



Peer-Review Report Form

Manuscript Title:	Phytochemical Screening and Antibacterial Activity of Fresh Leaf Extracts of Selected Philippine Medicinal Plants Against <i>Escherichia coli</i>
Type of Article:	Original Article

FIRST ROUND

Editor

<u>General Comments and Recommendations</u>
The reviewers acknowledge the significance of your manuscript and recommend several revisions to further improve its clarity, coherence, organization, and overall scholarly quality. In addition, you are advised to thoroughly review and validate all references included in the manuscript. Please ensure that all cited sources are accurate, accessible, and retrievable through Google Scholar to uphold the credibility and reliability of the study. Additionally, make sure to include the DOI or website link for every reference to facilitate verification and accessibility of sources. Furthermore, only sources that are cited within the manuscript should appear in the References section, and all entries listed in the References section must also be cited in the text. For example, if 20 unique sources are cited in the manuscript, then only those 20 sources should be included in the References section. Please note that repeated citations of the same source within the text are counted as a single reference entry; therefore, the total number of references may be lower than the total number of in-text citations.

Reviewer 1

<u>General Comments and Recommendations</u>
The manuscript presents an evaluation of five medicinal plants. The attempt to evaluate fresh leaf extracts is commendable, but the experimental design and statistical analyses possess flaws. The choice to extract with cold tap water guarantees an extremely low phytochemical yield, which explains the absolute zero activity in four of five plants. The statistical treatment of data with zero variance completely invalidates the ANOVA.

<u>Specific Comments and Recommendations</u>
Title: The title is acceptable but uses a noun derived from a verb in the original text. It reflects the content adequately.
Abstract: The abstract states aratilis and cefuroxime belong to the same statistical group. This conclusion is mathematically absurd. You absolutely cannot categorize a positive control that exhibits profound inhibition with a crude extract that barely deters bacterial proliferation.
Introduction: The prose lacks focus. The text jumps sporadically from global statistics to local lore without logical transitions. There are unacceptable typographical errors, such as 'cast



diversity' instead of vast diversity, and 'out healthcare' instead of our healthcare. The rationale for cold water extraction is practical for rural areas, but the authors fail to establish how this relates to strict pharmacological standards.

Methodology: This section contains severe structural deficits. The extraction yields are abysmal, such as 0.01 ml/g for banaba. This low yield is the direct result of manual maceration with cold tap water. Cold water is a demonstrably poor solvent for alkaloids and flavonoids. Furthermore, the experimental design compares these weak aqueous extracts to cefuroxime, a purified antibiotic. This comparison is inappropriate and highly unbalanced.

Results and Statistics: The statistical approach is completely invalid. Five of the seven treatments show exactly zero variance. ANOVA assumes homogeneity of variance. When researchers apply ANOVA to data where most groups have a standard deviation of zero, the F statistic becomes void of value. Duncan Multiple Range Test categorized cefuroxime and aratilis in group 'a' despite their massive numerical differences. A radius of 1.49 mm is statistically distinct from 7.59 mm.

Discussion and Conclusion: The authors admit the zero variance problem but proceed to interpret the flawed statistical categories anyway. They claim sustained antibacterial activity for aratilis, yet an inhibition zone of 1.49 mm at 24 hours is barely visible and borderline negligible. The discussion attributes activity to flavonoids and tannins, but cold water extraction barely pulls these compounds from the cellular matrix.

Please indicate your recommendation by checking the appropriate box below.

<u>Decision</u>	
	Accept the manuscript for publication.
/	Reconsider the manuscript after the authors have satisfactorily addressed and complied with the reviewers' comments and recommendations.
	Reject the manuscript, as it is not suitable for publication.

Reviewer 2

<u>General Comments and Recommendations</u>
Overall, I think the manuscript may still have value as a preliminary laboratory study, but the authors first need to establish that the observed antibacterial effect came from a reproducible and properly documented experiment. At present, several missing information in the assay procedure, bacterial strain, experimental replication, and preparation of the fresh extracts make the central finding difficult to independently verify.
<u>Specific Comments and Recommendations</u>
The antibacterial assay is not sufficiently standardized and reproducible. My biggest concern is that important experimental details are missing. The manuscript states that 6-mm filter paper discs were used, but it does not report how many microliters of each plant extract were loaded per disc, the actual concentration/dose represented by each disc, or the potency/concentration of cefuroxime used. Without these information, another researcher cannot reproduce the experiment. More importantly, the manuscript measures the radius extending beyond the disc edge, rather than reporting the usual inhibition-zone diameter. This makes the reported values



difficult to compare with established disc-diffusion work. Standard reference disc-diffusion procedures require controlled inoculum, incubation conditions, interpretation, and quality-control procedures, while results are normally expressed using zone diameters. I think the authors need to clearly reconstruct the exact assay procedure and justify every departure from a standard method. If these details were not controlled during the actual experiment, this may require repeating the antibacterial assay.

The 24-, 48-, and 72-hour measurements need serious reconsideration. The manuscript appears to incubate the plates and repeatedly measure the inhibition zones up to 72 hours. This is problematic because disc-diffusion susceptibility testing is not normally a longitudinal assay where increasing zones over several days are interpreted as stronger antibacterial effect. Standardized disc-diffusion testing uses defined incubation conditions and reading periods; CLSI, for example, describes the standard method around controlled incubation and interpretation rather than following one plate for several days. If the authors really want to demonstrate a time-dependent antibacterial action, a more suitable experimental design should be used, such as independent plates for predetermined time points or another microbial growth/time-kill approach. The present design cannot automatically establish that an increase from 1.49 to 7.10 mm represents increasing biological antibacterial activity.

The organism tested does not adequately match the major clinical problem used to justify the study. The Introduction is strongly built around UTI, uropathogenic *E. coli*, and antibiotic resistance. However, the Methods only state that a "standard laboratory strain of *Escherichia coli*" was used, without giving the strain number, source identification, susceptibility profile, or evidence that it is uropathogenic or antibiotic-resistant. This creates an important disconnect between the rationale and the experiment. Activity against an unspecified laboratory *E. coli* cannot directly support a claim concerning UTI-causing or resistant *E. coli*. The authors should provide the exact strain identification and substantially narrow the UTI/antimicrobial-resistance interpretation. Ideally, future validation should include characterized UPEC or relevant clinical isolates if the clinical framing will be retained.

The true experimental unit and replication are unclear. The Methods indicate three separately prepared extracts for each plant, with three technical discs coming from each extract. This seems to provide three biological replicates and nine technical observations per plant. However, Table 3 suddenly reports only $n = 3$. The reader cannot know whether the three technical discs were averaged first for each biological replicate, whether only three discs were analyzed, or whether technical replicates were treated as independent observations. This is a major issue because technical repetitions are not the same as independent biological experiments. The authors should provide the raw replicate-level data, explain exactly how the values were averaged, identify what constitutes n , and show the experimental layout. Otherwise, it is difficult to evaluate reliability of the reported means and SDs.

The fresh plant materials and extracts are still not described enough for reproducibility. Plant authentication is mentioned and a certificate was reportedly obtained, which is good. However, the manuscript does not sufficiently provide the collection location, collection date/season, voucher specimen or accession number, time between harvesting and extraction, exact refrigeration temperature, storage duration before testing, or a standardized concentration of the resulting fresh extracts. These factors become particularly important when using fresh plant material because two researchers can macerate the same mass of leaves but obtain preparations with quite different chemical composition and concentration. A plant identity certificate alone is not enough for someone to repeat the study.

I did not find photographs or figures in the submitted manuscript. In this particular study, some visual documentation would be very useful. I suggest adding a figure showing the authenticated leaves of the five plant species actually used in the experiment, properly labelled. More



importantly, the authors should provide representative original photographs of the Mueller-Hinton plates showing the negative control, cefuroxime, and especially aratilis, with a visible measurement scale. If the 24-, 48-, and 72-hour findings will remain, representative images from these observation periods would also help readers judge the reported change in inhibition. These photographs should be original, clear, and minimally processed.

Please indicate your recommendation by checking the appropriate box below.

<u>Decision</u>	
	Accept the manuscript for publication.
/	Reconsider the manuscript after the authors have satisfactorily addressed and complied with the reviewers' comments and recommendations.
	Reject the manuscript, as it is not suitable for publication.

SECOND ROUND

<u>Decision</u>	<u>Editor</u>	<u>Reviewer</u> 1	<u>Reviewer</u> 2
Accept the manuscript for publication.	/	/	/
Reconsider the manuscript after the authors have satisfactorily addressed and complied with the reviewers' comments and recommendations.			
Reject the manuscript, as it is not suitable for publication.			