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IN VITRO ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF DATURA INNOXIA EXTRACTS

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Abstract

The aim of this study was evaluated the Antimicrobial Activity of extraction of *Datura innoxia* (Seeds, leaves and roots). *Datura innoxia* Seeds, leaves and roots were collected to examine their antimicrobial activity. Extracts of different parts of the plant were tested against standard microorganisms by using the agar- well diffusion method. Extracts of methanol, and aqueous of seeds, leaves and roots were prepared and tested against four types of bacteria namely: *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Proteus vulgaris* and two types of fungi namely: *Aspergillus niger* and *Candida albicans*. The methanolic and aqueous extracts of leaves showed high activities against fungi (*A. niger)* and less effect on the all bacteria. The methanolic extracts of seeds showed high activities against all organisms except fungi (*C. albicanas*), while the aqueous extracts of seeds showed no activity on the bacteria. All organisms were examined against known standard antibiotics and then compare the results of plant extracts with standard antibiotics. The results indicated that the antibacterial drug is less active than the plant extracts, while the antifungal drugs are more active than the plant extracts.

Key words: *Datura innoxia, Antimicrobial activity, Antibiotic, Aspergillus niger, Candida albicans, Staphylococcus aureus*

Introduction

Medicinal plants have became the focus of intense study in terms of validation of their traditional uses through the determination of their actual pharmacological effects. Synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects. Therefore, there is need to search new infection fighting strategies to control microbial infections (Bhaskarwar, 2008).

It was further added that the use of plant extracts and phytochemicals with antimicrobial properties may be of importance in therapeutic treatments, whereas in the past few years, a number of studies have been conducted in different countries to prove such efficiencies (Salihu and Garba, 2008). *Datura innoxia* (Family: Solanaceae, locally known as Elsakran), widely speeded in AL Jazeera State and other States in Sudan. It is native to Central and South America, and introduce in Africa, Asia, Australia and Europe (Hans-Georg, 2002). All *Datura* plants contain tropane alkaloids such as scopolamine and atropine. The inventory of defensive chemistry also includes hyoscyamine, hyoscine, norscopalomine and meteloidine. The leaves contain 0.52% scopolamine, the calices 1.08%, stems 0.3%, roots 0.39%, fruits 0.77%, capsules 0.33%, seeds 0.44% and whole plant 0.52 - 0.62% (Hans-Georg 2002). All parts of the plant are anodyne, antispasmodic, hallucinogenic, hypnotic and narcotic. It has been used in the past as a pain killer and also in the treatment of insanity, fevers with catarrh, diarrhea and skin diseases (Emboden, 2008).Traditional medicine uses flowers and seed of *Datura innoxia* were used to treat skin eruptions, colds, and nervous disorders. It was mixed with cannabis in wine to use as a narcotic for surgical procedures. Several compounds, important for drug manufactory, are present in *Datura* plants. Daturine, hyoscyamine, atropine, scopolamineand essence materials used as antispasmodic, narcotic, neuro-sedative and anti-asthmatic drugs were found in *Datura* (Chiej, 1984). *Staphylococcus aureus* is characterized as facultative anaerobic, Gram-positive, has large round golden-yellow colonies (Ryan and Ray, 2004). Normal flora of humans found on nasal passages, skin and mucous membranes pathogen of humans It causes a wide range of supportive infections such as food poisoning and toxic shock syndrome (Todar, 2011). *Escherichia coli* is a Gram-negative, found in the normal flora of the intestinal tract of human and animals, also found in soil, water and vegetation. It causes Urinary tract infection; diarrheal disease; neonatal meningitits and wound infection (Cheesbrough, 2008). *Proteus vulgaris* is rod-shaped Gram-negative chemoheterotroph bacterium. It inhabits the soil, polluted water, raw

meat, gastrointestinal tracts of animals, and dust. In humans, Proteus species most frequently cause urinary tract infections, but can also produce severe abscesses (Hara *et al*, 2000).

Pseudomonas aeruginosa is a Gram-negative, commonly found in soil and water; it causes urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia, bone and joint infections, gastrointestinal infections and a variety of systemic infections, particularly in patients with severe burns and in cancer and AIDS patients who are immune-suppressed (Todar, 2011). *Candida albicans* is commensally and is among the gut flora, the many organisms that live in the human mouth and gastrointestinal tract, under normal circumstances, *C. albicans* lives in 80% of the human population with no harmful effects, although overgrowth results in candidiasis. Candidiasis also may occur in the blood and genital tract (Berman and Sudbery, 2002). *Aspergillus niger is* a fungus and one of the most common species of the genus *Aspergillus*. It causes a disease called black mold on certain fruits and vegetables such as grapes, onions, and peanuts, and is a common contaminant of food (Samson *et al*, 2001). Aspergillosis is the group of diseases caused by *Aspergillus*. The most common subtype among paranasal sinus infections associated with Aspergillosis is *Aspergillus* fumigates (Bozkurt *et al.,* 2008).

The objective of this study is to evaluation anti microbial activity of certain types of Gram positive & Gram negative bacteria (S. *aureus, E. coli, P. aerugions, P. vulgares*) and fungi (*C. albicans* and *A. niger*) by using extraction of seeds, leaves and roots of *Datura innoxia* with different concentrations.

Materials and methods

Datura innoxia was collected from AL Jazeera State-Sudan in February, 2013. The plant tissues (Seeds, leaves and roots) were cleaned, shade-dried and ground by a mechanical grinder. The microorganism used in the present study was kindly provided by Scientists at Khartoum National Health Laboratory as shown in Table (1). While Standard antibiotics such as Gentamicin and Nystatin were collected from Roussel Laboratories Ltd, England and Sigma chemical Company, U.S.A, respectively.

Table 1: Show Standard microorganisms used in this study

Organism	Bacterial				Fungal	
	Staphylococcus aureus	*Escherichia coli*	*Pseudomonas aeruginosa*	*Proteus vulgaris*	*Aspergillus niger*	*Candida albicans*
Code number	*ATCC/25923*	*ATCC/27853*	*27853/ATCC*	*NCTC/4175*	*ATCC/7596*	*ATCC9763*

* National Collection of Type Culture (NCTC), Colindale, England

* American Type Culture Collection (ATCC) Rockville, Maryland, USA.

Preparation of crude extracts

It was carried out according to the Soxhlet extraction technique (Kaushik *et al.*, 2007). A quantity of the fine powder for each separated leaves, seeds and roots (100g) was weighed and suspended into 500ml capacity conical flasks. Extraction was done by using petroleum ether (60 –80^{o}C) and methanol. These extracts were concentrated for further studies at reduced temperature and pressure in a rotary evaporator. Then the percentage yield of extraction was measured by using the following formula (Harborne, 1984).

Percentage yield extraction = (weight of extraction /weight of plant) X100.

In a conical flask, the plant residues were further extracted with distilled water over night at room temperature (25-30ºC), filtered and freeze dried (Mudaser, 2004).

Preparation of extract concentrations

The extracts were dissolved in dimethyl sulphoroxide (DMSO), while the aqueous extracts were dissolved in water at varying concentrations (Motsei *et al,* 2003). Stock solution of the methanol and petroleum ether extract were prepared by weighing two mg of extract and dissolved in 4 ml of dimethyl sulphoroxide (DMSO), and kept in plastic container to make 50% concentration; and 1ml from the Stock solution mixed with 1ml of dimethyl sulphoroxide (DMSO), kept in plastic container to make 25% concentration.

Preparation of media

Mueller Hinton agar for bacteria, Potato dextrose agar for fungi and peptone water for both were prepared according to the method described by Cheesbrough (2008).

Preparation of the inoculums

Peptone water (PW) were used to prepare broth cultures of bacteria and fungi, 3-5 well isolated colony of organisms selected from plate culture touched with a loop and transferred to tub contain 4-5 ml from PW, then incubated at 37°C for the bacteria, at 28°C for fungi for 24 hours.

Antimicrobial activity tests

Antimicrobial activity of the crude extracts was determined by Agar-well diffusion assay methods.

Agar-well diffusion method

Under sterile condition, swabs were dipped into the broth culture of the organism. Gently squeezed against inside of the tube to remove excess fluid; Mueller-Hinton agar plates and potato dextrose agar plates were streaked in three direction to insure complete spread of the organisms, then the plates were left (5min) to stand. The three equidistant wells of 10 mm in diameter made on the agar by using a sterile cutter. Then wells were labeled with the code numbers of the test crude extracts and controls. 0.1ml of dimethyl sulphoroxide (DMSO) in one well as control negative and 0.1ml of standard antibiotics (Gentamicine for bacteria and Nystatine for fungi) were added in two wells as control positive. 0.1ml of each extracts were added in three well. The treated plates were stored for at least 1 hour to allow diffusion of the extracts into the agar while arresting the growth of the test microbes. Then plates were incubated for 24 hours at 37°C for bacteria and Yeast, and 48 hours at 28°C for molds. Antimicrobial activity was determined by measuring the diameters of zones of inhibition by using ruler with three replicates, averages and the mean values were tabulated, then all cultures were discarded by autoclaving. Then evaluation of antimicrobial activity of Gentamicin and Nystatin was estimated by agar well diffusion method (Kinsbury and Wagner, 1990). 1 ml of 24 hr of incubated broth culture was distributed in to Mueller – hinton agar. The Gentamicin at concentration of 10 mg /ml was used as reference drug and distributed in the centre of Mueller –hinton agar and the plate was kept for 1 hr at room temperature. Then plates were incubated in the upright position at 37^{o}C for 18 hr. After incubation, the diameters of inhibition zones were recorded.

Statistical analysis

The significance of differences between means compared at each time point using Duncan's multiple range tests after SPSS for one-way classified data.

Results

Inhibition zone of 50 and 25% methanol extraction for seed of *D innoxia* for 4 examined bacteria. Figure (1) indicated that inhibition zones at 50% methanol extracted for seed of *D innoxia* are 25 mm against *S. aureus,* 21 mm against *E. coli,* 12 mm against *Ps aeruginosa and* 18 mm against *P. vulgars.* While that inhibition zone of all at 25% methanol extracted for seed of *D innoxia* are 13 mm against *S. aureus,* 13 mm against *E. coli* 15 mm against *Ps aeruginosa and* 11 mm against *P. vulgars* in which inhibition zone of concentration10 mg\ml antibiotic (control) are shown 22 mm against *S. aureus,* 23 mm against *E. coli,* 24 mm against *Ps aeruginosa* and 20 mm against *P. vulgars.* These results comparing with control are indicated that 50% methanol for *Ps aeruginosa* was inactive while for other examined bacteria were active. The findings are agreed with those results reported by Barry *et al*., (1970), who stated that microbial activity less than (15mm) are inactive

Inhibition zone of 50 and 25% aqueous extraction for seed of *D innoxia* **for 4 examined bacteria**

Figure (2) indicated that inhibition zones at 50% aqueous extracted for seed of *D innoxia* are 0.0 mm against all examined bacteria. While inhibition zone at 25% aqueous extracted for seed of *D innoxia* are 0.0 mm against *S. aureus,*0.0 mm against *E. coli* 14 mm against *Ps aeruginosa and* 0.0 mm against *P. vulgars.* These results were indicated that concentration 50 and 25% aqueous for all examined bacteria was shown inactive microbial for seed extracted.

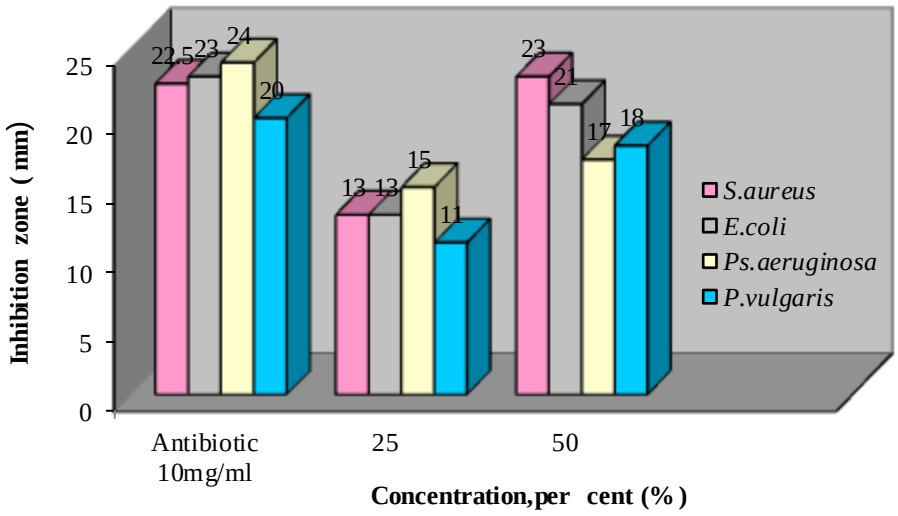

Fig .1.Antibacterial activity of *D. innoxia* seeds methanolic

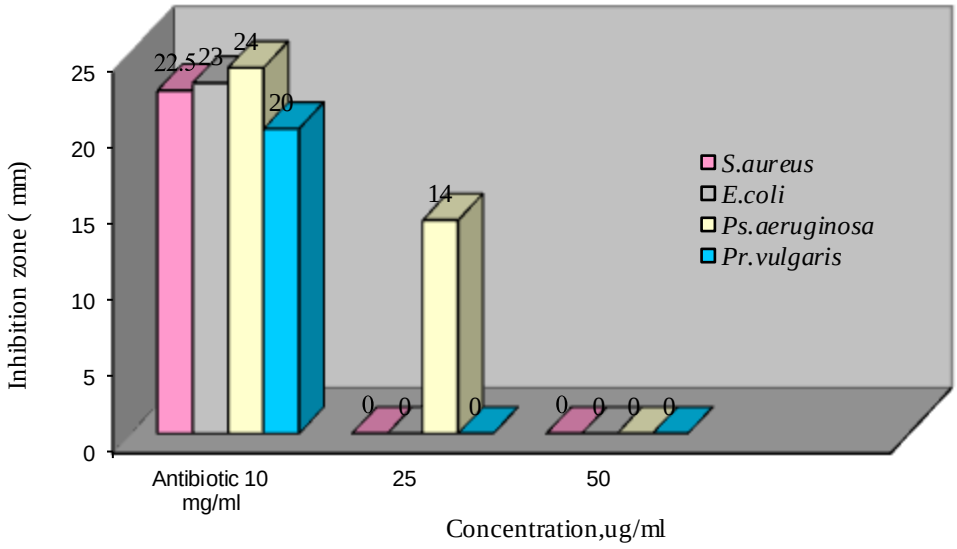

Fig .2.Antibacterial activity of *D.innoxia* seeds aqueous extracts against four microorganisms

Methanol extraction of *D. innoxia* for leaf in 50 and 25% concentration for bacteria

Figure (3) Indicated that inhibition zones at 50% methanol extracted for leaf of *D innoxia* are 24 mm against *S. aureus*, 21 mm against *E. coli*, 15 mm against *Ps aeruginosa and* 11 mm against *P. vulgars*. While inhibition zone at 25% methanol extracted for leaf of *D innoxia* are 11 mm against *S. aureus*, 16 mm against *E. coli* 14 mm against *Ps aeruginosa and* 16 mm against *P. vulgars*. These results indicated that 50% methanol for *P. vulgars* was inactive while for bacteria were active. The findings are agreed with those results reported by Barry *et al.*, (1970), who stated that microbial activity less than (15mm) are inactive.

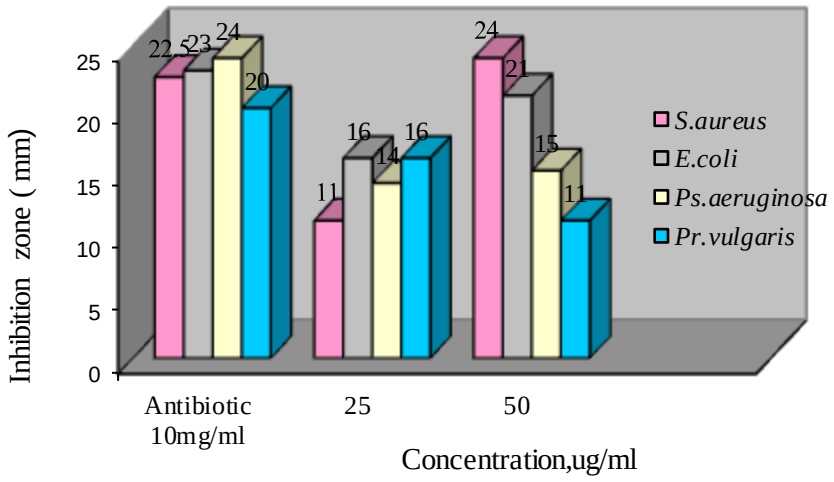

Fig .3.Antibacterial activity of *D.innoxia* leaves aqueous extracts against four microorganisms

Inhibition zone of 50 and 25% methanol and aqueous extraction of root, leaf and seed of *D innoxia* for *Aspergillus niger*

Figure (4) indicated that inhibition zone for 50 and 25 % methanol extraction for root are 17 mm and 0.0 mm against A. niger, respectively, while inhibition zone of 50 and 25 % aqueous extract for root are 20 mm and 18 mm against A. niger, respectively. Inhibition zone for 50 and 25 % methanol extraction for leaf are 25 mm and 19 mm against A. niger, respectively, but inhibition zone of 50 and 25 % aqueous extract for leaf are 26 mm and 20 mm against A. niger,

respectively. Inhibition zone for 50 and 25 % methanol extraction for seed are 19 mm and 15 mm against A. niger, respectively, inhibition zone of 50 and 25 % aqueous extract for seed are 23 mm and 18 mm against A. niger, respectively.

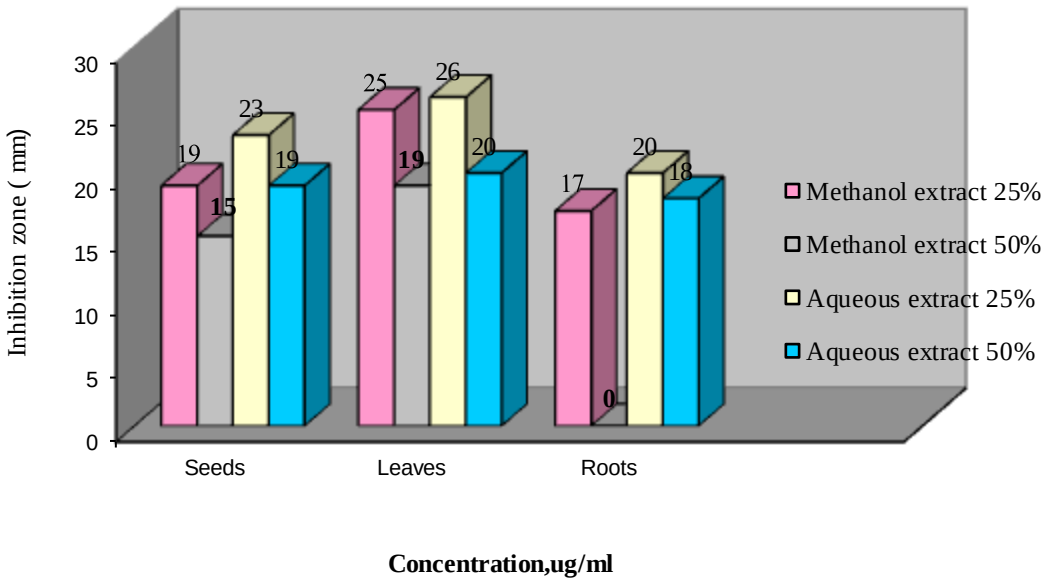

Fig .4.Antifungal activity of *D.innoxia* extracts *against A.niger*

Inhibition zone of 50 and 25% methanol and aqueous extraction of root, leaf and seed of *D innoxia* for Candida *albicans*

Figure (5) indicated that inhibition zone for both concentration(50 and 25 % methanol and aqueous extraction of root) are similar (0.0 mm) against *Candida albicans*, but inhibition zone for 50 and 25 % methanol extraction for leaf are similar (0.0 mm) against *Candida albicans*. While inhibition zone of 50 and 25 % aqueous extract for leaf are 12 mm and 14 mm against *Candida albicans*, respectively.

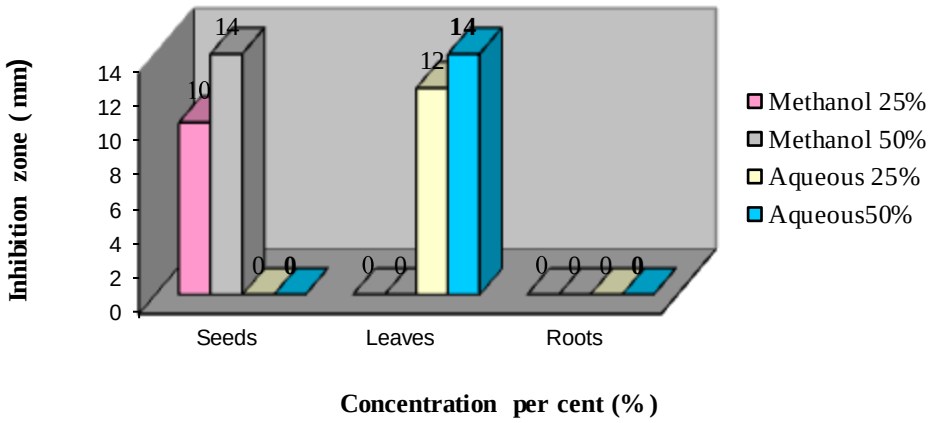

Fig .5.Antibacterial activity of *D.innoxia* extracts against *Candida albicanas*

Discussion

Although the *D. innoxia* seeds are used in traditional medicine in China, Indonesia and Africa for the treatment of different types of diseases, The leaves and seeds are widely used in herbal medicine as anesthetic, antispasmodic, bronchodilator and as hallucinogenic (Duke and Ayensu, 1985). The results of the present study on antibacterial activity of the *D. innoxia* seeds, leaves and roots extracts (methanol and Aqueous) used in different concentrations ranging from 25 - 50% indicated variations, to a great extent, depending on the type and concentration of the plant extract as well as the susceptibility of the examined bacteria. The methanol of *D. innoxia* seeds in concentration 50% was active against all examined bacteria and *Aspergillus* niger, but aqueous extract at concentration 25 and 50% were inactive. These findings supported the use of leaves for the treatment of wounds by which these pathogens are associated (Okigbo and Omodamiro, 2006). The aqueous extract of *D. innoxia* leaves in all concentrations used was active against all examined bacteria. The inhibition effect of *D. innoxia* on organisms may be the reason behind the use of *D. innoxia* in herbal medicine for the treatment of asthma, cough, catarrh, diarrhea, gonorrhea and skin diseases (Nadkarni, 1976; Duke and Ayensu, 1985). The

both methanol and aqueous extracts of *D. innoxia* roots in all concentrations used was inactive against all examined bacteria and fungi except *Aspergillus niger*. Methanolic and aqueous extracts of *D. innoxia* seeds, leaves and roots in different concentrations (25 and 50%) used was active against *Aspergillus niger*. but inactive against *Candida albicans.*

The indicated that inhibition zone that obtained by Gentamicine against *S. aureus* and *E. coli* similar to inhibition zone that obtained by seeds methanolic extraction and leaves aqueous extraction at concentration 50% (Figure 1 and 2). The inhibition zone that obtained by Nystain against *Aspergillus niger is large* more than inhibition zone that obtained against *A. niger* and *C. albicans* at concentrations (25 and 50%). The antibiotic (Gentamicine and Nystain) shows activity against all tested organisms at different concentrations.

Conclusion

The methanolic extract of *D. innoxia* seeds is more active than the aqueous extracts. Aqueous extract of *D. innoxia* leaves are more active than the methanolic extract. Methanol and Aqueous extracts of *D. innoxia* roots in all concentrations used was inactive against all test bacteria and fungi except *A. niger*. The antimicrobial activity of the different extracts was compared with standard antibiotics and found to be effective.

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