Effectiveness evaluation of *Pituranthos tortuosus* pneumatic parts against Some microbes cause of urinary tract infections.

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Abstract: Medicinal plants are considered one of the experimental methods for treating many diseases caused by microbes, especially after the emergence of strains resistant to antibiotics. Therefore, this study was conducted in laboratory of Faculty of Education / Department of Biology, to test inhibitory efficacy of hot and cold aqueous extracts of *Pituranthos tortuosus* at a concentration of (100, 200, 300, 400) mg/ml against some species of urinary tract infections pathogens (*Escherichia coli*, *Staphylococcus aureus*, *Klebsilla sp.*, *Proteus sp.*, *Candida albicans*), by a sensitivity test in form of agar well diffusion method, then compared with Ciprofloxacin. The results showed that *Pituranthos tortuosus* extract has good inhibitory activity against all tested microbes, and its effectiveness increased with increasing concentration, and aqueous extracts showed inhibitory activity superior to Ciprofloxacin for *Candida albicans* Inhibition at diameter (10.2) and (8.0)mm, for hot and cold extracts respectively, and antibiotic at concentration 5ug superior was on aqueous extracts against all bacterial species. *E.coli*, *Klebsilla sp.* Was most resistant to extracts and antibiotics, were *S.aureus* and *Proteus sp.* the most sensitive to extracts and antibiotics.

Key words: *Pituranthos tortuosus*, Urinary tract infections, Ciprofloxacin.

Introduction:

Urinary tract infections its one of the most common diseases in hospitals and outpatient clinics (Odongo *et al*., 2020). 150 million people around worldwide suffer from UTI (Terlizzi *et al*., 2017). The severity of infections varies with age and sex, where women and children are more susceptible to infection (Forsyth *et al*., 2018). The infections usually occur in one or more members of a urinary system and is often associated with urinary bladder (Flores-Mireles *et al*., 2015).

Bacteria are the major cause of more than 95% of cases of UTI (Ramesh *et al*., 2008). *Enterobacteriaceae* causes about 85% of UTI, especially *E.coli* and *Klebsilla pneumonia* (Khan *et al*., 2020). *Staphylococcus sp.*, *Proteus sp.*, and *Enterococcus sp.* are also known to be among a most common causes of urinary tract infections

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(Gajdacs and Urban, 2019). *Candida albicans* is the major cause of fungal urinary tract infections in hospitals (Jacqueline *et al*., 2010).

Urinary tract infection drug is mainly based on antibiotics (Ghaffar *et al*., 2019). Pathogenes became more resistant to antibiotics, which prompted researchers to explore natural alternatives represented in medicinal plants, for easy access and because of effective natural compounds they contain and for its few side effects (Shaheen *et al*., 2019). *Pituranthos tortuosus*, belongs to family *Apicaceae*, among medicinal plants are distributed in northeastern Libya (Abugarsa and Algosni, 2016). bushes wild-desert, an aromatic, of 30-80cm high (Assy *et al*., 2019). widely used in medicine traditional for treating UTI, asthma, rheumatism, diabetes and stings of scorpions (Krifa *et al*, 2011). Species of Genus *Pituranthos* vary according to geographical and regional location, but all of species are distinguished by the presence of essential oil, which is used to eliminate several types of microbes isolated from different infections, where indicated (Dahia *et al*., 2007) that essential oil of specie *P.Chloranthus* has good inhibiting activity against *Pseudomonas aeruginosa*, as noted (Abderrazak *et al*., 2013) the inhibitory effect of oil extracted from aerial parts of specie *P. scoparius* on *Staphylococcus aureus* and *Shigella sp.* and *Candida albicans*, a study conducted in Tunisia confirmed activity inhibition of oil extracted from fresh parts of two species *P. Chloranthus* and *P. tortuosus* against *Enterobacter aerogenes* (Mighri *et al*., 2015). It was also noted that acetone extract of *P. scoparius* was superior to all other extracts inhibited the tested bacterial (Houria *et al*., 2013).

Therefore, the study aimed to evaluate the efficacy of aqueous extracts of pneumatic parts, with a different concentrations of *Pituranthos tortuosus* on some microbes cause of urinary tract infections.

Material and methods:

Plant collection and preparation:

Samples of pneumatic parts of *Pituranthos tortuosus* were collected from Mertoba region east of Al-Bayda city / Libya, were classified in Biology Department/Faculty of Education / Omar Al-Mukhtar University, washed with distilled water, and dried inside laboratory under room temperature, then crushed by an electric grinder and saved until use.

Aqueous extraction:

add 200g of powder dry of plant to 1000 ml of cold sterilized distilled water for 24 hours at room temperature, then filter solution with filter papers (0.22um), put on into Oven at 40 ° C, store in refrigerator until use (Sani *et al*., 2014).

To prepare hot water extract, use the same previous method, replacing cold water with hot water 100 ° C.

Bacterial isolates:

- Isolates predefined were obtained from urinary tract patients reluctant to (Tiba-Alrazi-Alrahma Clinic) laboratories, Al-Bayda / Libya.

Antibacterial susceptibility testing:

After to growth of pathogenes bacteria on Mueller-Hinton agar medium, four wells were drilled in each petri dish with a sterilized cork borer with a diameter of 6 mm by equal distances. An appropriate volume was added to each well of a concentration prepared from extract. The dishes were incubated for 24 hours at a temperature of 37°C with three replications, were compared with Ciprofloxacin (Daoud *et al*., 2015), then a measure of diameters of inhibitory zones.

Antifungal susceptibility testing:

Was performed by well diffusion using Mueller-Hinton supplemented with glucose and methylene blue.(Mahboob *et al*., 2019).

Statistical analysis:

The study Experiences were designed according to the complete random design (CRD). Statistical analysis was performed using Minitab 17 program and ANOVA variance analysis tables. Were compared averages with the least significant difference LSD 0.05.

Results and discussion:

Sensitivity test antibiotic:

Effectiveness of Ciprofloxacin was tested with 5ug concentration against four species of bacteria and one species of fungi in patients with UTI, as one of antibiotics used in hospitals to control disease, from Table (1) shown results of resistance and sensitivity of pathogenic microbes to an antibiotic. was *Candida albicans* most resistant to Ciprofloxacin at diameter (4) mm. *Candida* Resistance returns to biofilm formation, through an intricate gene regulatory network genes and complex transcriptional factors, which are involved in cell surface regulation, hyphal formation, and development and virulence expression (Araujo *et al*., 2017).

E.coli was the most bacterial isolates resistant to antibiotic than other isolates, at a diameter of (6) mm. This resistance may be due to having a cell wall consisting of a multi-layer lipid (Melnyk *et al*., 2015), or because its cilia contain specialized genes that have the property of adherence to epithelial cells that The lumen of a urinary bladder is lined (Agarwal *et al*., 2012). Result agrees with (Fasugba *et al*., 2015; Reis *et al*., 2016), *E.coli* was one of bacterial species most to cause urinary tract infections have resisted Ciprofloxacin due to excessive use of antibiotics in all countries of a world, especially countries. Developing. *Klebsilla sp.* showed middle sensitivity to antibiotic with a diameter of (11) mm, our results agreed with (Grillon *et al*., 2016), while was *Proteus sp.* and *S.aureus* most sensitive to Ciprofloxacin with diameters of (15.7) and (17.1) mm, respectively, this was confirmed by (Kwiecinska-pirgo *et al*., 2016; Wasfi *et al*., 2012). Ciprofloxacin has high efficacy in destroying and removing cellular membranes of these bacterial species.

Sensitivity test of extracts (hot-cold):

Pituranthos tortuosus extract tests against pathogenic microbes showed a different effect depending on species of extract, a concentrations used and species of pathogens.

from Table (1) the results of aqueous extracts with a concentration of 100 mg/ml showed no inhibitory effect to studied isolates, except for *Proteus sp.* , which showed low sensitivity with a diameter (1.8) and (2.5) mm for cold and hot aqueous extract, respectively. This result agreed with (Allam *et al*., 2015). Aqueous extracts at a concentration of 200 mg/ml recorded low inhibiting activity against *S.aureus* and *Proteus sp.* with a diameter of (3.0) and (3.9) mm respectively, for cold extract, while inhibition diameters increased to (9.0) and (6.2) for hot extract, respectively. While did not have an inhibitory effect on *E.coli, Klebsilla sp.* and *Candida albicans*, result agree with (Houria *et al*., 2014) to there inhibiting activity of concentration 200 mg / ml for specie *P.scoparius*.

The concentration of 300 mg/ml showed good inhibition activity against most tested species with inhibition diameters ranging from (2.5-10) mm. Where was *Proteus sp.* most affected by this concentration of (hot-cold) extracts, while *Klebsilla sp.* Least affected, while this concentration did not affect *E.coli*.

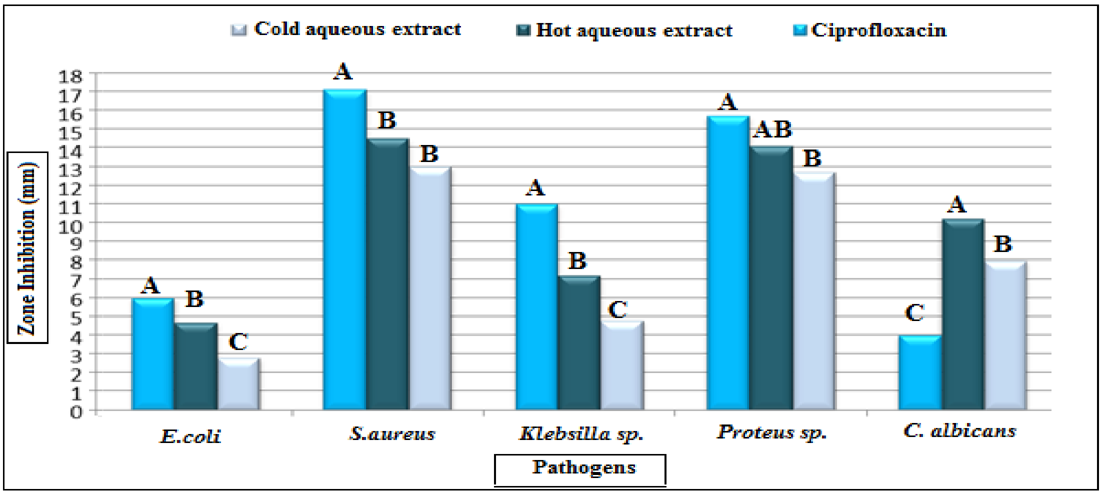
The concentration superiority of 400 mg/ml was also noted in recording the best inhibition rates on all previous concentrations of all tested species. *S.aureus* and *Proteus sp.* the most sensitive to extracts with diameters of (14.5) and (14.1) mm for hot extract, respectively, (13.0) and (12.7) mm for cold extract, respectively, while *Klebsilla sp.* and *E.coli* is most resistant to extracts with a diameter of (7.2) and (4.7) mm for hot extract, respectively, and (4.8) and (2.8) mm for cold extract, respectively. *Candida albicans* recorded a middle sensitivity with a diameter (10.2) and (8.0) mm for hot and cold extract, respectively. This results agreed with (Mansour et al., 2019; Houria *et al*., 2014; Lahmar *et al*., 2017), that Pituranthos sp. extracts have good inhibiting activity against pathogenes microbes.

The inhibitory activity of *Pituranthos tortuosus* aqueous extracts against some species of bacteria and fungi causing urinary tract infections, due it contains: anthocyanins, tannins, saponins, terpenoids and polypeptides (Saleem *et al*., 2010). Also contains β-myrcene, trans-iso-elemicin and terpinen4-ol (Abdallah and Ezzat, 2011). The activity mechanisms of plant extracts and their natural components are related to degradation of the cell wall, damage to cytoplasmic membrane and membrane proteins, leakage of intracellular contents, coagulation of cytoplasm, interference with active transport or metabolic enzymes, dissipate cellular energy in ATP form and depletion of proton motive force (PMF) and electron flow, which can cause cell death (Negi, 2012).

Table (1): Antimicrobial activity of *P. tortuosus* pneumatic parts extracts. (mean ± standard deviation)

Microbes / Extracts		*E.coli*	*S.aureus*	*Klebsilla sp.*	*Protuse sp.*	*Candida albincas*
Cold aqueous extract	**100**	0.0±0.0	0.0±0.0	0.0±0.0	1.8±0.0	0.0±0.0
	200	0.0±0.0	3.0±0.2	0.0±0.0	3.9±0.2	0.0±0.0
	300	0.0±0.0	8.5±0.6	0.0±0.0	9.0±0.2	4.3±0.1
	400	2.8±0.1	13.0±0.5	4.8±0.3	12.7±0.7	8.0±0.4
Hot aqueous extract	**100**	0.0±0.0	0.0±0.0	0.0±0.0	2.5±0.0	0.0±0.0
	200	0.0±0.0	9.0±0.5	0.0±0.0	6.2±0.3	0.0±0.0
	300	0.0±0.0	9.2±0.1	2.5±0.1	10.0±0.8	5.4±0.3
	400	4.7±0.4	14.5±0.7	7.2±0.2	14.1±1.0	10.2±0.7
Ciprofloxacin	**5ug**	6.0±0.3	17.1±0.6	11.0±0.5	15.7±1.0	4.0±0.0
L.S.D. 0.05 = 0.6008						

When comparing an inhibitory efficacy of *Pituranthos tortuosus* extracts and Ciprofloxacin. shown results in Table (1) and Figure (1) there was a significant difference at L.S.D (0.05), where Ciprofloxacin superior to aqueous extracts of all bacteria species, while aqueous extracts (hot-cold) superior at a concentration of 400 mg/ml on Ciprofloxacin against *Candida albicans* with diameters (10.2) and (8) mm, respectively, while hot aqueous extract was more efficient than cold aqueous extract against all pathogenic microbes, and differences in inhibitory capacity of *Pituranthos tortuosus* extracts were due to type of solvent used, amount of soluble active substances and species of pathogens.



Fig(1): Antimicrobial activity of *P. tortuosus* pneumatic parts extracts at concentration 400mg/ml.

Conclusion:

We conclude from this study that of *Pituranthos tortuosus* pneumatic parts can use to bio-control of pathogens of urinary tract infections, especially fungi. Were *E.coli* and *Klebsilla sp.* the most resistant to extracts and antibiotic. While *S.aureus* and *Proteus sp.* the most sensitive to extracts and antibiotic. So researchers recommend conducting future studies of these extracts, separate the active substances, and use them as alternatives to antibiotics.

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