BOHR Journal of Pharmaceutical Studies
2023, Vol. 1, No. 2, pp. 47–53
DOI: 10.54646/bjops.2023.07
www.bohrpub.com

RESEARCH

Methanolic seed extract of *Swietenia mahagoni*: Evaluation of antimicrobial, antioxidant, and *in-vivo* toxicity assessment in the brine shrimp model

Sourav Pal[1*], Subhajit Mandal[1], Sk Anabul Aktar[2], Arpan Kar[1], Nilanjan Adhikari[1] and Samyadip Singha Roy[1]

[1]P.G. Institute of Medical Sciences, Dhamkuria, India
[2]Calcutta Institute of Pharmaceutical Technology and AHS, Howrah, India

***Correspondence:**
Sourav Pal,
souravpal2525@gmail.com

Received: 30 November 2023; **Accepted:** 09 December 2023; **Published:** 14 December 2023

In light of the availability of phytochemicals with medicinal properties, herbal remedies are a valuable natural resource. *Sweetenia mahagoni* has been used for centuries in tropical places for a variety of medical purposes. Preclinical studies are essential; therefore, it is important to confirm their safety and answer any lingering doubts about their efficacy before they enter human trials. Methanol was employed to macerate the plant and get out its phytochemical constituents. The presence of antimicrobial properties of the extract was demonstrated through *in vitro* studies of antibacterial activity against Gram-negative bacteria such as *Escherichia coli* and *Salmonella abony*. It's possible that the extract can neutralize reactive oxygen species. Bioactive phytochemicals (limonoids, flavonoids, and terpenoids) have been linked to both antibacterial and antioxidant capabilities, according to trials testing their efficacy. The extract was found to be nontoxic to brine shrimp at concentrations up to 300 μg/mL in an *in vivo* lethality test.

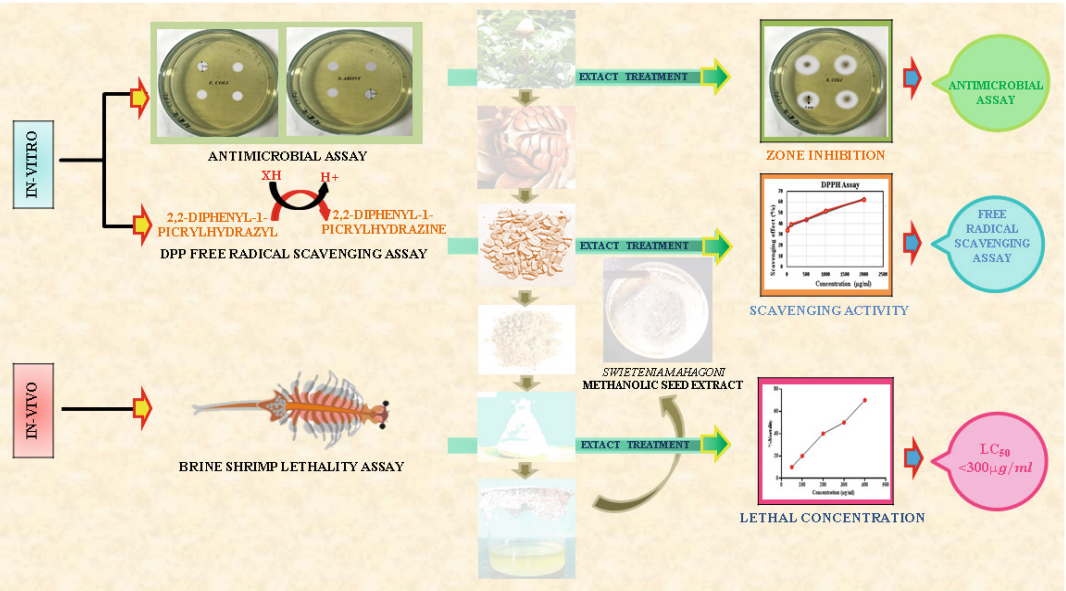
Keywords: *Sweetenia mahagoni*, antimicrobial assay, free radical scavenging assay, brine shrimp lethality assay

1. Introduction

Medicinal plants are valuable natural resources because they contain phytochemicals with therapeutic properties. Phytochemicals found in plants can be extracted and utilized for alleviating a wide range of illnesses (1). Many traditional or folk medicine systems rely heavily on medicinal plants for daily healthcare in developing countries. Antimicrobial susceptibility testing is an important tool in the field of medicine and pharmaceutical research (2). It involves testing the effectiveness of antimicrobial agents, such as antibiotics, against specific microorganisms, such as bacteria or fungi. These tests help researchers see how microbes react to different drugs. After big antibiotic classes such as tetracyclines, cephalosporins, aminoglycosides, and macrolides were found in the 1960s, microbes are now resisting them more (3). Unfortunately, we are running low on effective antibiotics to treat infections as many treatment options are not available these days (4). The issue of antibiotic resistance is a major global problem. When antibiotics stop working as well, it puts lives at risk. To keep antibiotics effective in the future, countries need to work together on solutions. We must protect the power of these important medicines (5).

Butylated hydroxytoluene (BHT) is a synthetic antioxidant that is used to treat issues caused by free radicals (6). While artificial chemicals are used in many products, some studies have shown they can negatively impact our health. Specifically, there are concerns that certain chemicals may cause liver toxicity or genetic mutations. Overall, it seems artificial ingredients could lead to unwanted side effects if not

Abbreviations: BHT, butylated hydroxytoluene; SMME, *S. mahagoni* methanolic extract; ZOI, zone of inhibition; AAI, antioxidant activity index; OD, optical density, ROS, reactive oxygen species.



GRAPHICAL ABSTRACT

properly evaluated (7). People are more interested in natural sources of antioxidants these days. Many tropical plants seem to have good defense against damage from oxidation and stress. Scientists think that the high antioxidant levels of tropical plants may help protect from health issues such as microbial diseases (8).

1.1. Brine shrimp as an alternative animal model for biomedical research

The brine shrimp test involves exposing brine shrimp larvae to a compound to see how toxic it is. It is also called the *Artemia* nauplii bioassay. They just see if the little brine shrimp survive when placed in the substance they are testing (9). So, this test looks at whether something is toxic by seeing if it kills brine shrimp larvae. They think that if it kills the shrimp, it may also kill other living things. To do the test, they put the thing they are testing into the water with baby brine shrimp. After some time, they count how many shrimps died. If a lot of them died, then they concluded that whatever they tested was probably toxic. In this study, they used brine shrimp to test something for how toxic it is to living things. They also looked at whether it had antioxidant effects to fight damage in the body, and antibacterial effects against germs (10).

2 Methodology

2.1. Plant materials

The province Paschim Medinipur of West Bengal is the place source of the *Swietenia mahagoni* seeds. The seeds have been washed thoroughly with running water to remove any traces of dirt before being dried. After being hacked up into smaller pieces, the seeds were dried at a temperature of 40°C for a week. The seeds were ground up in a blender to produce the powder (11).

2.2. Extract preparation

Maceration with methanol was used to extract the powdered seeds for 4 days. After filtering the extract with Whatman filter sheets, we collected the filtrate and concentrated it in a rotary evaporator at 40°C; 3 days were spent drying the concentrated extract in a 40°C oven before it was stored in the fridge for later usage (**Figure 1**) (12).

2.3. *In vitro* study

2.3.1. Antimicrobial assay

2.3.1.1. Preparation of media. Nutrient agar was the medium of choice (13). The specified volume was measured and prepared as per the manufacturer's instructions (28 g/L) before being transferred into conical flasks covered with nonabsorbent cotton wool, and coated in aluminum foil, before being autoclaved at 121°C for 15 min to kill any bacteria. The glasses were rinsed thoroughly and then placed in an autoclave for 15 min at a temperature of 121°C.

2.3.1.2. Verification of living organisms' viability. The Central Drug Laboratory, Howrah, Kolkata provided the *Escherichia coli* and *Salmonella abony* clinical strains used in the viability tests. To test for growth, *E. coli* and *S. abony*

FIGURE 1 | Extraction procedure of *S. mahagoni* methanolic seed extract.

were subcultured into nutrient agar plates and placed in an incubator for 24 h (14).

2.3.1.3. Analysis of S. mahagoni methanolic extract (SMME) for antimicrobial activity using the agar well diffusion technique. Cotton wool dipped in ethanol was used to wipe out the work surface. Conical flasks containing the prepared media (nutrient agar) were autoclaved at 121°C for 15 min, after which the contents were cooled, put into labeled Petri plates through the pour plate method of dispensing, and allowed to harden. Selected organisms were looped onto the appropriate solidified medium using a sterile wire loop. The zone of inhibition (ZOI) was confirmed by observing the plates, where the clear zones appeared around the wells (15). After the wells were numbered, 100 μL of SMME was poured into each one. These zones of inhibition were measured for their diameter and documented in millimeters (16).

2.3.2. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

The DPPH assay was used to evaluate the ability to quench free radicals (17). Using Blois's outlined procedures, we were able to calculate a quantitative estimate of radical scavenging activity. Concentrations of *Swietenia mahagoni* crude methanolic (SMCM) seed extract in the range of 10—2000 g/mL were mixed with 5 mL of a 0.04% DPPH radical solution. Keeping the mixture in the darkroom for 30 min, the combinations were given a final stir using a vortex mixer. At a wavelength of 517 nm, the optical density (OD) was determined. As a standard of comparison, methanol was employed. The vitamin C ascorbic acid is used as a reference standard. The following equation was used to determine the concentration of DPPH radicals:

$$\text{Scavenging effect (\%)} = \frac{\text{A0} - \text{A1}}{\text{A0}} \times 100\%$$

A_0 represents the absorbance of the control reaction, while A_1 represents the absorbance when the tested extracts are present. The half-maximal inhibitory concentration (IC_{50})—the concentration at which 50% inhibition occurs—was determined by visually analyzing a calibration curve within the linear range. This involved plotting the concentration of the extract against the matching scavenging effect. The antioxidant capacity was quantified using the antioxidant activity index (AAI) (18), which was computed as follows:

AAI is calculated by dividing the final concentration of DPPH ($mg{\cdot}mL^{-1}$) by the IC_{50} ($mg{\cdot}mL^{-1}$). Therefore, the AAI was determined by factoring in the weight of the DPPH and the weight of the extract being evaluated in the reaction. The studied extract's AAI is categorized as poor when AAI < 0.5, moderate when AAI is between 0.5 and 1.0, strong when AAI is between 1.0 and 2.0, and very strong when AAI > 2.0.

2.4. *In vivo* study

2.4.1. Brine shrimp (Artemia salina) lethality assay

2.4.1.1. Extract preparation. After macerating the ground seeds in methanol for 4 days, we got an extract. To concentrate the extract at 40°C, it was filtered through Whatman filter sheets. The concentrated extract remained refrigerated for 3 days after being dried in an oven at 40°C. Extract from SMME seeds was treated in a 4:1:4 mixture of propylene glycol, Tween 80, and water for use in subsequent research. Every sample used in the experiments was made fresh on the day of the tests (9).

2.4.1.2. Brine shrimp lethality assay. Eggs of the brine shrimp (*A. salina*) species were incubated in synthetic seawater made with 38 g/L of commercial sea salt (19). To coax the newly hatched prawns closer to the tank wall, a lamp was hung above the transparent tank side. After 24 h, the nauplii (*A. salina*) prawns were fully developed and ready for testing. Extract from SMME seeds was subjected to the usual bioassay for lethality using brine prawns. The

TABLE 1 | Antimicrobial activity of SMME.

S. No.	*E. coli*		*S. abony*	
	Inhibition	Diameter of ZOI (mm)	Inhibition	Diameter of ZOI (mm)
1	+	3	–	0

Key: "+" = inhibition, "–" = no inhibition.

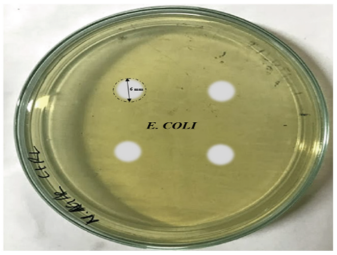
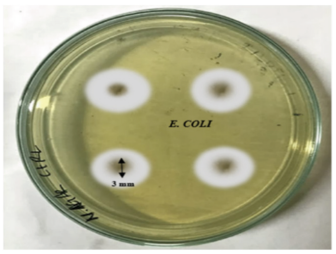
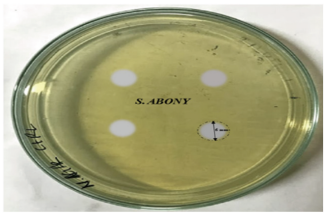
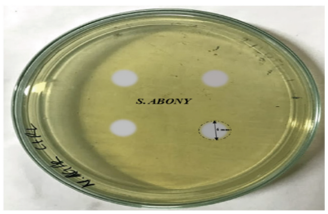
Microorganisms	Initial	SMME Treated (After 24h of treatment)
Escherichia coli (Plate 1)		
Salmonella abony (Plate 2)		

FIGURE 2 | Antimicrobial assay of SMME.

content of the crude extract was determined by dissolving 20 mg of the extract into 1 mL of Propylene glycol/Tween 80/water (4:1:4). The concentration range of the test solution was determined by diluting a stock solution of 10 mg/mL with salt water by a factor of two. Each concentration was examined 3 times. As a negative control, we utilized 5 mL of salt water mixed with propylene glycol and Tween 80 (4:1:4) in a test tube. The concentrations of 0.01−5 mg/mL were obtained by serially diluting a solution of potassium dichromate (as positive control) in propylene glycol, Tween 80, and water (4:1:4). Each test tube had 0.1 mL of a larvae solution added to it, with an estimated 10–15 larvae in it. After 6, 12, and 24 h, the test tubes were inspected, and the number of dead larvae in each bottle was recorded. Shrimp in each bottle were counted and reported as a whole. Using statistical methods, we calculated the LC_{50} and the percentage of deaths (20).

$$\text{Percentage of death (\%)} = \frac{\text{Total naupii} - \text{Alive naupii}}{\text{Total naupii}} \times 100$$

3 Results

3.1. *In vitro* study

3.1.1. Antimicrobial assay

Our research was the first of its kind to test the seed extract of *S. mahagoni* for antibacterial activity. Disk diffusion assays were used to test the extract's antibacterial efficacy against Gram-negative bacteria. SMME's antibacterial efficacy against a few different pathogenic microbes was tested *in vitro* using the agar well diffusion method. Gram-negative organisms (*E. coli* and *Salmonella abony*), respectively as shown in plates 1 and 2 with an initial diameter of 6 mm. The microbial growth inhibition of SMME is summarized in **Table 1**. After 24 h of extract exposure, SMME had clear zones of inhibition on *E. coli* (3 mm), but it has no effect on *S. abony*. The methanolic extract inhibited only *E. coli* with of inhibition of 3 mm and the colony of *S. abony* was not affected (**Table 1** and **Figure 2**).

TABLE 2 | DPPH radical scavenging assay.

S. No.	Sample	Concentration (μ g/mL)	Scavenging effect (%)	IC_{50} (mg/mL)	AAI
1	SMME	10	34.23	1.00015	1.999
2		100	39.57		
3		500	43.67		
4		1000	51.72		
5		2000	62.24		
6.	Ascorbic acid	500	88.43	0.468	1.068

3.1.2. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

To measure the free radical scavenging ability of different samples, including plant extracts, the scavenging of the stable DPPH radical is commonly employed. **Table 2** displays the results of the tests on the scavenging activity against DPPH radicals. Concentrations of 10, 100, 500, 1000, and 2000 g/mL of SMME seed extract showed

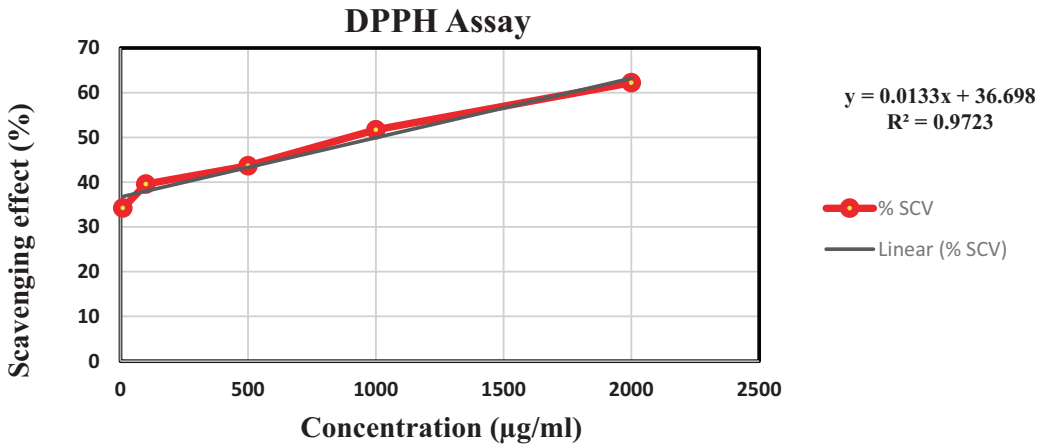


FIGURE 3 | Graph of DPPH radical scavenging assay of SMME. SCV, Scavenging activity.

TABLE 3 | Brine shrimp lethality assay of SMME.

Concentration (µ g/mL)	Log C	Number of brine shrimp taken	Number of brine shrimp dead	Number of shrimp alive	% Mortality (LC_{50})
50	1.69897	10	1	9	10
100	2	10	2	8	20
200	2.30103	10	4	6	40
300	2.477121	10	5	5	50
400	2.602059	10	7	3	70

antioxidant activities of 34.23, 39.57, 43.67, 51.72, and 62.24%, respectively. When comparing the ascorbic acid (500 µg/mL) to the extract at the same dose, the scavenging effect was found to be 88.43 and 43.67%, respectively (**Figure 3**). Extracts demonstrated some proton-donating activity and could function as free radical inhibitors or scavengers, possibly acting as major antioxidants; however, their DPPH radical scavenging activities were substantially lower than those of ascorbic acid. **Table 2** displays the IC_{50} values that were used to evaluate the antioxidant potency of the various extracts. For SMME seed extract, the IC_{50} value was found to be 1.00015 mg/mL. While most antioxidants had an AAI between 1.0 and 2.0, the SMME seed extract had an AAI of 1.999, indicating very significant antioxidant activity.

3.2. *In vivo* study

3.2.1. Brine shrimp (Artemia salina) lethality assay

Results on *A. salina* nauplii mortality after different SMME concentrations for 24 h are shown in **Figure 4**. The extract is moderately hazardous to brine shrimp in a dose-dependent manner, according to the results of the brine shrimp lethality test. Mortality rates (LC_{50}) at extract concentrations of 50, 100, 200, 300, and 400 g/mL were 10, 20, 40, 50, and 70, respectively. The highest rate of nauplii mortality is seen with SMME at a dose of 400 g/mL (**Table 3** and **Figure 4**).

4 Discussion

The initial stage in assessing a medicinal plant's efficacy is determining its potential side effects and the severity at which they manifest (21). In the tropics, where *S. mahagoni* is common, these medicines can be administered continuously for months or years without worrying about reaching a threshold that will have adverse effects. As a result, there has been a rise in the indiscriminate use of these kinds of drugs.

Microbial infections are currently a significant clinical concern, causing substantial morbidity and mortality. This is mostly attributed to the emergence of microbial resistance to existing antimicrobial treatments (22). Compounds such as thymol and carvacrol, found in *Thymus vulgaris*, have potent antibacterial effects (23). Both fungi and bacteria, including some strains that are resistant to antibiotics, are vulnerable to thyme oil's antimicrobial effects. *Melaleuca alternifolia*, the plant used to make tea tree oil, has strong antibacterial capabilities (24). Various fungi, including *Candida* species, and bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA), are no match for it. There are a number of compounds in *S. mahagoni* that have been shown to have antibacterial effects (25).

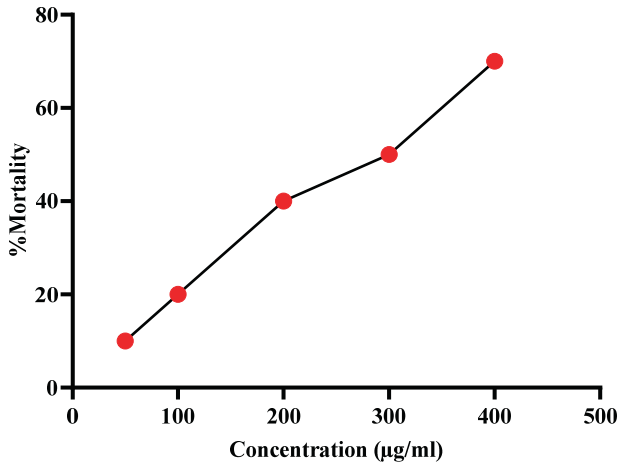


FIGURE 4 | Graph of brine shrimp lethality assay of SMME.

Inhibiting bacterial development, disrupting microbial cell membranes, and interfering with important microbial enzymes are just some of the effects of phytochemicals like limonoids, flavonoids, and triterpenoids (26). The antibacterial activity was studied against *E. coli* and *S. abony* as Gram-negative bacteria. In our study, the diffusion test showed that the *S. mahagoni* seed extract was active against *E. coli*. However, no significant activity showed against *S. abony*. It denotes our extract may have some antimicrobial properties.

The free radical scavenging experiment was used to determine if the methanolic extract of *S. mahagoni* (L.) seeds possessed any antioxidant activity. With an IC_{50} value of 1.00015 mg/mL, at which point 50% of the initial DPPH free radical concentration had been decreased, the seed extract showed considerable antioxidant capability. The extract showed strong scavenging action compared to the positive control (IC_{50} value of ascorbic acid = 0.468 mg/mL). Protecting animals and humans against oxidative damage, this extract has the potential to neutralize ROS such as peroxyl radicals, hydroxyl radicals, superoxide anions, peroxynitrite, and hypochlorous acid (27). Bioactive phytochemicals (limonoids, flavonoids, and terpenoids) may be responsible for the connection between antibacterial and antioxidant characteristics (28), this is corroborated by the results of antimicrobial assays.

The brine shrimp lethality assay, commonly known as the *A. salina* assay, is a popular bioassay for determining the toxicity or lethality of a substance. Brine shrimp larvae (nauplii) are used because of their extreme sensitivity to harmful chemicals in the assay (29). *Phyllanthus acidus* was highly hazardous with an LC_{50} of 3.12 and 12.5 µg/mL, and 70% mortality at 50 µg/mL. It indicates cytotoxic components and significant bioactivity of the plant extract (30). *A. nauplii* mortality after 24 h of SMME exposure is dose dependent. The brine shrimp lethality assay demonstrates the extract may be safe up to 300 µg/mL. Therefore, the study's outcome suggests that, with additional research, the extract could be established as a potential therapeutic agent.

5. Conclusion

In this study, an antibacterial assay using Gram-negative bacteria such as *E. coli* and *S. abony* suggests our extract may have antibacterial benefits. The outcome of the DDPH assay demonstrates that the extract is effective at quelling the production of reactive oxygen species. Antimicrobial experiments suggest bioactive phytochemicals (limonoids, flavonoids, and terpenoids) link antibacterial and antioxidant properties. The findings of *in-vivo* toxicity test on a brine shrimp model suggest that the extract would be safe at concentrations of up to 300 µ g/mL.

Authors' contributions

Manuscript writing and data analysis were performed by the SP, SM, and NA; SR, SA, and AK contributed to the construction of figures and tables; NA and SP contributed in revising the manuscript to its definitive form. All authors equally contributed to the article and approved the submitted version.

Acknowledgments

We are thankful for the support extended by the P.G. Institution of Medical Sciences, Chandrakona Town, Paschim Medinipur, West Bengal, India and permitting us to work on the project with all the facilities available in the institute.

References

1. Radha, Kumar M, Puri S, Pundir A, Bangar S, Changan S, et al. Evaluation of nutritional, phytochemical, and mineral composition of selected medicinal plants for therapeutic uses from cold desert of Western Himalaya. *Plants.* (2021) 10:1429.
2. Gajic I, Kabic J, Kekic D, Jovicevic M, Milenkovic M, Mitic Culafic D, et al. Antimicrobial susceptibility testing: a comprehensive review of currently used methods. *Antibiotics.* (2022) 11:427.
3. Uddin TM, Chakraborty AJ, Khusro A, Zidan BR, Mitra S, Emran TB, et al. Antibiotic resistance in microbes: history, mechanisms, therapeutic strategies and future prospects. *J Infect Public Health.* (2021) 14:1750–66.
4. Tarín-Pelló A, Suay-García B, Pérez-Gracia MT. Antibiotic resistant bacteria: current situation and treatment options to accelerate the development of a new antimicrobial arsenal. *Expert Rev Anti Infect Ther.* (2022) 20:1095–108.
5. Bhatia R, Katoch VM, Inoue H. Creating political commitment for antimicrobial resistance in developing countries. *Indian J Med Res.* (2019) 149:83.
6. Bisht R. Antioxidants: a brief review. *J Drug Deliv Ther.* (2018) 8:373–6.

7. Taslimi P, Gulçin I. Antioxidant and anticholinergic properties of olivetol. *J Food Biochem.* (2018) 42:e12516.
8. Arunachalam K, Yang X, San TT. Tinospora cordifolia (Willd.) Miers: protection mechanisms and strategies against oxidative stress-related diseases. *J Ethnopharmacol.* (2022) 283:114540.
9. Yadav AR, Mohite SK. Toxicological evaluation of *Psidium guajava* leaf extracts using brine shrimp (*Artemia salina* L.) model. *Res J Pharm Dosage Forms Technol.* (2020) 12:258–60.
10. Ntungwe NE, Dominguez-Martin EM, Roberto A, Tavares J, Isca VM, Pereira P, et al. Artemia species: an important tool to screen general toxicity samples. *Curr Pharm Design.* (2020) 26:2892–908.
11. Ervina M. The recent use of *Swietenia mahagoni* (L.) Jacq. as antidiabetes type 2 phytomedicine: a systematic review. *Heliyon* (2020) 6:e03536.
12. Sahgal G, Ramanathan S, Sasidharan S, Mordi MN, Ismail S, Mansor SM, et al. Brine shrimp lethality and acute oral toxicity studies on *Swietenia mahagoni* (Linn.) Jacq. seed methanolic extract. *Pharmacogn Res.* (2010) 2:215.
13. Lu Z, Guo W, Liu C. Isolation, identification and characterization of novel *Bacillus subtilis*. *J Vet Med Sci.* (2018) 80:427–33.
14. Alekish M, Ismail ZB, Albiss B, Nawasrah S. *In vitro* antibacterial effects of zinc oxide nanoparticles on multiple drug-resistant strains of Staphylococcus aureus and *Escherichia coli*: an alternative approach for antibacterial therapy of mastitis in sheep. *Vet World.* (2018) 11:1428.
15. Kannan K, Radhika D, Nikolova MP, Sadasivuni KK, Mahdizadeh H, Verma U, et al. Structural studies of bio-mediated NiO nanoparticles for photocatalytic and antibacterial activities. *Inorganic Chem Commun.* (2020) 113:107755.
16. Saquib SA, AlQahtani NA, Ahmad I, Arora S, Mohammed Asif S, Ahmed Javali M, et al. Synergistic antibacterial activity of herbal extracts with antibiotics on bacteria responsible for periodontitis. *J Infect Dev Countr.* (2021) 15:1685–93.
17. Xiao F, Xu T, Lu B, Liu R. Guidelines for antioxidant assays for food components. *Food Front.* (2020) 1:60–9.
18. Sharma KR, Khanal NR. Comparative investigation on antihyperglycemic and antioxidant activity of Zingiber officinale growing in different regions of Nepal. *Sci World.* (2022) 15:33–43.
19. Hirpara AB, Chaki SH, Kannaujiya RM, Khimani AJ, Parekh ZR, Vaidya YH, et al. Biological investigation of sonochemically synthesized CZTS nanoparticles. *Appl Surface Sci Adv.* (2022) 12:100338.
20. Nerdy N, Lestari P, Sinaga JP, Ginting S, Zebua NF, Mierza V, et al. Brine shrimp (*Artemia salina* Leach.) lethality test of ethanolic extract from green betel (Piper betle Linn.) and red betel (Piper crocatum Ruiz and Pav.) through the soxhletation method for cytotoxicity test. *Open Access Macedonian J Med Sci.* (2021) 9:407–12.
21. Lankatillake C, Huynh T, Dias DA. Understanding glycaemic control and current approaches for screening antidiabetic natural products from evidence-based medicinal plants. *Plant Methods.* (2019) 15:1–35.
22. Pachori P, Gothalwal R, Gandhi P. Emergence of antibiotic resistance *Pseudomonas aeruginosa* in intensive care unit; a critical review. *Genes Dis.* (2019) 6:109–19.
23. Vinciguerra V, Rojas F, Tedesco V, Giusiano G, Angiolella L. Chemical characterization and antifungal activity of *Origanum vulgare*, *Thymus vulgaris* essential oils and carvacrol against Malassezia furfur. *Natural Product Res.* (2019) 33:3273–7.
24. Yasin M, Younis A, Javed T, Akram A, Ahsan M, Shabbir R, et al. River tea tree oil: composition, antimicrobial and antioxidant activities, and potential applications in agriculture. *Plants.* (2021) 10:2105.
25. Syame SM, Mohamed SM, Elgabry EA, Darwish YA, Mansour AS. Chemical characterization, antimicrobial, antioxidant, and cytotoxic potentials of *Swietenia mahagoni*. *AMB Express.* (2022) 12:77.
26. Suriyaprom S, Mosoni P, Leroy S, Kaewkod T, Desvaux M, Tragoolpua Y, et al. Antioxidants of fruit extracts as antimicrobial agents against pathogenic bacteria. *Antioxidants.* (2022) 11:602.
27. Pisoschi AM, Pop A, Iordache F, Stanca L, Predoi G, Serban A, et al. Oxidative stress mitigation by antioxidants-an overview on their chemistry and influences on health status. *Eur J Med Chem.* (2021) 209:112891.
28. Aziz M, Ahmad S, Iqbal MN, Khurshid U, Saleem H, Kashif-ur-Rehman, et al. Phytochemical, pharmacological, and *in silico* molecular docking studies of *Strobilanthes glutinosus* Nees: an unexplored source of bioactive compounds. *S Afr J Bot.* (2022) 147:618–27.
29. Suryawanshi V, Yadav A, Mohite S, Magdum CS. Toxicological Assessment using Brine shrimp lethality assay and antimicrobial activity of *Capparis grandis*. *J Univ Shanghai Sci Technol.* (2020) 22:746–59.
30. Mali SN, Pandey A. Synthesis of new hydrazones using a biodegradable catalyst, their biological evaluations and molecular modeling studies (Part-II). *J Comput Biophys Chem.* (2022) 21:857–82.