

Review of: "Antimicrobial Sensitivity of Plant Extracts of *Acacia arabica*, *Prosopis juliflora*, *Abutilon indicum*, and *Bryonia laciniata* on *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*"

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Potential competing interests: No potential competing interests to declare.

Abstract

Background: "In the recent era, biological treatment using therapeutic microbes or phytochemicals has proven more beneficial than conventional methods due to several reasons - permanent control of weeds, host-specific control, cost-effectiveness, and low health risk." It is not clear how controlling weeds has any connection with the antimicrobial activity of plant extracts, if you have used a species that is a weed, then better to describe that specifically.

In the Background and Methods sections there is a repetition, sort of overlapping. "This study determined the antimicrobial sensitivity profile of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* against plants like *Acacia arabica*, *Prosopis juliflora*, *Abutilon indicum*, and *Bryonia laciniata*." and "the primary purpose of this study was to determine the antimicrobial sensitivity of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* using extracts from plants like *Acacia arabica*, *Prosopis juliflora*, *Abutilon indicum*, and *Bryonia laciniata*. Antimicrobial properties of plant extracts were analyzed by determining the *Zone of Inhibition (ZOI)*". Better to resolve and avoid these repetitions.

In the methods section, the statement "The antibiogram pattern of isolated *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* was observed to be Susceptible, Intermediate, and Slightly Resistant to *Acacia arabica*, *Prosopis juliflora*, *Abutilon indicum*, and *Bryonia laciniata*." is not clear. It would be better to specify which microorganism is susceptible to which plant extract and so on. You shouldn't include the result or outcome in the Methods section but include this in the Results section.

Introduction

Since this article corresponds to the antimicrobial properties of plant extracts, it would be appropriate to remove details about cardiovascular diseases, cancer, and diabetes mellitus as they don't have any significant relationship with antimicrobial activity.

It is also more suitable to present 1.1. Vernacular Name and 1.2. Taxonomic Classification in 2 separate tables rather than sub-sections and merge 1.4. Phytochemical Constitution and 1.5. Antimicrobial Properties sections and discuss these details collectively under the titles of four species of medicinal plants (e.g. 1.1 *Acacia arabica*, 1.2 *Prosopis*

juliflora, 1.3 *Abutilon indicum*, and 1.4 *Bryonia laciniosa*). Even here, you should focus more on reported antimicrobial activity rather than other therapeutic effects of the plant species.

In section 1.5.b. *Prosopis juliflora*, the statement “The several alkaloid constituents present in *Prosopis juliflora* were assessed for their antibacterial property using the disc diffusion method on several Gram-negative and Gram-positive bacterial strains like *E. coli* and *Staphylococcus aureus*.” doesn't make any sense. It should be corrected as “The several alkaloid constituents present in *Prosopis juliflora* has been assessed for their antibacterial property using the disc diffusion method on *E. coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) bacteria.” In the follow-up, you should also report the figures of the above study to clarify the significance of the antimicrobial properties of the plant. Also please provide the reference corresponding to this information.

In section 1.5.c. *Abutilon indicum* “The seed oil of *Abutilon indicum* and *Abutilon muticum* demonstrated broad-spectrum activity as they were active against both Gram-positive and Gram-negative bacteria.” you should clarify which bacterial species specifically.

Further down, the statement “The highest larval mortality was found in the petroleum ether extract of *A. indicum*.” doesn't make sense at all. There's no cohesion with this sentence in that paragraph. Please be more descriptive and make sure your story is flawless.

In section 1.5.d. *Bryonia laciniosa*, the statement “The aqueous extract of the polysaccharide component isolated from the *B. laciniosa* leaf was examined for antibacterial activity against *Staphylococcus aureus*, *S. pyogenes*, *E. coli*, and *K. aerogenes* at dosages of 1.25 mg/ml, 3.12 mg/ml, 6.25 mg/ml, and 12.5 mg/ml.” is bit confusing. It is difficult to apprehend whether all bacterial species were susceptible to each concentration of plant extract or whether each bacterial species was susceptible to different concentrations. The sentence immediately after “The extract showed antibacterial activity against *E. coli* at a minimum dosage of 6.25 mg/ml.” makes it even more confusing to understand. Please resolve these issues before submitting the article for publication.

Material and Methods

In section 2.1 Collection of Plant Samples, please incorporate the details about the criteria for collecting plant samples (e.g. do the collected plants were in the same maturity levels or different, why you selected the specified geographical area, how you identified each plant species, etc.).

In section 2.2 Preparation of Plant Extracts, please mention the drying/atmospheric conditions. Also, the equipment used for pulverizing and filtration of the samples should be mentioned. Did you use manual homogenization? If so it should be clearly mentioned. Under which conditions did you store the extracts? These details are very important in your method section and must be included.

In the same section, the statement “A weighed quantity of 50 mg/ml of each species was taken” doesn't make any sense as you can't weigh 50 mg/mL, which is a concentration. May need to correct.

In section 2.3 Isolation and Identification of Bacterial Samples, you should provide identification details of the bacterial cultures (e.g. ATCC number).

In section 2.4, you should have stated the reason why the plate diffusion assay method was preferred over the diffusion method because you have referenced a report which favors the later method.

Results

In section 3.2, you mentioned that “A zone of inhibition greater than 4 mm in diameter was considered to have significant activity against a particular bacterium.” In the methods section, you have mentioned the criteria for measuring ZOI. Accordingly, the diameter of ZOI needed to be considered as significant activity is 8 mm (the diameter of the well itself is 6 mm). Therefore the following statement “Both the aqueous and ethanolic extracts showed significant antimicrobial activity against all three bacterial species.” is not an appropriate claim at all.

You haven’t clearly mentioned how many replicates and repeats you have done which is a very important factor in establishing your claims. According to the available results, it is evident that no replicates nor any repeats were done, which questions the available results. When closely observed, the “3.3 Portrayal Representation of ZOI on Nutrient Agar Plates” it was evident that the plates were not properly labeled at all. Therefore, the readers can’t easily follow the description given in the methods section. With regards to the controls, ethanol treatment in the control plates should be the positive control and water has to be the negative control. Ethanol is generally used as a sanitizing agent. Thus, it is not a suitable candidate to be used for positive control. Instead, you should have used a current antimicrobial agent such as Amoxicillin, Gentamycin, Penicillin, etc. On the other hand, you should also mention the concentration of ethanol used in the assay (e.g. 100%, 50%, or 25%). Another issue is that, you have tested only a single concentration of plant extracts (50 mg/mL). In order to have a better understanding of the antimicrobial activity of the plant extracts, you should use at least 3 different concentrations of each extract.

It is confusing how the headings of the figures and sections were labeled. The sections, 3.3 Portrayal Representation of ZOI on Nutrient Agar Plates and 3.4. Graphical Representation of ZOI on Nutrient Agar Plates, should have been represented as figures.

Discussion

For a better comparison, it would be beneficial to state the concentrations of different plant extracts applied by the investigators in the studies corresponding to references 36 and 43. It is clear that the results of the other studies (references 41 and 42) are far superior (e.g. larger inhibition zones for much lower concentration of plant extracts applied) than the results reported in the current study. In addition, they have evaluated more bacterial species and have tested different concentrations of plant extracts than the current study. The results of the current study has been discussed barely compared to the results of the other studies.

There’s no significant evidence in the results to support the following statement “In the present study, it has been observed that the extracts of *Acacia arabica*, *Prosopis juliflora*, *Abutilon indicum*, and *Bryonia laciniosa* demonstrate

impressive antimicrobial activity when compared with the ethanol and water controls.”

The authors also state that “This investigation confirms that *Acacia arabica* leaves extract exhibits remarkable antimicrobial susceptibility towards all three bacterial strains, while relatively lower inhibitory activity was recorded by *Acacia arabica* seed extract. Based on the results, it is indicated that the extracts of *Acacia arabica*, *Prosopis juliflora*, *Abutilon indicum*, and *Bryonia laciniola* have the potential to be used in the management of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* related diseases.” Considering the (1) depth and breadth of the experiments carried out, (2) lack of evidence to confirm the results with sufficient repeats, and (3) the available results presented in the article, doesn’t support the claims stated in the discussion and conclusion sections at all. Just having a <5 mm inhibition zone doesn’t really mean that the plant extract has superior inhibitory activity or is suitable to be used for drug development. The antimicrobial activity has to be thoroughly investigated on a range of microbial species at different concentrations before arriving at claims or declaring the extracts as superior antimicrobial substances. It is also extremely important to confirm the results by repeats, otherwise, you can’t consolidate your results, or the claims based on them.

Overall

Although, there are 2 tables (Table 2.3 and 3.4) and a single figure (Figure 2.2), only Table 3.4 has been referenced in the text. Others have been left stand-alone without incorporating them into context. When you include a table or figure, you need to reference it properly in the text so that the reader can easily follow. On the other hand, the tables and figures in the manuscript should have been labeled as Table 1, Table 2, and Figure 1. However, there are more figures and tables presented in this manuscript that need to be properly presented. Please refer to the comments regarding sections 1.1 and 1.2 under the introduction and comments regarding sections 3.3 and 3.4 under the results.

When you use an abbreviation, it should be introduced at the first instance and thereafter you can use the abbreviation throughout the text. In this manuscript, some abbreviations have been used without proper introduction. Moreover, there is no abbreviations section included in the manuscript.

Summary

In my opinion, a lot has to be improved with this manuscript. The range of methods/results discussed is very narrow. As a suggestion, this manuscript could be improved by incorporating more dimensions to the results (i.e. Minimum Inhibitory Concentration, screening more bacterial strains) of this study.