

The paper Ms_EJMP_46487 is very preliminary but the data is impressive. As the multidrug-resistant bacteria is spreading and most antibiotics are becoming useless, I argue the paper must be accepted for propaganda of utility of the plant. The apple like fruit is edible and thus leaves must be non-toxic. The author may try in molly fishes or rats to check the toxicity. English is very poor and no activity on literature search to demonstrate the antibiotic void. Please accept the paper only after extensive revision. Few experiments must be repeated. If the author has isolated the bacteria, then source must be given and 16S rRNA sequencing and GenBank accession no should be mentioned. Data with 100, 200, 400, 600 mg extract sometime look same or negative value with increasing concentration. How that could be possible? So lower values of extract concentrations must be assayed??? MIC determination is little bit hard so either you give long data with graph or omit it. Determination of chemicals needs expertise and details addition of reagents and substance must be recorded so that other can do similar experiment. Author can do TLC and cut the colour band and UV shadowed white band from TLC and recover in ethanol by centrifugation. Then assay the individual fraction to give a idea of drug commercialization as herbal drug as well as comparable allopathic drug. That way you can authenticate the utility of plant extract.

Specific suggestions are:

Title

1. The Title "ANTI-GASTRO-INTESTINAL BACTERIA OF CHRYSOPHYLLUM ALBIDUM LEAF EXTRACT" is incomplete. The title may be written as " Anti- bacterial effect of Chrysophyllum albidum phyto extract"
2. chrysophyllum albidum must be Chrysophyllum albidum.

Summary

3. Remove or rewrite. "by the in vitro cup-plate method of agar diffusion technique with concentration of about 10^{-5} cells/ml of the selected bacteria" . "100 μ l over night culture " may be written.
3. Remove or rewrite. "if not for anything but its availability and non toxicity for human consumption".
4. In Introduction, " sapoyaceae family" may be corrected to "Sapotaceae family"
5. Write in two sentences. "In an effort to expand the spectrum of antibacterial agents from natural resources, *Chrysophyllum albidum* has been selected for study .The specific objectives of this study are as to determine the antimicrobial activity of *chrysophyllum albidum* leaves on five selected Gastro-intestinal bacteria, to investigate the phytochemical constituents present in *Chrysophyllum albidum* leaves, to determine the effectiveness of the ethanolic, methanolic and aqueous leaves extract of *Chrysophyllum albidum* on five selected Gastro-intestinal organisms; namely; *Salmonella*

typhimurium, *Shigella dysenteriae*, *Clostridium perfringens*, *Vibrio cholera* and *Escherichia coli* and compare them with tetracycline and metronidazole; so as to validate and justify the rationale behind the use of the leaves of *Chrysophyllum albidum* by local traditional health service provider for the treatment of stomach-ache and diarrhoea.”

As for example, “ Due to wide use in ayurvedic medicine in Africa, we design to study the antibacterial potential of *Chrysophyllum albidum* leaves extract.”

Methods

6. (0.45µ/m) may be corrected to (0.45µm)

7. “Mueller Hinton agar was prepared for the test according to manufacturer prescription. Test organisms were first subculture and incubated for 24hrs”.

Remove “subculture” and 24hrs must be 18hrs or over night.

8. “Simultaneously, tetracycline (30mg) and metronidazole (30mg) were used as positive control.” Please correct as 30 µg or write 30µl of stock antibiotics (30mg/ml).

Result

9. The *Ethanolic*, methanolic and aqueous extracts are dark green, light green and brown respectively. Ethanollic = ethanolic

10. Steroid or alkaloid????? Give the method of assay in details

11. In 3.2.1 if 100mg/ml extract gave 31mm diameter zone, then why you used other concentration. Show the original result with 20, 50, 100 mg as well as control tetracycline 20, 50, 100 mg. Give zone formation data side by side. Use mobile phone to record original data, gmailed to computer, fix with word, then assemble with Paint and transfer into original text file.

12. Determine MIC carefully and give graphical representation only. Give 100mg data only. This world shaped in nanogram. So if not inhibited at 100mg, then it is not a inhibitor?????

13. Omit 3.2.4 table. Water is not inhibitory is known by all????? Write in one line in the below of the table 3.2..3.

Discussion

14. “The methanolic extract showed the following zones of inhibition; for the *Salmonella typhimurium* the maximum inhibition concentration is 35.5mm at 500mg while the minimum inhibition concentration is 33mm at both 200mg and 400mg;”

200mg and 400mg are huge difference , how be same 33mm zone. I hope you have not done any experiment. With any plant extract getting 30-40mm zone is a dream to any scientist. If you did, then I should do the experiment to develop the drug.

15. “The antibiotics used showed that the following zones of inhibition; for tetracycline, the maximum inhibition is 39.5mm on *Shigella dysenteriae* at 30mg; while the minimum inhibition concentration was 33mm on *Escherichia coli* at 30mg; for Metronidazole the maximum inhibition is 34.5mm on

Escherichia coli at 30mg while the minimum inhibition concentration was 28mm on *Vibrio cholerae* at 30mg.”

Repeat the data with more dilution. What is your hole size and how you determine diameter.

16. In method or discussion, you have not mentioned how you got the bacteria. Please give accession nos of 16S rRNA sequencing data. Or you have to give ATCC no and write how you collected.

Reference

17. You have searched literature

Add the 20 more references below and discuss multi-resistance and need for phyto-extract according to ancient Indian and Chinese Civilization?

Sun W, Weingarten RA, Xu M, Southall N et al. (2016), Rapid antimicrobial susceptibility test for identification of new therapeutics and drug combinations against multidrug-resistant bacteria. *Emerg Microbes Infect.* 5(11): e116.

Sahoo N, Manchikanti P, Dey SH, 2011. Herbal drug patenting in India: IP potential. *J. Ethnopharmacol.* 137:289-297.

Chakraborty AK, Poria K, Saha D, Halder V, Das S, Nandi SK. (2018) Multidrug-Resistant Bacteria with Diversified MDR Genes in Kolkata Water : Ganga Action Plan and Heterogeneous Phyto-Antibiotics Tackling Superbug Spread in India, *American J Drug Deli Ther.* 5: 1-9.

Cowan MM (1999) Plant products as antimicrobial agents. *Clinical Microbiology Reviews.* 12: 564 – 582.

Swaminathan S, Sundaramurthi JC, Palaniappan AN, Narayanan S. (2016) Recent developments in genomics, bioinformatics and drug discovery to combat emerging drug-resistant tuberculosis. *Tuberculosis (Edinb).* 101:31-40.

Chakraborty, AK (2015). High mode contamination of multi-drug resistant bacteria in Kolkata: mechanism of gene activation and remedy by heterogenous phyto-antibiotics. *Indian J Biotechnol.* 14, 149-159.

Medina E, Pieper DH (2017) Tackling threats and future problems of Multidrug-Resistant bacteria. *Curr Top Microbiol Immunol.* 398: 3-33. doi: 10.1007/82_2016_492.

Xu ZQ, Flavin MT and Flavin J. (2014). Combating multi-drug resistant gram-negative bacterial infection. *Exp Opin Invest Drugs.* 23: 163-182.

Chakraborty AK (2017). Enzybiotics, a new class of antimicrobials targeted against multidrug-resistant superbugs. *Nov Appo Drug Des Dev.* 2(4): 555592.