of nutrient agar, Sabouraud dextrose agar was used.

**Cytotoxicity screening**

***Brine shrimp bioassay***

Brine shrimp bioassay experiment was performed according to the procedure described by Meyer et al. (1982).

***Artemia salina***

*Artemia* cysts, batch number DE RP 33801, was purchased from JBL GmbH and Co.KG (Neuhofen, Germany) and the product was labelled as JBL Artemio Pur Brand. The *Artemia* cysts had been harvested from Great Salt Lake, Utah, USA.

**Preparation and hatching of brine shrimp (*A. salina*)**

Brine shrimp eggs (*A. salina*) were hatched in shallow glass vessel covered with a foil to divide it into two unequal holes compartment (dark large part and light small part) and full with artificial sea water prepared from sodium chloride salt (NaCl 7% = 35 g was dissolved in 500 ml of distilled water).

The smaller compartment was illuminated by tungsten filament light and gently spared with air. After 24 h, hatched were transferred to fresh artificial seawater and incubated for further 24 h in a warm room 25 to 29°C.

**Sample preparation of *Cordia* sinensis extracts**

Samples for the experiment were prepared by dissolving 20 mg of the different extracts in each 5 ml of Dimethyl Sulfoxide (DMSO). An appropriate amounts of DMSO solution (5, 50 and 500 µl to give concentrations of 10, 100 and 1000 ppm respectively) (Figure 1), were transferred into 10 ml vials (3 vials for each dose and 1 for control). The Control was prepared from DMSO only. The experiment was done in triplicate.

**Figure 1.** *Cordia sinensis* tree shape.

**Bioassay of *A. salina***

For the toxicity tests, ten nauplii were selected and transferred into each sample vial by means of a 23 cm disposable Pasteur pipette from the lighted side, having been separated by the divider from the shells and the final volume in each vial was adjusted to 5 ml using artificial seawater. The vials were maintained under illumination. The surviving nauplii were counted with the aid of a 3x magnifying glass, after 24 h, the percentage of deaths at the three dose levels and control were determined. In case of the control no deaths were observed to occur in the control after 48 h (Nguta et al., 2012).

Abbott's formula (Pelka et al., 2000) was used to calculate the percentage of deaths:

% deaths = [(Test-control)/control × 100].

**$LC_{50}$ determinations**

The lethal concentration fifty ($LC_{50}$), 95% confidence interval and slope were determined from the 24 h counts using the probit analysis method . $LC_{50}$ is indicative of toxicity level of a given plant extract to the brine shrimp larva.

Control discs were prepared using only methanol. Five replicates were prepared for each dose level. To begin the bioassay, brine shrimp eggs (obtained from Directorate of Fisheries, Bahrain) were hatched in a shallow rectangular dish (22 to 32 cm) under the same conditions described in the literature except artificial sea water instead of neutral. According to Meyer et al. (1982), crude plant

**Table 1.** Crude extracts form, colour and yield.

| Solvent (extractant) | Form | Colour | Yield (g) | Yield (%) |
|---|---|---|---|---|
| Petroleum ether | Fatty | Yellow | 0.64 | 8.7 |
| Chloroform | Soft | Dark green | 1.7 | 23 |
| Ethyl acetate | powder | Green | 0.54 | 7.3 |
| Methanol | Sticky | Brown | 4.52 | 61 |

**Table 2.** Phytochemical constituents of four solvents extracts of bark of *Cordia sinensis.*

| Phytochemicals | Reagents | Petroleum ether | $CHCl_3$ | Ethyl acetate | Methanol |
|---|---|---|---|---|---|
| Alkaloids | Dragendorffs test | ++ | + | + | - |
| | Mayer's test | + | + | - | - |
| | Wagner's test | + | + | - | - |
| Coumarins | KOH/U.V. | - | - | - | - |
| Flavonoids | NaOH test | - | + | + | + |
| | Mg test | + | + | + | ++ |
| | $ALCL_3$ test | - | - | + | ++ |
| | $NH_3$ test | + | + | ++ | +++ |
| Anthraquinone | | - | - | - | + |
| Saponins | Foam test | - | - | - | - |
| Sterols/ Triterpene | Salkowski test | +++ | ++ | + | + |
| | Liebermann test | ++ | + | + | + |
| Tannins | Ferric chloride test | - | - | - | +++ |
| | Salts gelatin test | - | - | - | ++ |
| Cardiac glycosids | Baljet's reagent | + | + | ++ | +++ |

+++: high amount, ++: moderate amount, +: low amount, - not detected.

extract is toxic (active) if it has an $LC_{50}$ value less than 1000 µg/ml while non-toxic (inactive) if it is greater than 1000 µg/ml.

## RESULTS

### Crude extracts colour and yield

The crude extracts colour and yield from the different solvents were shown in Table 1.

### Phytochemical screening

Phytochemical screening of crude extracts revealed the presence of alkaloids, flavonoids, sterol/triterpene, tannins and anthraquinone as shown in Table 2.

### Antimicrobial activity

The effects of two different concentrations of four plant extracts on the growth of tested microorganisms by disc diffusion method assay are presented in Tables 3 and 4.

### DPPH scavenging activity

The percentages of DPPH radical scavenging activity of plant extracts are presented in Table 5.

### Cytotoxicity bioassay

The cytotoxicity of extracts on brine shrimps is shown in Table 6. Data were processed using probit analysis to

**Table 3.** Antibacterial activities of the plant extracts (inhibition zone diameter in mm).

| Solvent | Concentrations of the extract mg/ml | Bacillus | Pseudomonas | Staphylococcus | Klebsiella |
|---|---|---|---|---|---|
| Methanol | 25 | 10 | 12 | 10 | 14 |
| | 50 | 17 | 24 | 18 | 23 |
| Ethyl acetate | 25 | 0.7 | 10 | 0.6 | 0.8 |
| | 50 | 13 | 14 | 11 | 15 |
| Chloroform | 25 | 0.6 | 0.7 | 10 | 11 |
| | 50 | 10 | 12 | 12 | 13 |
| Petroleum ether | 25 | - | - | - | - |
| | 50 | - | - | - | - |

Sensitive >18, Intermediate 15 – 18, Resistance < 15.

**Table 4.** Antifungal activities of the plant extracts (inhibition zone diameter in mm).

| Solvent (extractant) | Concentrations of extract (mg/ml) | *Candida albicans* | *Aspergillius niger* |
|---|---|---|---|
| Methanol | 25 | 12 | 11 |
| | 50 | 19 | 18 |
| Ethyl acetate | 25 | 10 | o.9 |
| | 50 | 13 | 11 |
| Chloroform | 25 | 0.7 | 0.6 |
| | 50 | 11 | 10 |
| Petroleum ether | 25 | - | - |
| | 50 | - | - |

Sensitive >18, Intermediate 15 – 18, Resistance < 15.

**Table 5.** Effect of plant extracts on DPPH method.

| Sample number | Solvent (extractant) | % of activity |
|---|---|---|
| 1 | Methanol | 60 ± 0.05 |
| 2 | Ethyl acetate | 52 ± 0.04 |
| 3 | Chloroform | 48 ± 0.03 |
| 4 | Petroleum ether | 32 ± 0.08 |
| 5 | Standard | 87 ± 0.04 |

50% ≤ active compound.

estimate $LC_{50}$ values at 95% confidence interval for statistically significant comparisons of activities.

## DISCUSSION

### Extracts form, colour and yield

Four solvents were used to extract secondary metabolites from bark of the plant under investigation. Methanol and petroleum ether solvents gave higher extractability than chloroform and ethyl acetate (Table 1). There are variations in forms, colours and yields as shown in Table 1. This indicated different components in each extracts.

### Phytochemical screening

Phytochemical screening of plant extracts provides information about the chemical constituents which can leads to a discovery of novel drugs. The qualitative tests

**Table 6.** Effects of bark crude extracts on brine shrimps.

| Solvent (extractant) | Con. ppm | Dead | Survivors | $LD_{50}$ | Cytotoxicity |
|---|---|---|---|---|---|
| | 10 | 0 | 10 | | |
| Methanol | 100 | 3 | 7 | 132.969 | Moderate |
| | 1000 | 10 | 0 | | |
| | 10 | 0 | 10 | | |
| Ethyl acetate | 100 | 2 | 8 | 147.932 | Moderate |
| | 1000 | 10 | 0 | | |
| | 10 | 0 | 10 | | |
| chloroforms | 100 | 2 | 8 | 167.119 | Moderate |
| | 1000 | 10 | 0 | | |
| | 10 | 0 | 10 | | |
| Pet. ether | 100 | 1 | 9 | 262.138 | Moderate |
| | 1000 | 10 | 0 | | |

$LD_{50}$ from 0 - 100: strong, 100 - 500: moderate, 500 - 1000: weak, < 20: very actives.

for all the four extracts showed the presence of metabolites. Sterols/triterpene were found in high amount in petroleum ether extract, in an intermediate amount in chloroform and in low amount in ethyl acetate and methanol extracts; alkaloids were present in low amount in petroleum ether and chloroform extracts; tannins were present in high amount in methanol extract and anthraquinones is present in low amount in methanol extract. The flavonoids and cardiac glycoside were present in high amount in methanol extract and in an intermediate amount in ethyl acetate extract and in low amounts in petroleum ether and chloroform extracts. Most of these results are in agreement with the finding of Saadabi and Moglad (2011) and Mohamed et al. (2010). These variations in the results may be due to the differences in the experiment conditions, the methodology, or the area of collection of the plant sample. The presence of these metabolites probably explains the various uses of the bark of this plant in traditional medicine.

## Antioxidant activity

Natural antioxidants present in herbs and spices are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. The antioxidant activities of bark extracts and the standard were assessed on the basis of the free radical scavenging effect of the stable DPPH free radical activity. The results are expressed as percentage activity. It is evident from Table 5 that the methanolic extract has the highest and is followed in a descending order by ethyl acetate, chloroform, and petroleum ether as free radical scavenging and these results is in agreement with the phytochemical screening in Table 2. The antioxidant capacity of plant extract may be due to the hydrogen donating ability of poly-phenols and flavonoids present in it. This finding revealed the high antioxidant potential of *C. sinensis* bark. This may explain its role in altering the oxidative stress, its usefulness in the treatment and management of many diseases.

## Antimicrobial activity

The strong antibacterial effects of the methanolic extract (50 mg/ml) against the two types of bacteria *Pseudomonas aeruginosa* (24 mm), *Klebsiella pneumonia* (23 mm), and the moderate with gram positive *Staphylococcus aureus* (17 mm), and *Bacillus subtilis* (18 mm) as shown in Table 3 suggest that it may contain remarkable therapeutic action in the treatment of diarrhoea and gastrointestinal infection. The methanolic extract (50 mg/ml) has been shown to possess high antimicrobial against the two types of fungal *A. niger* (18 mm) and *C. albicans* (19 mm). This result points out that methanolic extract could be useful in controlling the development of tested fungal. Petroleum ether extract in both concentrations (25 and 50 mg/ml) did not show any activity towards the tested microorganisms. The tested microorganisms showed resistance towards ethyl acetate and chloroform extracts. These results agreed with Tapsell et al. (2006) and this confirm the use of bark extract in traditional medicine.

## Cytotoxicity bioassay

The evaluation of the toxic action of plant extracts is

indispensable in order to consider a treatment safe; it enables the definition of the intrinsic toxicity of the plant and the effects of acute overdose (Padmaja et al., 2002). In bioactivity evaluation of plant extracts by brine shrimp bioassay, an $LC_{50}$ value lower than 1000 µg/ml is considered cytotoxic (Meyer et al., 1982). The cytotoxic activity was considered weak when the $LC_{50}$ between 500 and 1000 µg/ml, moderate when the $LC_{50}$ between 100 and 500 µg/ml, strong when the $LC_{50}$ ranged from 0 to 100 µg/ml and designated as nontoxic when the $LC_{50}$ value greater than 1000 µg/ml. In the current study all extracts demonstrated $LC_{50}$ values between 100 and 500 µg/ml, this mean that they have a moderate activity. The degree of brine shrimp lethality was found to be directly proportional to the concentration of the extracts. In the evaluation for general toxicity of the extracts using *brine shrimp*, maximum mortalities took place at a concentration of 1000 µg/ml where as least mortalities were at 10 µg/ml concentration.

## CONCLUSION

Antimicrobial resistance is reported to be on the increase due to gene mutations of the disease causing pathogens. It is believed that new antibiotics with activities and structures different from those in current use could be found through ethnobotanical route. *C. sinensis* was chosen for this study because of their reputation in folklore medicine as antimicrobial, antioxidant agents and usage of different parts in many diseases. Methanolic extract of park of *C. sinensis* was found to be having potent effect against tested microorganisms. This effect is increased by increasing the quantity of the extract, therefore chromatographic separation is necessary to isolate and characterize the active compounds. From the results obtained in this study, it is concluded that methanolic extract contains large amounts of phenolic compounds which shows high antioxidant and free radical scavenging activity. These finding indicate that this plant extracts might be a source of natural antioxidant that can help in preventing oxidative stress progress. The results of the study demonstrated the moderate cytotoxic activity of the bark extracts. The presence of flavonoids and alkaloids may be responsible for the observed brine shrimp lethality activities of the extracts. Due to the moderate toxicity obtained by extracts of *C. sinensis* bark, the present study suggests it may have 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.

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