

## Evaluation of in vitro activity of *Calvatia craniiformis* extract against bacteria isolated from pregnant women with asymptomatic bacteriuria

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### Summary:

**Background:** Urinary tract infection (UTI) is the most common disease associated with pregnant women due to several anatomical and hormonal changes in pregnant women lead to ureteral dilatation and urinary infection, many of microorganisms such as fungi, viruses, and bacteria have numerous virulence factors that enhance their ability to colonize in pregnant women. Some bacteria have resistance toward many antimicrobial agents for treatment has led to therapeutic difficulties worldwide, in order that many investigators try to test fungal extract to reduce bacterial infection.

**Objectives:** To assess the effectiveness of extract of *Calvatia craniiformis* on different types of bacteria which was isolated from pregnant women with asymptomatic bacteriuria.

**Materials and methods:** One hundred five vaginal swabs were collected from pregnant women with asymptomatic bacteriuria attending to Al-Batool hospital for maternity and children in Diyala Governorate during the period from 1<sup>st</sup> /April /2012 till 1<sup>st</sup> /July/2012.

All specimens were streak on blood agar and MacConke ager, then identified according to standard bacteriology and biochemical criteria. The susceptibility patterns toward 6 antimicrobial agents were done by disc diffusion methods, 40% *Calvatia craniiformis* extract used for study the effect of this extract against different types of bacteria .

**Results:** Fifty five out of 105 samples (52.38%) were demonstrated negative bacteria growth while 50 out of 105 samples (47.61%) demonstrated positive bacteria culture. The isolated revealed that 22 *Escherichia coli*, 15 *Klebsiella*, 11 *Proteus*, and 2 *Staphylococcus saprophetec*. The majority of bacterial isolates were sensitive to 40% concentration of extract of *Calvatia craniiformis*.

**Conclusion:** Extract of *Calvatia craniiformis* was effective as antibacterial against antibiotic resistant strain.

**Keywords:** Urinary tract infection (UTI), pregnant women, antibiotic, *Calvatia craniiformis*.

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### Introduction:

Urinary tract infections are the second most common type of infection in the body, accounting for about 8.1 million visits to health care providers each year [1]. Women are especially prone to UTIs for anatomical reasons. One factor is that a woman's urethra is shorter, allowing bacteria quicker access to the bladder. Also, a woman's urethral opening is near sources of bacteria from the anus and vagina. For women, the lifetime risk of having a UTI is greater than 50 percent [2].

Urinary tract infections are more concerning in pregnancy due to the increased risk of kidney infections. During pregnancy, high progesterone levels elevate the risk of decreased muscle tone of the ureters and bladder, which leads to a greater likelihood of reflux, where urine flows back up the ureters and towards the kidneys. While pregnant women do not have an increased risk of asymptomatic

bacteriuria, if bacteriuria is present they do have a 25-40% risk of a kidney infection [3].

Thus if urine testing shows signs of an infection even in the absence of symptoms treatment is recommended. Cephalexin is typically used because they are generally considered safe in pregnancy [4].

*Calvatia* (Basidiomycetes, Lycoperdaceae) is a cosmopolitan gasteromycetous genus of about 35-45 species of mostly medium to large sized epigeous puffballs. The genus is characterized by stalked or sessile, globose, ubglobose, turbinate, pyriform or agaricoid fruit-bodies that dehisce by irregular fragmentation of the peridium (spore-sac wall) and not by an apical pore [5].

The use of *Calvatia* species in Chinese and Japanese traditional medicine has served as an additional motivation for continued pharmaceutical investigation of these fungi and has led to the isolation of ergosterol From *C.excipuliformis* (as *C. saccata*) [6]. A polyoxygenated ergosterol derivative, various other steroids as well as

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a calvatic acid derivative from *C.nipponica* Kawam as *Lasio-sphaera nipponica* (Kawam.) Kobayasi [7]. And the novel steroids calvasterone, cyathisterone, cyathisterol, calvasterol A and B from *C. cyathiformis* [8].

Kim et al. Observed that protein-bound polysaccharides extracted from cultured *C. craniiformis* mycelium suppressed the growth of sarcoma 180 in mice by up to 74.1%. The anti-tumour activity of at least one of the extracted fractions, referred to as calvatan, was believed to be as a result of immunopotential rather than cytotoxicity [9].

The present study was undertaken to investigate the antibacterial activity of fungal extract against bacteria isolated from pregnant women with asymptomatic bacteriuria.

**Materials and methods:**

**Sample collection:** One hundred five vaginal swabs were collected from pregnant women with asymptomatic bacteriuria attending to Al- Batool Hospital for Maternity and Children in Diyala Governorate, during the period from 1<sup>st</sup> /April /2012 till 1<sup>st</sup> / July/2012.

**Culture of bacteria:** The work of present study was done in department of microbiology - College of Medicine - Diyala University. For isolation, agars were prepared according to the manufacturer company; clinical specimens were collected using sterile capped swabs inoculated on the blood agar and MacConkey agar, then incubated at 37°C for 24 hours [10]. In next day many bacteriological and biochemical tests performed to recovered types of bacteria. **Antibiotic sensitivity test:** Kirby and Bauer test were done to determine the susceptibility patterns toward 6 antimicrobial agents include cefalexin, cefotaxime, erythromycin, amoxicillin, nalidixic acid and nitrofurantoin, were done by disc diffusion method [11]. According to this method, bacterial suspension of 0.1 X 10<sup>6</sup> CFU concentration was distributed on the surface of Muller-Hinton agar media for all bacterial species, then the antibiotic disc were put on the surface of culture media by sterile forceps. The plates were incubated under aerobic condition at 37°C for 24 hours. The results were read by measuring the inhibition zone in mm.

**Preparation of *Calvatia craniiformis* extracts:** The mushroom were diagnosed according to morphological characteristic of species [12, 13]. The air-dried fruity body of *Calvatia craniiformis* mushroom, then crushed in sterile petri dish to obtain a yellow-brown powder, 40 mg of mushroom powder were dissolved with 10 ml ethanol, dried by incubate overnight. Then 40 mg of the mushrooms powder were dissolved with 100 ml sterile distilled water and considered as standard stock solution for prepared serial dilution include 2, 4, 8, 12, 16, 20 mg/ml. Filtered and autoclaved and finally keep in refrigerator at 4°C.

Bacterial sensitivity to *Calvatia craniiformis* solution. The bacteria which were resistant to the preceding antibiotics, which was selected according to the diameter of inhibition zone for testing the extract of *Calvatia craniiformis* (40mg/ml stock solution, equal 40% concentration) by agar dilution method[14]. Using bacterial cell suspension which equilibrated their concentration to a 0.5 McFarland standard. Each bacterial suspension (0.2ml) was spread on Mueller-Hinton agar plate, the plate were incubate at 37°C overnight. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the *Calvatia craniiformis* solution Were also investigated for the bacterial strains which were determined as sensitive to the compounds in disc diffusion assay.

Data analysis: Chi-Square test was used to obtain statistically significant differences in the present study.

**Results:**

The result of current study demonstrated that 50 out of 105 vaginal swabs (47.61%) were positive for bacterial culture, while 55 out of 105 vaginal swabs (52.38%) were negative for bacterial culture. On the other hand these results occur after culturing.

According to standard bacteriological and biochemical tests. *E.coli* constitute the higher percentage 22 out of 50 (20.95%) among other types of bacteria, followed by *Klebsella*15 (14.28%), *Proteus* 11 (10.47%), and finally *Staphylococcus saprophytic* 2(1.90%). As showed in (Table1).

**Table 1: Number and type of bacteria isolate from pregnant women without clinical UTI.**

Result of bacteria culture	Type of bacteria	Number and percentage
50 with bacterial growth (47.61%)	<i>E. coli</i>	22 (20.95%)
	<i>Klebsella</i>	15 (14.28%)
	<i>Proteus</i>	11(10.47%)
	<i>Staphylococcus saprophytic</i>	2 (1.90%)
55 without bacterial growth (52.38%)	0	0

Effect of antibiotic on bacterial growth which demonstrated that, the majority of bacterial isolate show resistant to more than one antibiotic. *E. coli* show resistant to nalidixic acid, amoxicillin and nitrofurantoin was (81.81%, 90.90% and 95.45%) respectively. While *Klebsella* shows resistant to nitrofurantoin, amoxicillin and nalidixic acid was (73.33%, 86.66% and 93.33%). *Proteus* as show nitrofurantoin, amoxicillin and in table (2).

**Table 2: Number and percentage of the bacterial isolates resistant to different antibiotics.**

Antibiotic	<i>E. coli</i> (n=22)	<i>Klebsella</i> (n=15)	<i>Proteus</i> (n=11)	<i>Staphylococcus saprophytic</i> (n=2)	Chi-Square
Cefalexin	9(40.90%)	7(46.66%)	5(45.45%)	0	1.65 p=0.648
Cefotaxime	3(13.63%)	5(33.33%)	4(36.36%)	0	3.57 p=0.312
Erythromycin	12(54.54%)	7(46.66%)	9(81.81%)	1(50%)	3.5 p=0.320
Amoxicillin	20(90.90%)	13(86.66%)	10(90.90%)	1(50%)	3.02 p=0.388
Nalidixic acid	18(81.81%)	14(93.33%)	8(72.72%)	0	10.06 =0.018
Nitrofurantoin	21(95.45%)	11(73.33%)	11(100%)	0	17.8 =0.0001

Three bacterial isolates were taken from each bacterium which shows high resistant to antibiotics for testing their sensitivity to *Calvatia craniiformis* extract. All concentration of the fungal extract solution show more effect on bacteria than distal water in different percentage.

Regarding to the values of MIC and MBC as shown in (table 3), the results of present work which demonstrated that fungal extract was more effective as antibacterial agent against *Klebsella* and *Staphylococcus saprophytic* comparing with *E. coli* and *Proteus*.

**Table 3: Values of MIC and MBC of *Calvatia craniiformis* extract.**

Type of bacteria	<i>Calvatia craniiformis</i> extract	
	MIC mg/ml	MBC mg/ml
<i>E. coli</i>	8	12
<i>Klebsella</i>	2	4
<i>Proteus</i>	20	40
<i>Staphylococcus saprophytic</i>	2	4

**Discussion:**

We found that the percentage of urinary tract infection in pregnant women admitted to the Al-Batool Hospital for Maternity and Children was 47.61%. And this higher percent could be explained by smaller sample size of the present work. In this study, *E.coli* was the commonest organism causing urinary tract infection (20.95% of cases) followed by *Klebsiella* (14.28%), *Proteus* (10.47%) and lastly *Staphylococcus saprophytic* (1.90%). By comparison to the study performed by Nicolle [15].Who indicate that, percentage of *E. coli* in urinary tract infections is 80-85%. *Escherichia coli* bearing adhesins of the Dr/Afa (Dr afimbrial adhesins) family frequently causes urogenital infections during pregnancy in humans and has been associated with mortality in pregnant rats. Two components of the adhesin, Dra/Afa E and Dra/Afa D, considered virulence factors, are responsible for bacterial binding and internalization [16].

The change in immune responses may result from the increase in progesterone level during pregnancy, which modifies maternal immune responses in order to protect semiallogenic fetal antigens from rejection, the increased progesterone level might encourage infection with Dr/Afa+ *E. coli*, up-regulating the expression of DAF, which acts as an epithelial receptor for this bacterium [17,18].

Other bacterial causes include: *Klebsiella*, *Proteus*, *Pseudomonas*, and *Enterobacter*. These are uncommon and typically related to abnormalities of the urinary system or urinary catheterization [19].

The percentage of *Staphylococcus saprophytic* was (1.90%) agreed with study performed by Nicolle [15]. Who found that the *Staphylococcus saprophytic* is cause of 5-10% of urinary tract infections cases in his study. Research funded by the National Institutes of Health (NIH) suggests that one factor behind recurrent UTIs may be the ability of bacteria to attach to cells lining the urinary tract. One NIH-funded study found that bacteria formed a protective film on the inner lining of the bladder in mice [20]. If a similar process can be demonstrated in humans, the discovery may lead to new treatments to prevent recurrent UTIs. Another line of research has indicated that women who are “nonsecretors” of certain blood group antigens may be more prone to recurrent UTIs because the cells lining the vagina and urethra may allow bacteria to attach more easily. A nonsecretor is a person with an A, B, or AB blood type who does not secrete the normal antigens for that blood type in bodily fluids, such as fluids that line the bladder wall [21]. The majority of bacterial isolates show resistant to more than one antibiotics. *E. coli* and *Klebsella* show resistant to amoxicillin, nalidixic acid and nitrofurantion. While *Proteus* show resistance to nitrofurantion, amoxicillin and erythromycin and *Staphylococcus saprophytic* show resistance to amoxicillin and erythromycin (table 2). One the other hand, cefotaxime and cephalexin antibiotics show good activity toward all bacterial isolates. This resistant related with the widely used of these antibiotic [22]. In addition, the development of the bacterial resistant due

to change in the site of antibiotic activity and bacterial membrane permeability or may be enzymatic resistant [23, 24]. Cefotaxime was the most common antibiotics to which the organisms were sensitive to pregnant women without urinary tract infection followed by cephalexin, and lastly erythromycin. The cause of high bacterial resistant to the used antibiotics was the widely used of these antibiotics [22].

The results of present study demonstrated that *Calvatia craniiformis* extract was relatively effective as antibacterial agent different bacterial types, according to the decrease in MIC and BCC. This may be related with compounds of *Calvatia craniiformis*. Chung et al., [25]. Who showed that tannins substance have inhibitor effect against *Staphylococcus aureus* and *E.coli*. Since many plant phenolics have been found to be responsible for several biological properties, including antimicrobial properties [26, 27, 28]. It was expected that the antimicrobial activity of this plant species would be related to its phenolic compounds. *Calvatia craniiformis* contain Gallic acid its one type of phenolic compound. Also the *Calvatia craniiformis* contains the carbohydrate polymers known as  $\beta$ -glucans exert potent effects on the immune system usually stimulating antitumour and antimicrobial activity by binding to receptors on macrophages and other white blood cells and activating them. Although glucans are known to bind to receptors, such as complement receptor 3 [29]. Other compounds such as Ergothioncine has antioxidant activity it's maintain human cell [30]. In conclusion. Urinary tract infection in pregnant women are considered the most serious health problems facing the world, the *E.coli* bacterium is the main bacteria that cause UTI in pregnant women. Fungal extract was effective against selected isolate and can be used for treatment. Bacteria was the most sensitive to the action of fungal solution are *Klebsella* and *Staphylococcus saprophytic* while the *Proteus* and *E. coli* was the lest sensitive to it.

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