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In-vitro Antibacterial Activities of Different Extracts from *Conocarpus lancifolius* Engl. against Some Clinical Pathogens

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The antibacterial activities of methanolic crude extracts from wood, bark, leaves, branches and fruits of *Conocarpus lancifolius* Engl. were bio-assayed against four clinical pathogens namely; *Micrococcus luteus*, *Staphylococcus aureus*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. The inhibition zones were ranged between 8-18 mm at a concentration of 2000 µg/mL. The study showed that the extracts from the branches had a good antibacterial against the studies pathogens.

Key words: *Conocarpus lancifolius*; antibacterial activity; clinical pathogens.

Genus *Conocarpus* contains two species; *Conocarpus lancifolius* Engl., and *C. erectus* belongs to family Combretaceae native to coastal and riverine areas of Somalia, Djibouti, and Yemen and found throughout East Africa, the Arabian Peninsula, and South Asia (Redha *et el.*, 2011). Al-Kandari *et al.*, (2009) reported that the tree is flourishes under the semi-arid conditions. Most of the research studies focused on its responses to environmental stress like drought and salinity stress (Redha *et al.*, 2012; Al-Kandari *et al.*, 2009). Previously, the extracts of *C. lancifolius* were shown antiprotozoal activity (Al-Musayeib *et al.*, 2012), *C. erectus* showed high free radical scavenging activity (Abdel-Hameed *et al.*, 2012). Recently, the alkaloidal extract of *C. lancifolius* was active at all concentrations tested (5-200 µg/mL) against *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Agrobacterium tumefaciens* except *Erwinia amylovora* (Ali *et al.*, 2013). The presence of alkaloids in the extracts has been shown to possess an antimicrobial and antioxidant activities (Erdemoglu *et al.*, 2007; Salem *et al.*, 2013). Karou *et al.*, (2006) reported that there are varied in the susceptibility of bacteria to plant extracts according to strains and species. Omega-3 fatty acid, linolenic acid was the predominant fatty acid present at 61.32% in the 10% PEG-treated plants *C. lancifolius* (Redha *et al.*, 2012).

For the first time, in this study, the extract from different parts (wood, bark, leaves, branches and fruits) of *Conocarpus lancifolius* Engl., was assayed for their antibacterial against some clinical pathogens.

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MATERIALS AND METHODS

Plant material

Samples of different parts from *C. lancifolius* were collected from the garden of the Faculty of Agriculture, university of Alexandria during the pruning process as a residue. The different parts of the tree as shown in Figure 1 (wood, bark, branches, leaves and fruits) were air-dried at room temperature and pulverized into powder (40-60 mesh) with small laboratory mill. The plant was kindly identified at the Department of Forestry and Wood Technology, Faculty of Agriculture, Alexandria University.

Preparation of the extracts

The fresh leaves were washed, air-dried under shade at room temperature and then milled into powder using a laboratory small mill. The air-dried powder (100 g) was soaked with 200 mL of methanol (80%). After one week of soaking the solution was passed through activated charcoal to remove the chlorophyll and filtrated. After filtration the residue was processed similarly with the same amount of solvent (Salem *et al.,* 2012). The crude methanol extract was concentrated to dryness using a rotary evaporator under reduced pressure at 45°C. The extract was lyophilized and stored at 4°C in the refrigerator until further use. The methanolic crude extract obtained was then weighed and prepared for stock solution at a concentration of 2000 µg/mL by diluting the crude extract in 99.5% Dimethylsulfoxide (DMSO, Sigma-Aldrich) and distilled water (1:1 v/v).

Antibacterial activity

The antibacterial activity was carried out on the methanoilc extracts of wood, bark, leaves and branches of *C. lancifolius*. The extracts were prepared at a concentration of 2000 µg/mL and studied against the growth of human pathogenic bacteria; *Pseudomonas aeruginosa, Staphylococcus aureus, Acinetobacter baumannii* and *Micrococcus luteus*. Nutrient agar (NA) medium was used for maintenance of the tested bacterial organisms. Mueller Hinton agar (MHA) was used in all bioassays applying the disc diffusion method.

Disc diffusion susceptibility

The Kirby-Bauer disc diffusion susceptibility test (NCCLS, 1997) method was applied for the antibacterial activity assay. The tested bacteria (1 mL of 10^5 CFU/mL) were spread over the surface of solid media plates. Discs (Filter paper) with 5 mm in diameter were suspended with 20 µL of the extracts and placed on the inoculated plates and the plates were incubated at 37°C for 24 h. The diameters of the inhibition zones were recorded in millimeters.

RESULTS AND DISCUSSION

The quantity of methanolic extracts from the wood, bark, leaves, branches and fruits were 5.40, 7.10, 7.52, 5.12 and 9.43 g/ 100 dry matters of *C. lancifolius*. The Antibacterial activity of the extracts of *C. lancifolius* was measured by the diameters of the inhibition zones of the growth of some human pathogenic bacteria (Table 1).

The bark extracts did not show any activity against the studied bacterial strains (*P. aeruginosa*, *S. aureus*, *A. baumannii* and *M.*

Table 1. Antibacterial activity of extracts from different parts of C. *lancifolius* against the growth of some pathogenic bacteria

Tree part	Inhibition zones (mm)			
	M. luteus	*S. aureus*	*A. baumannii*	*P. aeruginosa*
bark	na	na	na	na
leaves	10	na	12	14
branches	18	7	12	10
fruits	11	8	na	na
wood	na	10	na	na
Tetracycline *	22	20	21	25
DMSO	na	na	na	na

Inhibition Zone (mm) including disc diameter of 5 mm at 2000 µg/mL; na: Not active; n.t. Not tested. * Tetracycline (20 µg/disc).

luteus) at a concentration of 2000 µg/mL.

Leaves extracts showed good antibacterial activity against *P. aeruginosa* (inhibition zone value of 14 mm), *A. baumannii* (inhibition zone value of 12 mm), and *M. luteus* (inhibition zone value of 10 mm), and did not show activity against *S. aureus.*

In this study the extracts (2000 µg/mL) of branches were shown good antibacterial activity against all the studied bacterial strains; *P. aeruginosa* (inhibition zone value of 10 mm), *S. aureus* (inhibition zone value of 7 mm), *A. baumannii* (inhibition zone value of 10 mm) and *M. luteus* (inhibition zone value of 18 mm).

The fruits methanolic extract showed activity against *M. luteus* (inhibition zone value of 11 mm) and *S. aureus* (inhibition zone value of 8 mm). On the other hand, the fruits extract did not

Branches

Fruits

Leaves

Bark

Wood

Fig. 1. Different parts from *Conocarpus lancifolius* Engl

show any activity against the growth of *P. aeruginosa* and A. *baumannii* at the concentration of 2000 µg/mL.

At the concentration of 2000 µg/mL, wood extract not shown effect against the studied bacterial strains except against the growth of *S. aureus* (inhibition zone value of 10 mm). It could be expected that the extracts of wood have a selectively activity against this pathogen.

Recently, Ali *et al.,* (2013) reported that the biological activity of extracts from *C. lancifolius* is very limited. The precipitated alkaloids hade varied inhibition zones within the levels of the trunk against the same bacterium (Ali *et al.,* 2013). Also, the alkaloids have been shown to possess an antimicrobial and antioxidant activities (Erdemoglu *et al.,* 2007; Salem *et al.,* 2013).

The precipitated alkaloids results from the leaves of *C. lancifolius* had shown different degrees of inhibition zones except against *E. amylovora* (Ali *et al.,* 2013) which observed a resistance to all the concentration. On the basis of inhibition zones, there are many variations in the susceptibility of bacteria to extracts according to strains and species (Karou *et al.,* 2006).

In the present the search for new drugs from nature for controlling the infections diseases caused by clinical pathogen could be achieved. Previously, *S. aureus* were reported to cause of hospital-acquired infections. Primary cause of lower respiratory tract infections and surgical site infections (Richards et al., 1999a,b). Secondly cause of nosocomial bacteremia (Wisplinghoff *et al.,* 2004; Diab *et al.,* 2012), pneumonia, and cardiovascular infections (Richards *et al.,* 1999a,b). Infections with *S. aureus* are especially difficult to treat because of evolved resistance to antimicrobial drugs (Klein *et al.,* 2007). *S. aureus* is resistance to penicillin and newer narrow-spectrum β-lactamase–resistant penicillin antimicrobial drugs (e.g., methicillin, oxacillin) appeared soon after they were introduced into clinical practice in the 1940s and 1960s, respectively (Lowy, 2003; Klein *et al.,* 2007).

P. aeruginosa is one of the leading pathogens among patients suffering from cystic fibrosis, diffused panbronchitis and chronic obstructive pulmonary disease (Lieberman, 2003; Registry, 2005; Adonizio *et al.,* 2008). The success of this organism is attributed to ability to form biofilms (Smith and Iglewski, 2003; Tang *et al.,* 1996) and innate antibiotic resistance (Fisher *et al.,* 2005).

M. luteus is not a severe pathogenic bacterium, however for many years it has served as the model system for bacterial cell wall study (Deng *et al.,* 2010). The TUA polymer of *M. luteus* is acidic and has some similarity with that of the so-called capsular polysaccharides (CPS) which are usually considered to be essential and virulent factors for pathogenic bacteria, such as *Streptococca* and *Staphylococca* (DeKimpe *et al.,* 1995; Monodane *et al.,* Wessels *et al.,* 1991).

Acinetobacter can cause suppurative infection in any organ or tissue, colonize almost any human body, and in the lungs, has been associated with multilobar infection, cavitation, and pleural effusion (Urban *et al.,* 2003). Host contributions to pathogenicity include a history of alcoholism, smoking, and chronic lung disease (Talbot *et al.,* 2006).

CONCLUSIONS

The different parts of *C. lancifolius* can be promising sources of potential antibacterial against the studied clinical pathogens. The branches showed good activity. The present results could be promise a basis for further work related to the bioactivity against the pathogens.

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