e-ISSN 2320 –7876 www.ijfans.org
Vol.11, Iss.11, Nov. 2022

ANTIMICROBIAL ACTIVITY OF DAEDALEOPSIS FLAVIDA AGAINST FOUR HUMAN PATHOGENIC MICROORGANISMS.

Prasad Y. Lamrood[1], Sagar S. Bawake[1] and Rajendra G. Choure[2]

[1]Department of Botany, Ahmednagar College (Affiliated to Savitribai Phule Pune University), Ahmednagar – 414001
[2]Department of Microbiology, Institute of Science,(Lead Institute of Dr. Homi Bhabha State University, Mumbai) Mumbai-400032
Email :prasadlamrood@gmail.com,

Abstract
The antibacterial activity of acetone, methanol, and petroleum ether extract of *Daedaleopsisflavida* was investigated against four human pathogenic bacteria: *Escherichia coli* (ATCC:8739), *Pseudomonas aeruginosa* (ATCC:9027), *Bacillus subtilis* (ATCC:6051) and *Salmonella enterica* (ATCC:14028). Minimal inhibitory concentration (MIC) was evaluated for each extract. Antibacterial activity was performed using agar well diffusion assay. This is the first report investigating the antibacterial activity of this mushroom. Petroleum ether and methanol extract significantly inhibited *E. coli* and *P. aeruginosa* as compared to the control (Streptomycin) while inhibition of *S. enterica* and *B. subtilis* was more or less the same for petroleum ether while methanol extract exhibited lesser activity. Acetone extract, however, exhibited more significant activity as compared to control, petroleum ether and methanol extract. Significant inhibitory activity was observed against *P. aeruginosa* (23.67± 0.58 mm) followed by *E. coli* (19.33 ± 1.15 mm), *B. subtilis* (18.00 ± 1.00 mm) and comparatively lower activity against *S. enterica* (15.33 ± 0.58 mm). Further investigation of acetone extract would reveal the bioactive compound.
Keywords: Antibacterial activity, *Daedaleopsisflavida*, Human Pathogenic bacteria, Snuff Fungus

Introduction

Overuse and misuse of antibiotics have led to the development of drug resistance by many human pathogenic bacteria not only in the clinical field but also in the veterinary and agricultural fields as well (Clericuzio et al., 2021). Bacteria can modify or inactivate the drug, modify the targets or binding sites or even alternate the cell permeability (Fair and Tor, 2014).

Furthermore, other survival strategies of the bacteria include the formation of biofilm covered with an exopolysaccharide layer that protects against antibiotics, and immune system activity through bacterial communication via signalling (Sharma et al., 2019). These strategies have led to the development of antimicrobial resistance which is currently one of the most serious threats to public health worldwide (Peterson and Kaur, 2018). It is estimated that resistant bacteria cause about 60,000 deaths per year of which 33,000 are in the EU/EEA Countries, and 29,500 are in the United State and by 2050, 2.5 million people will die due to infection caused by drug-resistant bacteria (Clericuzio et al., 2021).

To overcome the problem of antimicrobial resistance, World Health Organization (WHO) released a Global Action Plan in 2015 (Clericuzio et al., 2021) which was updated in 2019 focusing on the clinical development channel for antibacterial compounds (Morel et al., 2021).

These circumstances have led researchers to search for new and effective antimicrobial compounds against pathogenic microorganisms (Alves et al., 2012). Amongst the natural resources that have been exploited, the mushroom could serve as a potential source of new antimicrobials.

Mushrooms are well known not only for their test and nutritional values but also for their medicinal values (Ragupathi et al., 2018). Various secondary metabolites produced by macromycetes as a survival strategy in an adverse environment, exhibit potent antimicrobial activity. Suay et al. (2000) reported antimicrobial activity of 317 isolates belonging to 204 species of which 109 showed significant activity. While Ranadive et al. (2013) compiled information on the antimicrobial activity of 316 species belonging to 150 genera (45 Basidiomycetous, 21 Ascomycetous fungi (of which 6 were lichenized, 15 non-lichenized and 3 *Incertaesedis*). This indicates the antimicrobial potential of macromycetes.

According to an estimate, out of 140 000 mushroom species, only 22 000 (5%) are known and have been investigated. This presents a much wider scope to explore mushroom properties and potential application as an antimicrobial agent (Alves et al., 2012)

D.flavida (Lev.) Roy & Mitra is commonly called 'snuff fungus' in some places of western Maharashtra (Vaidya and Rabba, 1993). It is a saprobic, inedible fungus belonging to the Polyporaceae family (Hossena et al., 2021). In recent years, researchers have been investigating the potential of *D.confragosa* for its nutritional and medicinal potential, including antimicrobial activity (Chandrawanshi et al., 2018), the work on D.flavida remains scanty or missing.

The present study aims to investigate the antibacterial activity of acetone, methanol and petroleum ether extract against four human pathogenic bacteria - *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Salmonella enterica*.

Materials and Methods

Collection and Identification

Fresh basidiocarps of *D.flavida* were collected from Udadawane village of Akole Taluka, Ahmednagar which were growing on coppiced wood. The specimens were brought to the laboratory, dried in shade, and powdered by grinding. A part of the sample was preserved for the macro- and microscopic study.

Micro-morphological characters of the basidiocarp were studied by taking freehand thin sections of the fruiting body. Sections were stained using the method of Martin (1934). Semipermanent slides were prepared using lactoglycerin with 1% cotton blue and were sealed with nail polish (Foroutan and Vaidya, 2007). Identification was done using the key suggested by Rathod (2011).

Preparation of Extracts

Finely ground powder (10 g) of the mushroom was extracted using acetone (A), methanol (M), and petroleum ether (PE) for 24 h. at room temperature in a sealed Erlenmeyer flask separately. The extracts were filtered and transferred to a clean Petri plate and the solvent was allowed to evaporate at room temperature. The fine powder of extract thus, obtained was weighed and kept at 4ºC in an airtight container until use (Balakumar et al., 2011). The extracts were dissolved in 5% dimethyl sulphoxide (DMSO).

Isolation and maintenance of bacterial strains

Human pathogenic strains of *E. coli* (ATCC:8739), *P.aeruginosa* (ATCC:9027), *B. subtilis* (ATCC:6051) and *S. enterica* (ATCC:14028) were obtained from the Microbial Type Culture Collection (MTCC), The Institute of microbial Technology. Chandigarh, India. Cultures were stabilized by incubating in BOD incubator(Tempo TI – 90 F S/G, Tempo Instruments Pvt. Ltd., Mumbai) at 27ºC and were revived and maintained on Mueller Hinton Agar medium (MHA) (HiMedia Laboratories, Thane (W), India) by periodic subculturing.

Antibacterial activity

The antibacterial activity of different extracts was determined using agar well diffusion method (Avci et al., 2014). Bacterial cultures were grown at 37ºC for 24 h first on nutrient broth and then on MHA. Suspension of culture was made in saline and the density was adjusted to the 0.5 McFarland standard,~10^8 CFU/ml. (Kosanic et al., 2013). The

suspensions (10 µl.) were inoculated separately on Petri plates containing MHA (10 ml.) to form an even density lawn. 100 µl of extracts dissolved previously in DMSO was transferred to a 6 mm agar well. An equal amount of DMSO was used as the negative control and streptomycin(0.1% w/v) as the positive control (C) (Kosanic et al., 2013). All plates were kept in the refrigerator for 1/2 hr. to allow proper diffusion and then incubated at 37ºC for 24 h. After the incubation was over, the zone of growth inhibition formed surrounding each well was observed and the diameter (in mm) of each zone was recorded. All experiments were performed in triplicate.

Minimum Inhibitory Concentration (MIC)

The MIC of only acetone extract was determined (since it showed maximum antibacterial activity as compared to other extracts) by the agar plate method (NCCLS, 1998). A series of dilutions with concentrations ranging from 0.1µg to1.0 µg/ml for extracts were used against each bacteria tested. A starting solution was obtained by dissolving a certain quantity of extract in DMSO to afford a concentration of 1mg/ml. Two-fold dilutions of extract were prepared in MHA (Giri et al., 2012). 10µL of each bacterial suspension, equivalent to 0.5 McFarland standard to approximately 10^8 CFU/ml. was streaked onto MHA and 10µL solution of each concentration of the acetone extract was added separately into a well punched. The plates were then incubated at 37ºC for 24h. (Asri et al., 2019). MIC was determined as the lowest concentration (i.e. highest dilution) of the extract that showed no visible growth of the test bacteria (Nwachukwu and Uzoeto, 2011, Kosanic et al., 2013)

Statistical analysis

Statistical analysis was performed using R 4.2.2 (https://www.r-project.org). All tests were expressed as mean ± SD. The significance of the antibacterial activity of mushroom extracts was determined against the control using Kruskal Test, followed by Dunnet's t-test (2-sided) and Dunken Multiple range test. A Wilcox test was performed for pairwise comparison followed by Dunn's and Friedman's tests. Values of $p < 0.05$ were considered significant.

Results

Antibacterial Activity

The present study revealed the antibacterial activity of a lesser-known medicinal mushroom *D. flavida* against four human pathogenic bacteria. The results of antibacterial activity were presented in **table 1** and **fig 1**. It was revealed that acetone extract showed maximum activity as compared to petroleum ether and methanol extract against the test bacteria. Maximum (23.67± 0.58) inhibitory activity was reported against *P. aeruginosa* followed by *E. coli* (19.33 ± 1.15) and *B. subtilis* (18.00 ± 1.00). The acetone extract was ineffective against *S. enterica.* Petroleum Ether extract also showed maximum activity against *P. aeruginosa* (17. 33 ± 0.58) and *E. coli* (17.00 ± 1.00). Whereas, methanol extract was effective against *P. aeruginosa* (16.67 ± 0.58) only. Since acetone extract was effective as compared to methanol and petroleum ether extract, it was further evaluated for the determination of MIC.

Table 1: Antibacterial activity of different extract of *Daedaleopsisflavida* through determination of zone of inhibition.

Organism	Streptomycin[#]	Petroleum Ether	Methanol	Acetone
Salmonella enterica	15.67 ± 0.58[*]	14.33 ± 1.15	13.67 ± 0.58	15.33 ± 0.58
Escherichia coli	16.33 ± 0.58	17.00 ± 1.00	15.33 ± 1.15	19.33 ± 1.15
Bacillus subtilis	13.33 ± 0.58	13. 67 ± 0.58	12.67 ± 0.58	18.00 ± 1.00
Pseudomonas aeruginosa	14.33 ± 0.58	17. 33 ± 0.58	16.67 ± 0.58	23.67± 0.58

[#]Streptomycin used as a positive control

[*]The diameter of zone of inhibition was expressed in millimetres (mm) as mean ± standard deviation (SD) (n = 3).

A significant difference ($p<0.0000149$) in the activities was observed by Dunken Multiple range test. A significant difference in the activities of the solvents was also observed by Dunnet's Test($p<0.0001$) and Kruskal-Wallis test ($p<0.0008777$). A post-hoc Dunn test was carried out for pairwise comparison and multiple comparison *p*-values adjusted with the Holm method. The results are depicted in the following **table 2**.

Table 2: Kruskal-Wallis multiple comparison p-values

Comparison	Z	P. unadj	P. adj
A - C	3.3207330	0.0008978139	0.004489069
A - M	3.6888852	0.0002252388	0.001351433
C - M	0.3681522	0.7127597384	0.712759738
A - PE	2.4739829	0.0133616097	0.053446439
C - PE	-0.8467501	0.3971344296	0.794268859
M - PE	-1.2149023	0.2244033523	0.673210057

No significant difference was observed for Methanol, and Petroleum Ether. For the present investigation a large effect size was detected, eta2[H] = 0.308. Pairwise comparison using Dunn's test and Wilcoxon's test also revealed the significant effect of acetone extract with $p < 0.000898$ and $p < 0.001$ respectively as compared to streptomycin.

Furthermore, to gain confidence, the data was finally subjected to Friedman's test with pairwise comparison using the Durbin-Conover test. The results are presented in **fig 1**.

A pairwise comparison revealed that, *P. aeruginosa* showed more sensitivity against the acetone extract followed by *B.subtilis* and *E. coli*. *S.enterica* however, did not show any significant inhibition**(fig. 2)**.

The different MICs of acetone extract for each bacterium are represented in **table 3**.

Table 3: Minimum inhibitory concentrations determinations of acetone extract.

Organism	**MIC (µg/ml)**
S. enterica	0.4 ± 0.017
E. coli	0.4 ± 0.010
B. subtilis	0.8 ± 0.015
P. aeruginosa	0.1 ± 0.008

The results indicated that acetone extract was best to potentiate the antibacterial activity against *P. aeruginosa* with MIC 0.1 µg/ml. The MIC value was 0.4 µg/ml for both *S. enterica* and *E. coli*.MIC value was comparatively larger (0.8 µg/ml) for *B. subtilis*.

Discussion

This is the first report investigating the antibacterial activity of *D, flavida* against human pathogenic bacteria. The fungus is commonly found in the western ghats of Maharashtra and the western parts of the Ahmednagar district. *Daedaleopsis* spp. Belongs to the Polyporaceae family and are inedible owing to their hard woody texture (Shahtahmasebi et al., 2108). Amongst the species of *Daedaleopsis,D. confragosa* has been screened for medicinal properties such as antioxidant (Vidovic et al. 2011, Chandrawanshi et al., 2018) and antidiabetic activity using methanolic extract (Chandrawanshi et al., 2018).

The medicinal activity of the species was attributed to the presence of a large number of phenolic compounds (Shahtahmasebi et al., 2108, Audrey et al. 2020). Okwulehie et al. (2014) reported a comprehensive account of phytochemical, proximate, vitamins, minerals and heavy metals composition in *D. confragosa*. Yet another species, *D. tricolour* was reported to exhibit potential antifungal, antibacterial, and antioxidant properties (Zhao et al. 2013). Elkhateeb et al. (2019) reported the antiviral activity of an aqueous extract of mycelia against avian influenza and human influenza A viruses. Furthermore, total polysaccharide fractions fully suppressed the infectious activity of the West Nile virus as well as the inhibitory activities of the human immunodeficiency virus.

We observed significant anti-bacterial activity of Petroleum Ether, Methanol and Acetone extract against *E. coli* which is more than the activity reported by Fakoya et al. (2013) for *D. confragosa*Petroleum Ether (19.0 ± 0.02 mm) and aqueous extracts (12.0 ± 0.01 mm). They also reported the antibacterial activity against *S. aureus* and *P. vulgaris*. Culliao et al. (2020) reported inhibition of *E. coli* (Strain ATCC 25922) by chloroform extract (12.33 ± 1.15 mm) of *D. confragosa*. Inhibition of Helicobacter pylori (strain 51) was observed using n-hexane extract of *D. confragosa* (Na et al., 2022).

Conclusion

In this study, we studied the antibacterial activity of *D. flavida*. The study revealed significant activity in acetone extract, indicating the presence bioactive compound. Amongst the bacteria investigated, significant inhibition was observed against *P. aeruginosa*with MIC value of 0.1 ± 0.008µg/ml. Further investigation of the acetone extract to identify the bioactive compound would present a novel molecule to the scientific community.

Conflict of Interest: The authors declare that there is no conflict of interest.
Authors Contribution: All the authors have equal contribution.
Acknowledgement:
PYL and SSB would like to thank Prin. Dr. R. J. Barnabas for providing necessary facilities and constant support. RGC would like to thank the authorities of Department of Microbiology and Institute of Science.

References

1. Clericuzio, M., Bivona, M., Gamalero, E., Bona, E. *et al.* 2021. A Systematic Study of the Antibacterial Activity of Basidiomycota Crude Extracts. *Antibiotics***10 (11)**:1424
2. Fair, R.J., Tor, Y. 2014. Antibiotics and Bacterial Resistance in the 21st Century. *Perspect. Med. Chem.***6**, 14459.
3. Sharma, D., Misba, L., and Khan, A.U. 2019. Antibiotics versus Biofilm: An Emerging Battleground in Microbial Communities. *Antimicrob. Resist. Infect. Control***8**: 76.
4. Peterson, E. and Kaur, P. 2018. Antibiotic Resistance Mechanisms in Bacteria: Relationships Between Resistance Determinants of Antibiotic Producers, Environmental Bacteria, and Clinical Pathogens. Front. *Microbiol*. **9**: 2928.
5. Morel, S., Vitou, M., Masnou, A., Jumas-Bilak, E. *et al.* 2021. Antibacterial Activity of Wild Mushrooms from France. *International Journal of Medicinal Mushrooms***23(1)**: 79 – 89.
6. Alves, M.J., Ferreira, I.C.F.R., Martins, A. and Pintado, M. 2012. Antimicrobial activity of wild mushroom extracts against clinical isolates resistant to different antibiotics. *Journal of Applied Microbiology***113**: 466 – 475.
7. Ragupathi, V., Stephen, A., Arivoli, D. and Kumaresan, S. 2018. *In vitro* antibacterial activity of Methanolic extract of wild mushrooms from Southern Western ghats, India. *International Journal of Phytopharmacy Research***9(1)**: 32 – 39.
8. Suay, I., Arenal, F., Asensio, F.J., Basilio, A. *et al.* 2000. Screening of basidiomycetes for antimicrobial activities. *Antonie van Leeuwenhoek***78**: 129–139.
9. Ranadive, K.R., Belsare, M. H., Deokule, S.S., Jagtap, N.V. *et al.* 2013. Glimpses of antimicrobial activity of fungi from World. *Journal on New Biological Reports* **2(2)**: 142-162.

10. José, A. M., Ferreira, Isabel C. F. R., Dias, J., Teixeira, V., Martins, A. *et al.* 2012. Review on Antimicrobial Activity of Mushroom (Basidiomycetes) Extracts and Isolated Compounds. *Planta Med.***78**: 1707–1718.
11. Vaidya, J. G. and Rabba, A. S. Fungi in folk medicine. *Mycologist*, 1993, **7(3)**: 131 – 133.
12. Hossena, S. M. M., Hossaina, S. M., Akbara, S., Tahmidaa, U. *et al.* 2021. Wild mushrooms showed analgesic and cytotoxic properties along with phytoconstituent's binding affinity to COX-1, COX-2 and cytochrome P450 2C9. *Heliyon***7(9)**: e07997.
13. Chandrawanshi, N.K., Tandia, D. K. and Jadhav, S. K. 2018. Determination of Antioxidant and Antidiabetic Activities of Polar Solvent Extracts of *Daedaleopsisconfragosa* (Bolton) J. Schröt. *Research J. Pharm. and Tech.***11(12)**: 5623 – 5630.
14. Martin, G.W. Three new Heterobasidiomycetes. *Mycologia*, 1934, **26(3)**, 261-265.
15. Foroutan, A. and Vaidya, J.G. 2007. Record of New Species of *Phellinus* in Maharashtra India. *Asian Journal of Plant Sciences***6(4)**: 633 – 637.
16. Rathod, Mulchand M. 2011. Taxonomic studies on The Daedaloid and Hexagonoidpolypores form the forest of Western Maharashtra. *Recent Research in Science and Technology***3(5)**: 50-56.
17. Ramalingam, B., Elumalai, S., Devendiran, K., Sekaran, S. *et al.* 2011. Antibacterial and antifungal activity of fruit bodies of *Phellinus* mushroom extract. *International Journal of Biosciences* **1(3)**: 72-77.
18. Avci, E., Avci, G.A., and Köse, D.A. 2014. Determination of Antioxidant and Antimicrobial Activities of Medically Important Mushrooms Using Different Solvents and Chemical Composition via GC/MS Analyses. *Journal of Food and Nutrition Research* **2(8)**: 429-434.
19. Kosanic, M., Rankovic, B. and Dasic, M. 2013. Antioxidant and antimicrobial properties of mushrooms. *Bulgarian Journal of Agricultural Science* **19 (5)**: 1040 - 1046.
20. National Committee for Clinical Laboratory Standard. 1998. Methods for dilution in Antimicrobial Susceptibility Test. Approved Standard, National Committee for Clinical Laboratory Standard (NCCLS), Villanova, P.A.
21. Giri, S., Biswas, G., Pradhan, P., Mandal, S.C., and Acharya, K. 2012. Antimicrobial activities of basidiocarps of wild edible mushrooms of West Bengal, India. *International Journal of PharmTech Research* **4(4)**: 1554-1560.
22. Asri, R.M., Yahya, H., Rehan, M.M. and Yahya, H. N. 2019. Antibacterial properties of ethanolic extract of mushrooms sold in Malaysian local market. *East African ScholarsJ. Agri Life Sci.***2(11)**: 516-522.
23. Nwachukwu, E. and Uzoeto, H. O. 2011. Antimicrobial activity of some local mushrooms on pathogenic isolates. *International Journal of Current Research* **33(6)**: 1-5.
24. Kosanic, M., Rankovic, B. and Dasic, M. Antioxidant and antimicrobial properties of mushrooms. *Bulgarian Journal of Agricultural Science*, 2013, **19 (5)**: 1040-1046.
25. Shahtahmasebi, S.H., Pourianfar, H.R., Mahdizadeh, V., Shahzadeh, Fazeli S.A. *et al.* 2018. preliminary study on domestication of wild-growing medicinal mushrooms collected from Northern Iran. *Current Research in Environmental & Applied Mycology (Journal of Fungal Biology)***8(6)**: 606–623.
26. Vidovic, S., Zekovic, Z., Mujic, I., and Lepojevic, Z. 2011. The antioxidant properties of polypore mushroom *Daedaleopsisconfragosa*. *Central European Journal of Biology* **6**: 575–582.

27. Chandrawanshi, N.K., Tandia, D. K., and Jadhav, S. K. 2018. Determination of antioxidant and antidiabetic activities of polar solvent extracts of *Daedaleopsisconfragosa* (Bolton) J. Schröt. *Research J. Pharm. and Tech.***11(12)**: 5623-5630.
28. Culliao, Audrey Glenn L., Lumang-ay, Rhoda S., Kingat, Gloria Meryl T., Colallad, Tomasa P. *et al.* 2020. Preliminary Bioactivity Screening of Crude Extracts of Six Wild Macrofungi from Pine Forests in Benguet and Mt. Province, Philippines. *Manila Journal of Science* **13**: 74–88.
29. Okwulehie, I., Okorie, D. O. and Egekonye, T. A. 2014. Phytochemical, Proximate, Vitamins, Minerals and Heavy Metals Composition of Two Indigenous Mushrooms (*Daedaleopsisconfragosa*, (Polyporaceae) and RussuleGirolle (*Cantharelluscibarius*). *IOSR Journal of Pharmacy and Biological Sciences***9(3)**: 65-71.
30. Zhao, J.Y., Feng, T., Li, Z.H., Dong, and Z.J. 2013. Sesquiterpenoids and an ergosterol from cultures of the fungus *Daedaleopsistricolor*. *Natural Products and Bioprospecting* **3**: 271–276.
31. Elkhateeb, W. A., Daba, G.M., Elmahdy, E. M., Thomas, P. W. *et al.* 2019. Antiviral Potential of Mushrooms in the Light of their Biological active compounds. *ARC Journal of Pharmaceutical Sciences* **5(2)**, 45 – 49.
32. Fakoya, S., Adegbehingbe, K.T., and Ogundiimu, A.A. 2013. Biopharmaceutical assessment of active components of *Deadaleopsisconfragosa* and *Ganoderma lucidum*. *Open Journal of Medical Microbiology* **3**: 135-138.
33. Na, M.W., Lee, E., Kang, D. M., Jeong, S.Y., Ryoo, R., Kim, C. Y., Ahn, M. J., Kang, K.B., and Kim, K.H. 2022. Identification of antibacterial sterols from Korean wild mushroom *Daedaleopsisconfragosa* via Bioactivity and LC-MS/MS Profile-Guided fractionation. *Molecules***27**: 1865.

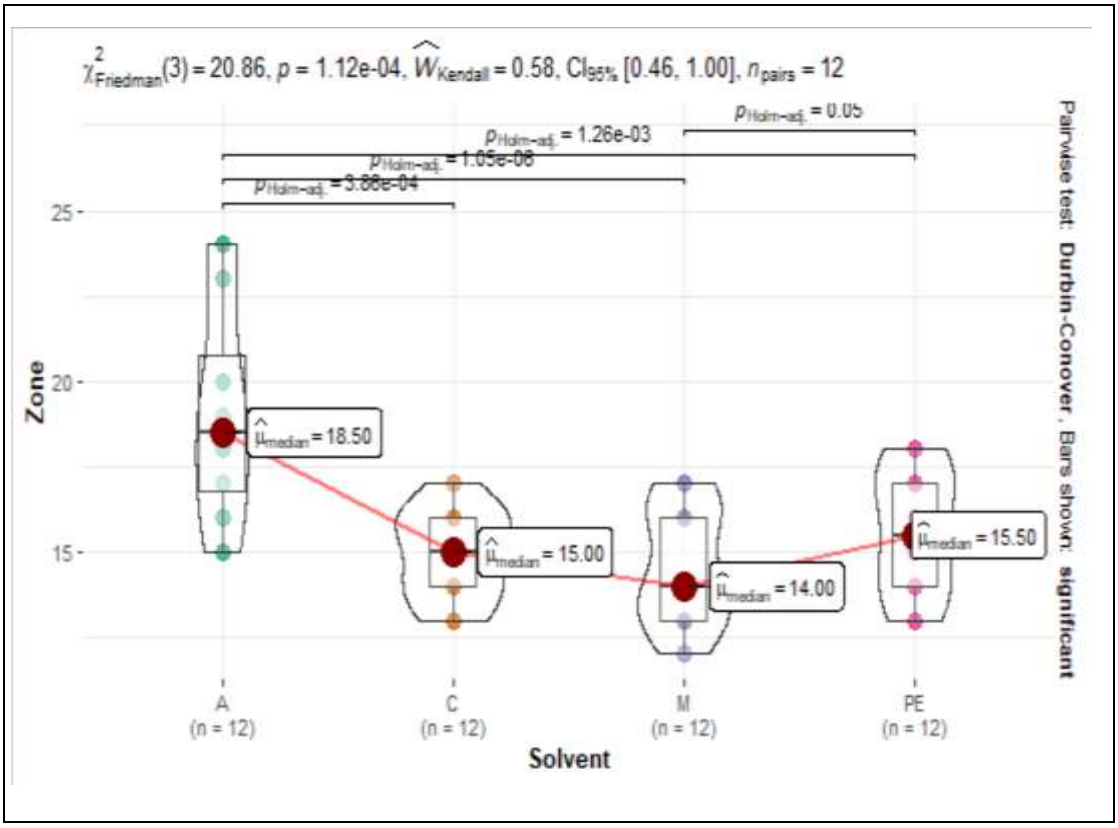

Fig 1: Pairwise comparison of the effect of the solvent using Friedman's test. Holm correction was used for the comparison. The Kendall Ŵ (0.58) indicate moderate agreement.

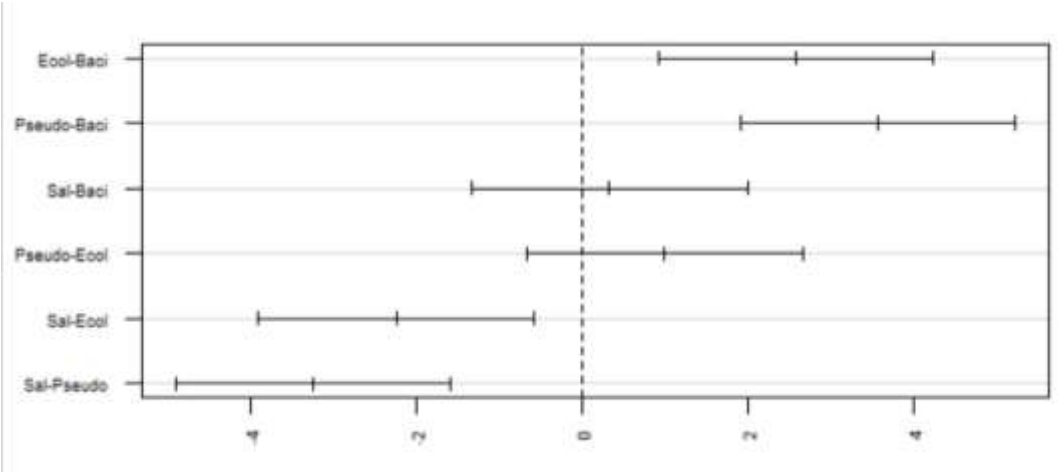

Fig 2: Pairwise comparison of differences in mean levels of organism (95% confidence interval)