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Antimicrobial and Larvicidal Efficacy of the Methanolic Extract of *Spinifex littoreus* (Burm F.) Merr.

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The increasing drug resistance of microbial populations is driving researchers to discover novel antimicrobial agents. Plants are the major reservoir of secondary metabolites which are capable of fighting against microorganisms. The current study focused on identifying a potent antimicrobial and larvicidal agent from the coastal plant Spinifex littoreus (Burm f.) Merr. The leaves of S. littoreus were extracted with methanol and used for further analysis. The antimicrobial assay of the methanolic extract of S. littoreus (SL-M) was conducted by agar well diffusion method for 6 bacterial (Escherichia coli, Propionibacterium acnes, Staphylococcus aureus, Aeromonas hydrophila, Streptococcus faecalis, Bacteroids fragilis) and 4 fungal (Cryptococcus neoformans, Aspergillus fumigatus, Aspergillus niger, Sporothrix schenckii) strains. Nutrient agar and Potato dextrose agar medium were used respectively, and different SL-M extracts were introduced. After incubation, the zone of inhibition was calculated. As for the larvicidal bioassay, the larvae of Aedes aegypti were grown in distilled water and they were treated with different concentrations of SL-M extracts. After 24 hours of incubation, larval mortality rate and LC50 values were calculated. The antimicrobial assay revealed that P. acnes and C. neoformans showed maximum inhibition than the other tested strains. The SL-M extract showed dose-dependent activity against larvae of Ae. aegypti. And the predicted LC50 value is 67.058 ppm. Considering the results of this current research the methanolic extract of S. littoreus can be an alternative source of antibiotics as well as a potent mosquito larval repellent.

Keywords: Antimicrobial; Aedes aegypti; Larvicidal agents; Secondary metabolites; *Spinifex littoreus* (Burm f.) Merr.

The environment is home to a diverse assortment of microorganisms. They are either beneficial or pathogenic. The number of beneficial organisms is relatively limited in comparison to pathogenic microbes. Furthermore, they often undergo significant evolution and can develop resistance to drugs[1]. Hence, finding new drugs is extremely necessary for mankind. Even today, one of the main sources of novel therapeutic compounds is natural ingredients such as plants, bacteria, eukaryotic microbes, and different animal species[2]. Various intricate and structurally varied compounds have been found in plants and other natural sources. The study of plant extracts, essential oils, isolated secondary metabolites, and newly synthesized compounds as possible

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antibacterial agents has attracted much attention lately[3,4,5]. Especially, the coastal plants have attracted researchers recently for their novel secondary metabolites[6].

Coastal plants survive under remarkably stressful environments such as high winds, hazardous ion concentrations, drought stress, excessive light, and inadequate nutritional availability[7,8]. Hence, these plants require specific adaptations, like thick or succulent and waxy coated leaves, sharp needle-like leaves, dry seeds, as well as solute accumulation to proliferate, endure, and infiltrate dunes[9]. Plant physiological activity is also altered by environmental stress. For example, the production of secondary metabolites is correlated with the physiological activity of the plant. The Secondary Metabolites function as crucial Primary Metabolites by considerably enhancing the development and endurance of plants in a variety of environmental conditions[10,11]. Since ancient times, secondary metabolites, a vital component of plants have dominated the medical industry and offered several therapeutic advantages. Many nations across the world still employ phytotherapy due to its low risk and cost-effectiveness in comparison to synthetic medications, which frequently have a wide variety of serious side effects[12]. Recently multiple studies have been conducted to discover suitable antimicrobial drugs due to the multidrug-resistance of pathogens. Keita and his colleagues (2022) listed the names of the plants and their antimicrobial effectiveness against the specific organism[13]. Keeping it all in consideration the current research work focused on discovering a new antimicrobial and larvicidal agent from the coastal grass *Spinifex littoreus* (Burm f.) Merr. *S. littoreus* is a key component of the ecology of coastal dunes. These perennial seashore plants help stabilize the sand and are prevalent on dunes across Africa, the Middle East, Asia, Australia, New Zealand, and New Caledonia[14]

MATERIALS AND METHODS

Plant collection

Spinifex littoreus (Fig.1) was collected from Kunthukal Beach, Pamban, Tamil Nadu, India (latitude: 9.25323; longitude: 79.21829). The collected plants were authenticated and submitted to the Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College, Tiruchirappalli. (Voucher nos. J.V 001 and J.V 002).

Plant extraction

The young leaves of *Spinifex littoreus* were separated from the plant, cleaned, and shade-dried. The dried *S. littoreus* leaves were mechanically pulverized. The powdered sample of *S. littoreus* was extracted with methanol through the Soxhlet apparatus for 16 hours. To concentrate the collected extract, it was subjected to a rotary evaporator[15].

Antimicrobial activity of the methanolic extract of *S. littoreus*

Microorganisms

The bacterial [*Escherichia coli* (MTCC 443), *Propionibacterium acnes* (MTCC 1951),

Fig. 1. Habit of *Spinifex littoreus* (Burm f.) Merr. (a). Male Inflorescence; (b). Female Inflorescence

Staphylococcus aureus (MTCC 902), *Aeromonas hydrophila* (MTCC 12301), *Streptococcus faecalis* (MTCC-439), *Bacteroids fragilis* (ATCC 25285)] and fungal species [*Cryptococcus neoformans* (ATCC 32045), *Aspergillus fumigatus* (MTCC 343), *Aspergillus niger* (MTCC 281), *Sporothrix schenckii* (ATCC 26327)] were utilized to examine the antimicrobial effect of the methanolic extract of *S. littoreus*. These microorganisms were purchased from the Microbial Type Culture Collection, Chandigarh, India.

Antimicrobial assay

The antibacterial and antifungal activity of the methanolic extract of *Spinifex littoreus* (SL-M) was tested using Nutrient Agar and Potato Dextrose Agar medium through agar well diffusion method. 20 ml of each medium was poured into a separate petri plate and let them solidify. After that, the surface of the agar medium was inoculated with the chosen microbial specimens over the entire surface of the agar medium. Then 6 mm diameter wells were created on the medium aseptically using a cork borer. To the well, 20 µL of different concentrations (500, 250, 100, & 50 µg/ml) of the methanolic extract of *S. littoreus* were introduced. Along with the test sample, the standard Gentamicin and Amphotericin B antibiotics were used as a positive control for bacterial and fungal species, respectively. Then the plates were incubated for 24 hours at 37°C for bacteria and 72 hours at 28°C for fungi. After the incubation period, the clear zone around the wells was measured and calculated the zone of inhibition[16,17].

Larvicidal bioassay methanolic extract of *S. littoreus*

The anti-larvicidal effect of SL-M extract was tested against the larvae of *Aedes aegypti*—the eggs of *Ae. Aegypti* were collected from the rice field stagnant water using an "O" type brush. The invitro larvicidal bioassay was conducted in

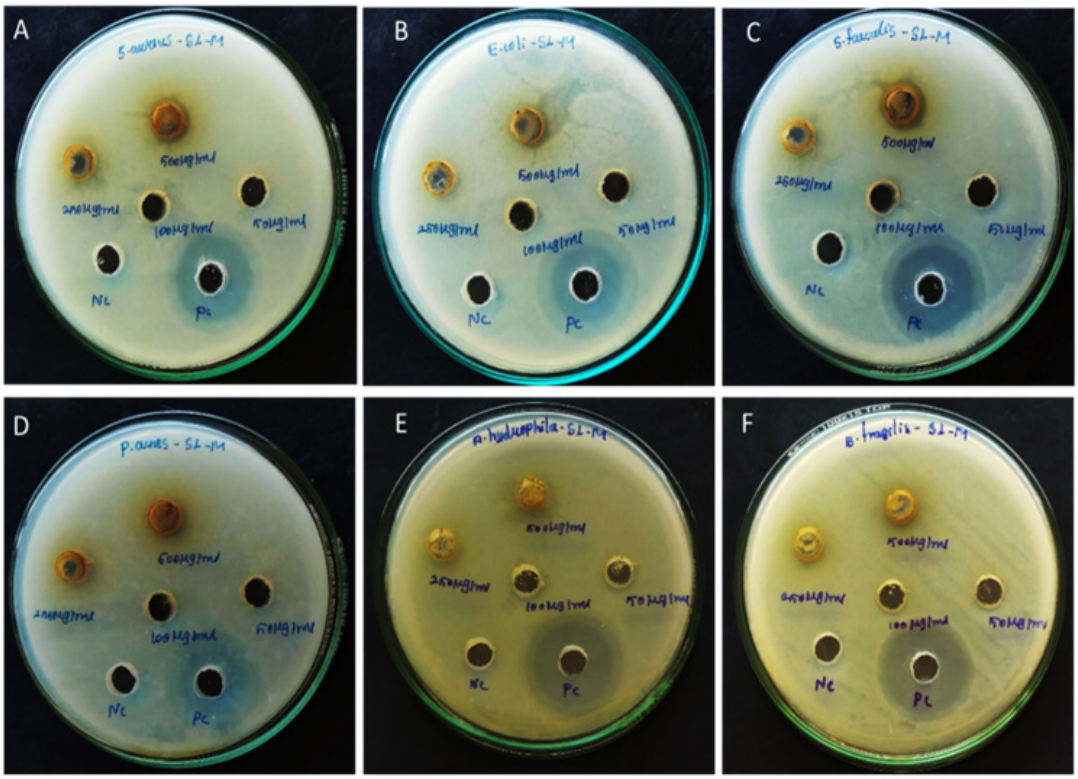

Fig. 2. Antibacterial assay of methanolic extract of *Spinifex littoreus* and Gentamicin as control indicated by zone of inhibition against (A) *Staphylococcus aureus,* (B) *Escherichia coli,* (C) *Streptococcus faecalis,* (D) *Propionibacterium acnes,* (E) *Aeromonas hydrophila,* (F) *Bacteroids fragilis.* The results were shown as independent experiment in triplicates

compliance with WHO guidelines[18]. Twenty-five larvae in their third or fourth instar were placed in beakers containing 100 ml of distilled water. The larvae were treated with *S. littoreus* methanolic extract at different concentrations (500 µg/ml, 250 µg/ml, 100 µg/ml, 50 µg/ml, 10 µg/ml) while a control group received no treatment. We conducted experiments three times for each concentration. The test containers had a 12:12 hour light/dark phase and were maintained at 25–28°C. Following a 24-hour exposure, larval mortality was noted. The mortality rate of the mosquito larvae was calculated

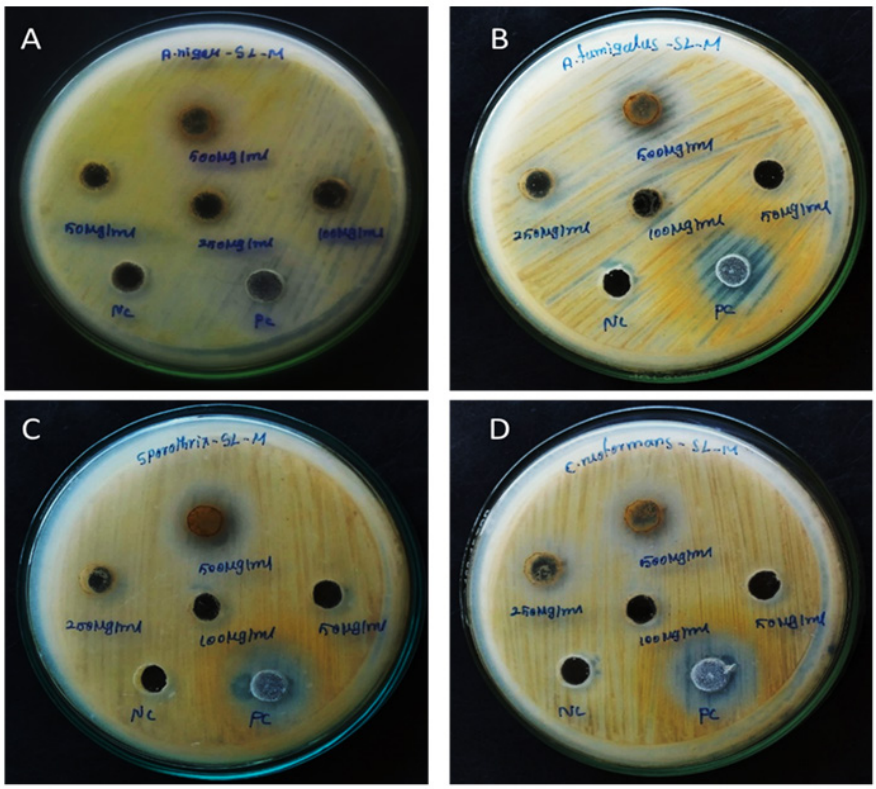

Fig. 3. Antifungal activity of methanolic extract of *S. littoreus* and Amphotericin-B as control showed by zone of inhibition against (A) *Aspergillus niger,* (B) *Aspergillus fumigatus,* (C) *Sporothrix schenckii,* (D) *Cryptococcus neoformans.* The results were shown as independent experiment in triplicates

Table 1. Antimicrobial activity of methanolic extract of *Spinifex littoreus*

No	Name of the test organism	Zone of inhibition (mm) Mean ± SD				
		500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	PC
1.	*Staphylococcus aureus*	14.5±0.7071	13.2±0.28	7.15±0.21	0	17.5±0.70
2.	*Escherichia coli*	14.5±0.7	5.15±0.21	0	0	16.75±1.06
3.	*Streptococcus faecalis*	13.75±1.06	6.35±0.49	5.25±0.35	4.05±0.07	15.5±0.7071
4.	*Bacteroids fragilis*	7.5±0.70	5.25±0.35	0	0	17.4±0.56
5.	*Propionibacterium acnes*	15.45±0.63	11.2±0.28	0	0	17.5±0.21
6.	*Aeromonas hydrophila*	12.5±0.70	6.35±0.45	0	0	18.5±0.7071
7.	*Aspergillus fumigatus*	12.5±0.707	5.25±0.35	0	0	19.5±0.7071
8.	*Aspergillus niger*	14.75±1.06	13.35±0.49	12.15±0.21	0	15.5±0.7071
9.	*Sporothrix schenckii*	14.35±0.49	0	0	0	15.75±1.06
10.	*Cryptococcus neoformans*	12.25±0.35	10.35±0.49	5.3±0.42	3.1±0.14	20.5±0.70

by the Graph Pad Prism program (USA) and the LC50 value was calculated by the Log- probit regression model developed by Dr. O.P. Sheoran.

RESULTS

Antimicrobial assay methanolic extract of *S. littoreus*

The antimicrobial analysis of the sample methanolic extract of *S. littoreus* extract showed dose-dependent activity for both bacteria and fungi. For the antibacterial study, the sample showed maximum inhibition for *Propionibacterium acnes* with 15.45 mm of the zone of inhibition (fig.2).

The least inhibition among the tested species was recorded in *B. fragilis* (7.5 mm). In the antifungal assay, *A. niger* (14.75 mm) and *S. schenckii* (14.35 mm) exhibited maximum inhibition than the other two tested organisms (fig.3).

Table 1 enlightens the name of the organisms and their zone of inhibition values (mean ± SD) of antimicrobial activity of methanolic extract of *S. littoreus*.

Anti-larvicidal activity of *S. littoreus* methanolic extract

The preliminary examination of the larvicidal effect of the methanolic extract of *S. littoreus* (SL-M) suggested dose-dependent activity on *Aedes aegypti* larvae. 100% of mortality was recorded in the 500 µg/ml of SL-M extract. The methanolic extract of *S. littoreus* showed considerable larvicidal activity with an LC50 value of 67.058 ppm.

Morphological changes of *Ae. Aegypti* after being treated with SL-M extract

The complete body of the treated and untreated larvae was examined under a light microscope after 24 hours. The SL-CH-treated larvae suffered significant harm. Through visual inspection, it was observed that the treated larvae's body motions and coiling were abnormal in the SL-M extract. Under a light microscope, they displayed clear morphological alterations that destroyed the thoracic and abdomen regions (oozing out of the contents of the digestive tract). In control larvae, no structural changes in their morphology were seen (Fig.4).

DISCUSSION

Plants generate a range of secondary metabolites according to their environment. These substances have traditionally been employed to treat chronic diseases and infectious[19]. Currently, scientists are concentrating on

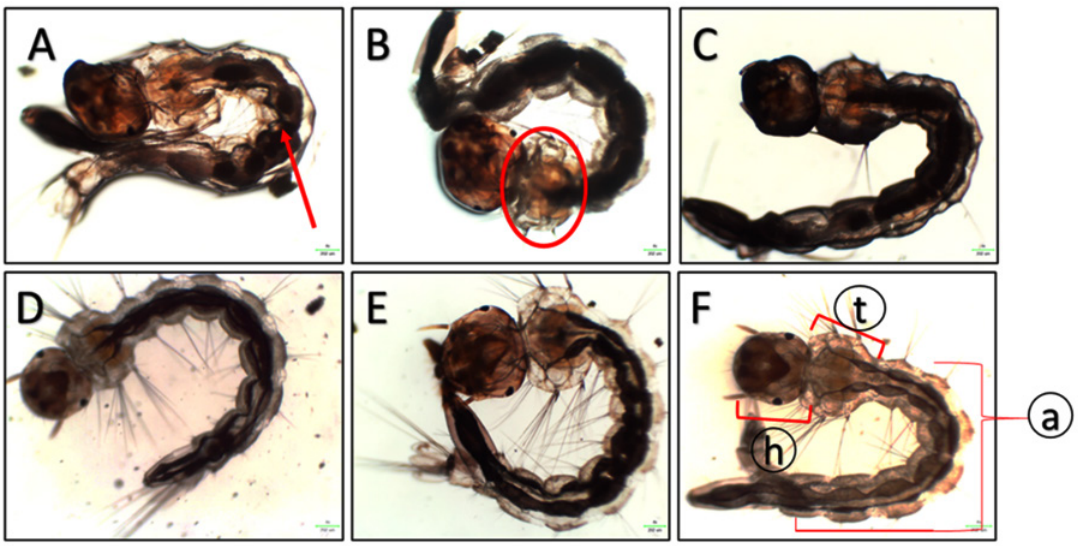

Fig. 4. Effect of SL-M extract on the larvae of *Aedes aegypti.* (A) shows intestine shortening on 500 µg/ml of SL-M treatment (Arrow); (B) Thoracic rupture and gut extrusion on 250 µg/ml (Circle); (C, D &E) shows gradual morphological changes and (F) control larva describes the structure. (h- head; t-thoracic; a – abdomen)

discovering a novel and significant antibacterial substance from plant extracts due to the emerging phenomenon of multidrug resistance of pathogens[20]. For example, alkaloids[21], flavonoids[22], tannins[23], terpenoids[24], phenolics[25], saponins[26], and steroids[27] are eventually reported as a potential antimicrobial agent against the pathogens listed as multidrug-resistant organisms. These plant secondary metabolites affect microbial cells in several ways by disturbing cytoplasmic membrane structure and functions, interacting with membrane proteins, and interrupting the transcription and translation processes[28,29,30]. The plant *S. littoreus* also showed the presence of therapeutically important secondary metabolites such as alkaloids, flavonoids, tannins, quinones, coumarins, and steroids[31].

In the current study, the antimicrobial assay results indicated that the bacteria *Propionibacterium acnes* and the fungal strain of *Cryptococcus neoformans* had a maximum zone of inhibition compared to the other tested species. The bacteria *P. acnes* are responsible for diseases like skin, joints, and lung infections[32] and the fungi *C. neoformans* can cause Cryptococcosis - It is fatal and injures the lungs or brain[33]. The chloroform extract of this *S. littoreus* also exhibited similar antimicrobial activities[34]

Mosquitoes are a major public health problem, carrying illnesses such as malaria, filariasis, dengue, and Japanese encephalitis, resulting in millions of fatalities each year[35]. This current investigation contributes to developing a novel larvicidal agent derived from *S. littoreus'* methanolic extract. Results of this study showed preferable anti-larvicidal activity against the larvae of *Ae. Aegypti.* Similar results were recorded by Rahuman., (2008), who conducted a larvicidal bioassay for the plant *Phyllanthus amarus* against *Aedes aegypti* larvae and the LC50 value is 90.92 ppm[36]. Plant-based extracts and their chemical components can be converted into environmentally friendly products for mosquito vector control owing to their selectivity, sustainability, and low toxic effect [37,38]. According to research by Thangam and Kathiresan (1991) marine plant extracts and synthetic insecticides work together to eradicate *Aedes aegypti* larvae[39]. In the study by Manh and his colleagues., (2020), essential oils from certain terrestrial aromatic plants have larvicidal effects on *Aedes aegypti* larvae[40]. Similar structural changes in mosquito larvae were also observed and documented by Ravi and team (2018) in the larvae of *Ae. Aegypti* when treated with *Azolla pinnata* extract[41].

CONCLUSION

The methanolic extract of the coastal plant of *Spinifex littoreus* (Burm f.) Merr. exhibits potential antibacterial, antifungal, and anti-larvicidal activities. Hence the plant *S. littoreus* can be the alternative therapeutics for diseases like skin infections, rheumatics, and Cryptococcosis.

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Conflict of Interest

The authors do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Authors' Contribution

Vedhamani John Nallaiyah and Paul Ajithkumar Issac Newton : participated in the study's design; Vedhamani John Nallaiyah : carried out all the experiments and drafted the manuscript; Paul Ajithkumar Issac Newton : supervised all the work and finalized the manuscript. All authors read and approved the final manuscript.

REFERENCES

1. Balouiri M., Sadiki M. and Ibnsouda S. K. Methods for in vitro evaluating antimicrobial

activity: A review. *J. Pharm. Anal.* 2016; 6(2): 71-9.
2. Berdy J. Bioactive microbial metabolites. *J Antibiot*. 2005;58(1):1-26.
3. Runyoro D. K., Matee M. I., Ngassapa O. D., Joseph C. C. and Mbwambo Z. H. Screening of Tanzanian medicinal plants for anti-Candida activity. *BMC Complement Altern Med*. 2006; 6: 1-0.
4. Mabona U., Viljoen A., Shikanga E., Marston A. and Van Vuuren S. Antimicrobial activity of southern African medicinal plants with dermatological relevance: from an ethnopharmacological screening approach to combination studies and the isolation of a bioactive compound. *J. Ethnopharmacol.* 2013; 148(1): 45-55.
5. Nazzaro F., Fratianni F., De Martino L., Coppola R. and De Feo V. Effect of essential oils on pathogenic bacteria. *Pharmaceuticals*. 2013; 6(12): cr1451-74.
6. Zidorn C. Secondary metabolites of seagrasses (Alismatales and Potamogetonales; Alismatidae): Chemical diversity, bioactivity, and ecological function. *Phytochemistry*, 2016; 124: 5-28. https://doi.org/10.1016/j.phytochem.2016.02.004
7. Hesp P.A. Ecological processes and plant adaptations on coastal dunes. *J. Arid Environ.* 1991; 21(2): 165-91.
8. Bermúdez R. and Retuerto R. Together but different: co-occurring dune plant species differ in their water and nitrogen-use strategies. *Oecologia*, 2014; 174: 651-63.
9. Larcher W. Physiological plant ecology: ecophysiology and stress physiology of functional groups. *Springer SBM.* 2003.
10. Kliebenstein D.J. Making new molecules—evolution of structures for novel metabolites in plants. *Curr. Opin. Plant Biol.* 2013; 16(1), 112-117. http://dx.doi.org/10.1016/j.pbi.2012.12.004.
11. Zandalinas S. I., Mittler R., Balfagón D., Arbona V. and Gómez Cadenas A. Plant adaptations to the combination of drought and high temperatures. *Physiol. Plant.* 2018;162(1), 2-12. http://dx.doi.org/10.1111/ppl.12540.
12. Wink M. Modes of action of herbal medicines and plant secondary metabolites. *Medicines*, 2015; (3), 251-286. https://doi.org/10.3390/medicines2030251
13. Keita K., Darkoh C. and Okafor F. Secondary plant metabolites as potent drug candidates against antimicrobial-resistant pathogens. *SN Appl. Sci.* 2022; 4(8),209.
14. Webb C. J., Sykes W. R. and Garnock-Jones P. J. Flora of New Zealand. Vol. IV. Botany Division, Department of Scientific and Industrial Research. 1988.
15. Redfern J., Kinninmonth, M., Burdass D. and Verran J. Using soxhlet ethanol extraction to produce and test plant material (essential oils) for their antimicrobial properties. *JMBE.* 2014; 15(1), 45-46. https://doi.org/10.1128/jmbe.v15i1.656
16. Hufford C. D., Funderburk M. J., Morgan J. M. and Robertson L. W. Two antimicrobial alkaloids from heartwood of *Liriodendron tulipifera* L. *J. Pharm. Sci.* 1975;64(5),789-792. https://doi.org/10.1002/jps.2600640512
17. Balouiri M., Sadiki M. and Ibnsouda S. K. Methods for in vitro evaluating antimicrobial activity: A review. *J. Pharm. Anal.* 2016;6(2),71-79.
18. World Health Organization. Guidelines for Laboratory and Field Testing of Mosquito Larvicides; World Health Organization: Geneva, Switzerland, 2005; 1–41. https://apps.who.int/iris/handle/10665/69101.
19. Boligon A. A., Agertt V., Janovik V., Cruz R. C., Campos M., Guillaume D., Athayde M. L. and dos Santos A.R. Antimycobacterial activity of the fractions and compounds from Scutia buxifolia. *Rev. Bras. Farmacogn.* 2012;22,45-52.
20. Dhama K., Tiwari R., Chakraborty S., Saminathan M., Kumar A., Karthik K., Wani M. Y., Amarpal S. S. and Rahal A. Evidence based antibacterial potentials of medicinal plants and herbs countering bacterial pathogens especially in the era of emerging drug resistance: an integrated update. *Int. J. Pharmacol.* 2014;10(1),1-43.
21. Özçelik B., Kartal M. and Orhan I. Cytotoxicity, antiviral and antimicrobial activities of alkaloids, flavonoids, and phenolic acids. *Pharm. Biol.* 2011;49(4),396-402.
22. Cushnie T. T. and Lamb A. J. Antimicrobial activity of flavonoids. *Int J Antimicrob Agents.* 2005,26(5),343-356.
23. Scalbert A. Antimicrobial properties of tannins. *Phytochemistry*, 1991;30(12),3875-3883.
24. Griffin S. G., Wyllie S. G., Markham J. L. and Leach D. N. The role of structure and molecular properties of terpenoids in determining their antimicrobial activity. *Flavour and Fragrance Journal*, 1999;14(5),322-332.
25. Sousa A., Ferreira I. C., Calhelha R., Andrade P. B., Valentão P., Seabra R., Estevinho L., Bento A. and Pereira J. A. Phenolics and antimicrobial activity of traditional stoned table olives 'alcaparra'. *Bioorganic & medicinal chemistry*, 2006;14(24),8533-8538.
26. Amraei S. and Ahmadi S. Recent studies on antimicrobial and anticancer activities

of saponins: a mini-review. *Nano Micro Biosystems*, 2022;1(1),22-26.

27. Taleb-Contini S. H., Salvador M. J., Watanabe E., Ito I.Y. and Oliveira D. C. R. D. Antimicrobial activity of flavonoids and steroids isolated from two Chromolaena species. *Revista Brasileira de Ciências Farmacêuticas*, 2003;39,403-408.
28. Anand U., Jacobo-Herrera N., Altemimi A., Lakhssassi N. A comprehensive review on medicinal plants as antimicrobial therapeutics: Potential avenues of biocompatible drug discovery. *Metabolites,* 2019; 9, 258.
29. Khameneh B., Iranshahy M., Soheili V., Bazzaz B.S.F. Review on plant antimicrobials: A mechanistic viewpoint. *Antimicrob. Res. Infect. Control.* 2019; *8*, 118.
30. Radulovic N. S., Blagojevic P. D., Stojanovic-Radic Z. Z., Stojanovic N. M. Antimicrobial plant metabolites: Structural diversity and mechanism of action. *Curr. Med. Chem.* 2013; 20, 932–952.
31. Yuvaraj D., Gokulnath M., Gayathri P. K., Chandran M., Vivek P., Kesavan D. Phytochemical Screening and Anti-Bacterial Studies in Salt Marsh Plant Extracts (Spinifex littoreus (BURM.F) MERR. and Heliotropium curassavicum L.). *Recent Trends in Biotechnology and Chemical Engineering*, 2014;6(9),4307-4311.
32. Perry A. and Lambert P. Propionibacterium acnes: infection beyond the skin. *Expert review of anti-infective therapy*, 2011;9(12),1149-1156.
33. Murakawa G. J., Kerschmann R. and Berger T. Cutaneous Cryptococcus infection and AIDS: report of 12 cases and review of the literature. *Archives of Dermatology*, 1996;132(5),545-548. https://jamanetwork.com/journals/jamadermatology/article-abstract/557735
34. Vedhamani J., Ajithkumar I. P. and Jemima E. A. Exploring the in vitro antimicrobial, antioxidant, and anticancer potentials of Spinifex littoreus Burm f. Merr. against human cervical cancer. *Plant Science Today*, 2024;11(2). https://doi.org/10.14719/pst.3011
35. Kolli G.R. and Sundararajan R. Evaluation of larvicidal activity of Pongamia pinnata extracts against three mosquito vectors. *Asian Pacific Journal of Tropical Biomedicine*, 2013;3(11),853-858. https://doi.org/10.1016/S2221-1691(13)60168-9
36. Rahuman A. A., Gopalakrishnan G., Venkatesan P. and Geetha K. Larvicidal activity of some Euphorbiaceae plant extracts against Aedes aegypti and Culex quinquefasciatus (Diptera: Culicidae). *Parasitology Research*, 2008;102,867-873. https://link.springer.com/article/10.1007/s00436-007-0839-6
37. Ghosh A., Chowdhury N., Chandra G. Plant extracts as potential mosquito larvicides. *Indian Journal of Medical Research,* 2012;135(5),581. https://journals.lww.com/ijmr/fulltext/2012/35050/plant_extracts_as_potential_mosquito_larvicides.3.aspx
38. Pavela R. History, presence and perspective of using plant extracts as commercial botanical insecticides and farm products for protection against insects–a review. *Plant Protection Science*, 2016;52(4), 229–241.
39. Thangam T. S. and Kathiresan K. Mosquito larvicidal activity of marine plant extracts with synthetic insecticides. *Botanica Marina*, 1991;34,537-539. https://doi.org/10.1515/botm.1991.34.6.537
40. Manh H. D., Hue D. T., Hieu N. T. T., Tuyen D. T. T. and Tuyet O. T. The Mosquito larvicidal activity of essential oils from Cymbopogon and Eucalyptus Species in Vietnam. *Insects*, 2020;11(2), p.128. https://doi.org/10.3390/insects11020128
41. Ravi R., Zulkrnin N.S.H., Rozhan N.N., Nik Yusoff N.R., Mat Rasat M.S., Ahmad M.I., Ishak I.H. and Amin M.F.M. Chemical composition and larvicidal activities of Azolla pinnata extracts against Aedes (Diptera: Culicidae). *PloS one*, 2018;13(11), p.e0206982. https://doi.org/10.1371/journal.pone.0206982