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Journal Name:	International Journal of Pathogen Research
Manuscript Number:	Ms_IJPR_42368
Title of the Manuscript:	Antimicrobial Efficacy of Chromolaena odorata Extract against Bacteria Isolated from Patients with Urinary Tract Infection in Ondo State, Nigeria
Type of the Article	Original Research Article

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound. To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

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PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Compulsory REVISION comments	Six hundred mid stream urine samples were analyzed for the presence the infection. Though the infection is more common in females due to their reproductive physiology, I would like to know the condition of females involved in the study? In case of females were they pregnant or not as the prevalence of the infection is high during pregnancy? Did the volunteers involved in the study have a past history of UTI? Were any of the patients involved in the study diabetic as UTI is also associated with diabetics? Was the infection symptomatic or asymptomatic? Were the patients asked to com for a follow up to understand the condition of the infection? Did the author come across any patient with pyuric condition (pus cells in urine)? Did these have a past history of UTI or were they diabetic? Apart from plate technique, did the author perform any other biochemical test to confirm the identity of the organism? Did the author perform any test to comprehend the biofilm forming ability of the organism if there were any as biofilm plays a vital role in causing the infection and majority of pathogens associated with UTI are biofilm producers? The study has highlighted the significance of a plant extract in inhibiting the pathogen. Did the study make any attempts to find out the various components present in the extract through any chromatography technique and reveal the exact component with anti microbial activity? The author has mentioned the MIC ranging from 5 to 50 mg/ml. I would like to know if any tests were performed to find out the maximum growth of the organisms [I mean at which hour or after how many hours the organism has reached its maximum density (is it after 12th hour or after 18 or 24 hrs) and I would like to know if the plant extract was able to inhibit the organism at its best hour of growth, when the density was maximum)? The author claims that the methanol extracts of the plant component was more effective than the chloroform extracts. Can the author site any specific reason for this? Does methanol enhances the efficacy of the plant extract or does any active component of the plant extract gets activated in presence of methanol which enhances the antimicrobial nature of the extract?	The patients were suspected to be symptomatic and showing symptoms of the infection. A few of the volunteers were pregnant but it was not recorded during the study. The Volunteers involved in the study showed some symptoms of UTI. However, UTI patients tend to be diabetic but none was established during the study. The patients were symptomatic. Hospital follow up report was not reported during the study. Some of the samples collected were seen to contain white blood cells or pus. But however, it was not established whether they had a past history of being infected with UTI. Apart from the plate technique method, other biochemical test to conform the identity of the organisms was performed. They include catalase, coagulase, spore, motility, oxidase, indole, methylred, urease and sugar fermentation test were performed to identity the organisms. Biofilm forming ability of the organisms were not performed or established. The various components present in the extract were not determined. Qualitative analyses of phytochemicals present in the extract were only performed. The MIC ranged from 5mg/ml to 50 mg/ml. All the broth cultures of the organisms were incubated for growth for 24hrs. After incubation, the tubes containing the broth were observed for growth. This could be because the active ingredients of the plant extract such as



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	In the result section the author discloses that the infection is nosocomial and is associated with hospitals as 42 positive cases are found in one of the hospital. What could be the reason for this? Does the author feel that hygienic aspects maintained at the hospitals are responsible for this extent of prevalence or is it because of resistance gained by the organisms towards commonly used antimicrobial drugs? Table 1 in the result section depict the locations and the extent of positive cases Does the author mean to say that UTI is endemic and it solely depends up on the geographical location and the ability of the pathogen in that location to confer the infection? Table 2 in the result section displays the different age groups and the extent of infection in these patients? Can it be generalized that the age of a person is directly related to the prevalence of the infection? The isolates like <i>E. coli</i>, <i>K. pneumonia</i> and <i>S. aureus</i> have been isolated from patients from 51 to 60 which confirm this. <i>S. aureus</i> is also known for compromising an individual's immune system. So is it right to come to a conclusion that age factor is vital in the prevalence of the infection? I would like to get the author's point of view. But as the age progresses the amount of isolates have reduced? Can the author provide his/her point of view on this aspect? Table 5 in the result section shows the antibiotic resistance pattern exhibited by different isolates of gram positive bacteria and it is obvious that erythromycin is better when compared to other agents as the percentage if resistance was low. Which method was followed by the author? Was it disc method or well method? What was the concentration of the antibiotics? Did that author try to mix up the antibiotic with the plant extract and check it efficacy on the microorganism? It is clear from the tables 5 and 6 that Gram positive isolates have shown a better degree of resistance towards antimicrobial agents when compared to Gram negative bacteria? What could be the reason for this? Is it the genetics of the organism that makes it tough or are there any other factors responsible for this behavior of the organism? In the discussion section in the 6th line, the author is mentions about six hormones during pregnancy and their role in UTI. What are these six hormones? Is it six hormones or sex	tannins and flavonoids were better extracted with methanol than the other solvents used in the study. In some cases, secondary metabolite or active ingredients could be activated. Moreover, these metabolites inhibit the growth of organisms by binding the bacterial cell wall and inhibit its biosynthesis, thereby enhancing the antimicrobial nature of the extract. 42 cases of UTI were found in a particular location in this study. This may be due to factors such as promiscuity, pregnancy, poor hygiene, profession, habits and low socio-economic status which are common among Nigerian young men and women leaving in rural or urban centers. And moreover, there are large geographical variations in the patterns of bacterial resistance properties depending on local practices of antimicrobial prescriptions. This resistance could vary from hospital to hospital settings. Antibiotics are routinely and largely used for the treatment of UTIs but however, poor hygienic practices, misuse, overuse and uncontrolled usage of antimicrobial agents may increase the risk of antimicrobial resistance to pathogens and hence increase the prevalence of the infection in particular hospital settings. UTI is commonly or regularly found among human populations living in a geographical area without any external inputs, therefore UTI could be endemic. But however for an infection to be endemic, the organisms in such area must have the ability to induce infections. In the study, Table 2 displayed the prevalence of UTI in Male and Female across different age groups. It could be suggested that the age of the patients were directly proportional to the prevalence of the infection. Often times, it has being reported that at age groups 21-30, 31-40, an individual tends to be sexually active. Sexually activities coupled with peer group influence and promiscuity are also factors that can incline the prevalence of UTI. Also in the study, it was observed that the prevalence declined in middle age group 41-50 but increased in age group 51-60. Risk factors such as diabetics coupled with poor glycemic control, poor history of urogynecologic surgery, urinary retention and urinary incontinence have been suggested to be factors responsible for UTI with these age groups. From this study, it can be concluded that age factor is vital in the prevalence of the infection. Disc method was used. The concentration of the antibiotics used are ; nitrofurantoin (300µg), gentamycin (10µg), cefuroxime (30µg), erythromycin (10µg), ciprofloxacin (5µg), streptomycin (10µg), ampicillin (30µg), amoxicillin (30µg), cotrimoxazole (25µg). A few of the resistant isolates were exposed to the leaf extract and the results are shown in Table 7. External factors such as misuse overuse and uncontrolled usage of antimicrobial agents in some hospital settings could be responsible for this
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	hormones? Clarify?	behavior of the organism. The genetics of these organisms may also be responsible for their resistance to antimicrobial agents. It is sex hormones. Sex hormones are a chemical substance that are produced in the ovaries, testes or adrenal cortex and affects the growth of the reproductive organs of the development of secondary sex characteristics. They also tend to affect sexual development or reproduction.
<u>Minor</u> REVISION comments		
<u>Optional/General</u> comments		