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SENSITIVITY OF ISOLATED PATHOGENIC BACTERIA FOR AQUEOUS AND ALCOHL EXTRACT OF CASSIA SENNA

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Abstract

In view of the fact that ancient time, plants have been a tremendous source of medicine. The knowledge of traditional medicine and medicinal plants and their study of scientific chemical principles may lead to the discovery of newer and cheaper drugs.Cassia was observed to have antibacterial activity and can be used to combat against vast flora of microorganisms. Senna (Cassia) has wide range of pharmacological actions hence present study was undertaken to evaluate the antibacterial potential of methanolic and aqueous extracts of dry flowers of Cassia using agar well, and disc diffusion methods. The microorganisms used include *Staphylococcus aureus*; *E. coli*; *Pseudomonas aeruginosa*; *Bacillus subtilius*; Klebsiella and Proteus. All extract established significant antibacterial activity against tested bacteria. Size of growth inhibition zone showed significant difference. Of present study revealed that in aqueous extract well method, Proteus showed the highest sensitivity, followed by each of *E. coli*, *Pseudomonas aeruginosa,* and Klebsiella, while Bacillus and Staphy. showed no significant difference .Aqueous disc method *Pseudomonas aeruginosa* and Proteus the most sensitive bacteria, other *E. Coli*, Bacillus, Klebsiella, *Staphylococcus aeureus* no significant changes. Aqueous extract in comparison between well and disc methods only Bacillus at 50 and 100 mg / ml, and Proteus art 100 was significantly different.Alcohol extract well method: E. coli was the most sensitive, followed by each of Bacillus, Staphylococcus, Pseudomonas, Klebsiella and Proteus which were sensitive at 100 mg / ml in comparison with 50 mg / ml. Disc method: Proteus was the most sensitive followedby Pseudomonas, then each of E. coli and Klebsiella. While Staphylococcus and Bacillus showed no significant difference. Sensitivity to alcohol extract in comparison between well and disc methods E. coli, Staphylococcus and Proteus were the most sensitive. While Pseudomonas was significantly sensitive only at 25 mg / ml. Bacillus and Klebsiella not show significant difference. In comparison between aqueous and alcohol extract the results revealed that in well method, Bacillus; staphylococcus; E. Coli and Klebsiella showed no significant differences; Proteus significant at the 25, 50 and 100 mg / ml. Pseudomonas showed significantly at 100 mg / ml .While in disc method all the test bacteria showed no significant differences.

Keywords: Cassia, Well Diffusion, Disc Diffusion, Methanol Extract, Aqueous Extract

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INTRODUCTION

Cassia italic (Mill).is a plant from order Fabales, belong to family Ceasapiniacae. in this family there are 5 tribs one of which is Cassiae, to which Genus Cassia belonged, this genus has 15 species, in Iraq present 8 species only, one wild and 7 cultivated . The plant is present in south east of Iraq (Guest, 1974). The emergence of organism resistance to nearly all classes of antimicrobial agents has become a serious public health concern in the past several years (Didem Dellorman Orhan, 2012). The plants that exhibit great activity could be considered as a source of potential antimicrobial compounds. Crude plant extract that were used in traditional folk medicine for their antimicrobial properties are still widely used to treat infection. Therefore, it is worthwhile to study plants and plant products for activity against microorganism (Kan *et al*., 2009).

The discovery of antimicrobial agents from plants based on the evaluation of traditional plant extracts is very important topic (Didem Dellorman Orhan, 2012). Senna obtusifolia commonly called sinameki (Turkey), its leaves, seeds and root are used medicinally, primarily in Asia. It is believed to possess laxative effect, as well as to be beneficial for the treatment of eye infections. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body (Hedges and Lister, 2007). Medicinal plants represent an important source of medically important compounds .since ancient time, medicinal plants are used to cure several types of health problems. Systemic analysis of the plants provides a variety of bioactive molecules for the development of newer pharmaceutical products .recently, there is a growing interest in the pharmacological evaluation of various plants used in different traditional system of medicine.

In last few decades, many of traditionally known plants have been extensively studied by advanced scientific techniques and reported for various medicinal properties viz, anticance, anti – inflammatory, anti diabetic, anthelmintic, antibacterial, antifungal, hepatoprotective, antioxidant, larvicvidal activity (Kumar, *et al.,* 2010). Now a days, medicinal plants have many applications in people's lives they can be used in the Pharmaceutical compounds,cosmetic, sanitary and nutritional industries (Ramtin *et al*., 2012).

MATERIALS AND METHODS

Specimens collection and cultures preparation

The aim of this study was to evaluated in vitro antibacterial activities of aqueous and alcoholic extracts of leaves of Cassia senna obtained from local market in Iraq.

Preparation of extracts

25 g of air dried powder of plant leaves was filled in the thimble and extracted successively with 300 ml of methanol using a Soxhlet extractor for 72 hours. The methanol was concentrated to near dryness under reduced pressure below 40 ^{o}C, after complete solvent evaporation the solvent extract was weighed and preserved at 4 ^{o}C in airtight bottles until use. 1 g of solvent residue was dissolved dimethyl sulfoxide solvent was used as the test extract for antibacterial activity assay (Karamasn *et al.,* 2003, Okeke *et al.,* 2001).

Preparation of concentrations

1 g of solvent residue was dissolved with 10% dimethyl sulfoxide (DMSO) solution and stored in air tight glass bottle in a refrigerator till further use (Mingarro *et al*., 2006). Dimethyl sulfoxide solvent was used as the test extract for antibacterial activity assay (Karamasn *et al.* 2003; Okeke *et al*., 2001). The concentrations depended in the experiment were, 25, 50and 100 mg/ ml.

Test bacterial strains

The following bacterial strains were used as test organisms:*Staphylococcus aureus, Bacillussubtilis; E. coli: Pseudomonas aeruginosa*: Klebsiella and Proteus. All the bacterial strains were obtained from Department of Microbiology, College of Veterinary Medicine, University of Diyala, Diyala, Iraq.

Antibacterial activities assay

Agar well diffusion method

The extract activities were carries by spreading 0.1 ml 0f bacterial suspension prepared according (Bauer *et al.,* 1966).which contain 1X10^{8} cells / ml over the surface of Muller – Hinton agar plate, to obtain uniform growth, left the plate to dry for 5 minutes. Then well were prepared by using Pasture pipette 5 mm diameter. These well were filled by 50 µl concentrated extract of either aqueous or alcoholic extract according to dilution used. Leave the medium to settle for 1 hour in laboratory condition. Then incubate for 24 h at 37 ^{o}C and zone of inhibition if any around the well were measured in mm. each treatment consists of four repeat (Karaman *et al*., 2003; Srinivasa *et al*., 2001; Masika and Afolayan, 2002).

Disc diffusion method

Antibacterial activity of aqueous and methanol extract were determined by disc diffusion method on Mueller – Hinton agar (Al_ Badrani, 2002) sterile Whatman filter disc (5 mm) were made using sterile cork borer (5mm), these discs were impregnated in the 50 µl of aqueous or alcoholic extract . Place in Petri dishes according to concentration for 24 hours. Inoculums containing 10^{8} CFU/ ml of bacteria were spread, wit sterile swab moistened with the bacterial suspension. The disc also impregnated in 50µl of solvent either distilled water or Dimethyl sulfoxide, served as a standard control. The plates were incubated for 24 h at 37 ^{o}C and zone of inhibition if any around disc were measured in mm. Each treatment consists of fourrepeat (Karaman *et al*., 2003; Srinivasa *et al*., 2001; Masika and Afolayan, 2002). Standard antibiotic disc Rifampin5; Doxycycline 30; Amoxicillin 25; Kanamycin 30 and Ampicillin – cloxacillin 30, for antibacterial activity test were carried out against bacterial strains in used.

Culture preparation

A loop full of 24 hr. surface growth on a NAS slope of each bacteria isolate was transferred individually to 5 ml of Brain heart infusion broth (PH 7.6) and incubated at 37 ^{o}C for 24 hr. Bacterial cells were collected by centrifugation at 3000 rpm for 15 min., washed twice and resuspended in 0.1% peptone water . Turbidity was adjusted to match that of as McFarland standard (108cfu/ ML). Then 1:10 dilution of the cell suspension was performed to give an inoculums concentration of (10^{7}CFU/ ml).

Statistical analysis

All values are expressed as the mean ± the standard error of the mean (SEM) .the data were analyzed by using one way analysis of variance ANOVA, and then the test of the least significant differences between the means of inhibitory zones (Steel and Torrie, 1985). The significant level of test was $P < 0.05$.

RESULTS AND DISCUSSION

The influence of aqueous and methanol extract of Senna leaves at 25, 50 and 100 mg / ml was tested by using disk and well diffusion methods against some pathogenic bacterial strains. The results revealed that aqueous extract of Senna in well method showed a significant difference in sensitivity of *E. Coli* at100mg / ml in comparison with 25 mg / ml. while in disc method no significant difference was exhibited .Also in comparison between well and disc method there was no significant difference in sensitivity of *E. Coli* .In case of Bacillus no significant differences was observed in both well and disc methods. In comparison between well and disc methods there were significant difference at 50mg / ml and 100 mg / ml. In case of *Staphylococcus aureus* there were no significant difference in well and disc methods.

In addition to no significant difference in sensitivity in comparison well with disc methods. *Pseudomonas aeruginosa* in well method there were significant difference at 100mg / ml in comparison with 25 and 50 mg / ml. in disc method the significance difference was at 50 and 100mg / ml in comparison with 25 mg / ml. Well with disc methods no difference was observed. In case of Klebsiella there were significant difference in well method between 100 mg / ml with 25 and 50 mg/ml. in disc method no significant difference. In well compared with disc no significant difference was observed. In Proteus in well there were significant difference in50 and 100 mg / ml in comparison with 25 mg / ml, and between 50 with 100 mg / ml .in disc method there was significant difference at 100mg / ml in comparison with 25 mg / ml. In comparison between well and disc methods there was significant difference at 100 mg / ml.(Table -1).The growth inhibition zone measured ranged from 10 -20 mm for all the sensitive bacteria. (Nayan *et al*., 2011).Cassia fistula exhibited significant antimicrobial activity and showed properties that support folkloric use in the treatment of some diseases as broad spectrum antimicrobial agents (Prashanth *et al*., 2006).

In disc method at 100 in comparison with 25 and 50 mg / ml. in comparison between well and disc methods the significance were at the 25, 50 and 100 mg /ml .In case of Bacillus in well method significant difference was between 100 and 25 mg /ml. . In disc method no significant difference. In comparison well with disc method no significant difference was observed. In case of staphylococcus in well at 50 and 100 mg / ml in comparison with 25 mg / ml. in disc method no difference was observed.

In comparison between well and disc there were significant difference at 25, 50 and 100 mg / ml. In case of Klebsiella in well method at 200 in comparison with 50 mg / ml. in case of pseudomonas aeruginosa in well method significance difference between 100 and 25 mg / ml , in disc method at 100 in comparison with 25mg / ml . Well in comparison with disc method at 25 mg / ml. In case of Klebsiella i8n well method significant difference was noted between 100 and 25 mg / ml. in disc method in between 100 and 25 mg / ml. in well compared with disc method no significant differences. Proteus in well method significant difference at 100 in comparison with 25 and 50 mg / ml.

Table 1. Showing the sensitivity of isolated pathogenic bacteria to aqueous extract of Senna in well and disc methods

	Well			Disc		
Bact. Spp.	Concentration			Concentration		
	25 mg / ml	50 mg / ml	100 mg / ml	25 mg / ml	50 mg / ml	100 mg / ml
E. coli	6.33±	7.33±	10.33±	7.00±	7.67±	7.67±
	1.20a	1.46a	1.20b	1.53a	0.67a	1.34a
Bacillus	8.33±	10.0±	11.33±	6.67±	7.67±	9.0±
	1.2aA	0.58Aa	0.34bA	1.34aA	0.88aB	0.58aB
Staphylococcus aureus	9.67±	11.0±	12.0±	8.33±	8.0±	9.33±
	1.34a	1.009a	1.53a	1.03a	1.523a	1.61a
Pseudomonas	7.00±	8.00±	9.67±	6.67±	9.00±	9.33±
aeruginosa	1.00a	0.58a	0.34b	0.67a	0.58b	0.67b
Klebsiella	8.33±	9.00±	12.0±	8.33±	8.67±	9.67±
	0.34a	0.58a	0.48b	1.03a	1.03	1.03a
Proteus	7.67±	9.67±	12.67±	7.00±	8.33±	9.33±
	0.34aA	0.67bA	1.46bcA	0.58aA	0.88aA	0.34bB

Values are M ± SEM: a, b significant difference at a level of P< 0.05 in comparison with in the same group. A, B significant in comparison between groups

Table 2. Showing the sensitivity of isolated pathogenic bacteria to alcoholic extract of Senna in well and disc methods

Bact. Spp.	Well			Disc		
	Concentration			concentration		
	25	50	100	25	50	100
E. coli	8.33±	10.0±	13.0±	6.0±	7.00±	9.67±
	0.34aA	0.58 bA	1.0bcA	0.58aB	0.58aB	0.67bB
Bacillus	8.67±	10.33±	12.67±	7.67±	8.08±	10.33±
	1.34a	1.41a	1.77b	1.86a	2.0a	1.67a
Staphylococcus aureus	9.343±	12.67±	13.0±	6.67±	7.67±	9.00±
	0.67aA	1.46bA	1.0bA	1.20aB	1.86aB	1.53aB
Pseudomonas	13.0±	13.67±	17.0±	6.0±	8.67±	11.33±
aeruginosa	1.55aA	1.1`8aA	1.22bA	0.58aB	1.46bA	1.86bA
Klebsiella	8.33±	9.33±	12.0±	8.0±	10.0±	11.67±
	0.34a	0.34a	1.53b	1.16a	1.31a	1.73b
Proteus	11.67±	14.33±	17.67±	5.67±	8.00±	9.33±
	1.20aA	1.20aA	1.34bA	0.67aB	0.58bB	0.34 bcB

Values are M ± SEM: a, b.c significant difference at a level of P< 0.05 in comparison with in the same

The results revealed that in alcohol extract of Senna showed: E. Coli in well method showed sensitivity which was significant at 50 and 100 in comparison with 25 mg / ml. and between 50 and 100 mg / ml.

In disc method there were significant difference between 50 and 100 in comparison with 25 and 100 in comparison with 50 mg / ml. in comparison well with disc method highly significant differenced at 25, 50 and 100 mg / ml. (Table 2).

Methanolic extract of Cassia auriculata has shown presence of carbohydrats (reducing sugars), saponin glycosides, flavonoids. alkaloids, tannins and phenolic compounds. The extract was found to have maximum activity against all organisms. The investigation confirmed the antimicrobial activity of flower extract of Cassia auriculata. Leaf extracts of Cassia auriculata exhibited significant broad spectrum activity against Bacillus subtilis and Staphylococcus aureus (Perumalsamy and Ignacimutu 2000).The extract of Cassia auriculata was found to have potent microbial activity against the E. Coli in poultry (Samy, 2000). Methanolic extract of flowers was found to have higher inhibitory activities against Staphylococcus aureus, Bacillus subtilis, E. coli and Salmonella typhoid. The minimum inhibitory concentration ranged between 12.5 mg / ml and 75 mg / ml depending on microorganism and extract (Doshi *et al*. 2011). The S. didymobotrya crude root extract inhibited all the organisms, with the best zone of inhibition been that of Bacillus cereus. Followed by P. Vulgaris, Salmonella typhi, E. coli, E. Aerogenes and Serratialiquefaciens. Inhibition against Bacillus Cereus was significantly higher than all the organisms (Anthoney *et al*., 2014).

Extracts of S.obtusifolia (L.)Demonstrated a broad – spectrum of activity against both gram – positive and gram – negative bacteria and fungi. The broad – spectrum antibacterial activities of the plant extract, possibly due to the identified alkaloids, further confirm its use as a health remedy in folklore medicine. (Doughari *et al*., 2008). It is obvious that the average activity of essence can be result of the reaction of its components because resultant of this reaction is positive or sometimes is negative .definitely, different effectiveness can be result of ecological, geographical, climatic factors and the age of plant on the mixing of various population of one or combined sort (Ramtinert *et al*., 2012).

It has been suggested that high resistant to plant extracts in gram negative bacteria is due to the outer membrane of their cell wall, acting as barrier to many substances including antibiotic (Marino *et al*., 2011). In comparison between aqueous and alcohol extract the results revealed that in well method, Bacillus ; staphylococcus ; E. Coli and Klebsiella showed no significant differences ; Proteus significant at the 25, 50 and 100 mg / ml . Pseudomonas showed significantly at 100 mg / ml.While in disc method all the test bacteria showed no significant differences.

Conclusions

The aqueous and methanolic leaves extract of Cassia senna had impressive antibacterial.

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