Ann. For. Res. 65(1): 826-844, 2022
DOI: 10.5281/zenodo.7256398

ANNALS OF FOREST RESEARCH
https://www.e-afr.org/

PLANT EXTRACTS FROM THE NILGIRI REGION: THE ACTION MECHANISM AND ANTIBACTERIAL PROPERTIES

Jitender Singh Hudda
Research Scholar, Department of Botany, Om Sterling Global University, Hissar
jitendersingh46@rediffmail.com

Dr. Devesh Kumar Mishra
Associate Professor, Department of Botany, Om Sterling Global University, Hissar

ABSTRACT
Since dangerous bacteria are naturally present in the environment, humans can easily be exposed to them. These microorganisms can withstand extreme settings and are resistant to conventional pasteurisation. Therefore, new environmentally friendly methods are required to prevent the growth of dangerous microorganisms and extend shelf life. Numerous academics have recently become interested in the potential use of specific plant extracts as effective natural preservatives. Some of the phytochemicals found in medicinal plants that have antibacterial and antioxidant properties include alkaloids, flavonoids, tannins & terpenoids. Numerous plant species have undergone substantial research to determine their antibacterial capacities. Making the most of these extracts as organic antimicrobials requires an understanding of the mechanisms of action of the medicinal plants found in the Nilgiri Bio Reserve.
Keywords: Standard pasteurization, Flavonoids, alkaloids, tannins, terpenoids, phytochemicals and incurable.

INTRODUCTION
Human illness brought on by microbes continues to have a significant global impact, even in developed nations. According to estimates, up to 40% of deaths in the human population occur each year as a result of numerous things, including illness brought on by microbes. The most prevalent forms of microorganisms that cause infections and sickness in humans are bacteria, yeast, and mould. [(1)] Several plant species and extracts have been extensively studied for their antibacterial properties. Knowing the Nilgiri Bio Reserve's medicinal plants' mechanisms of action is the first step in making the most of these extracts as organic antimicrobials.

METHODS & METHODOLOGY
EXTRICACTION
Based on their historical use in folk medicine, the medicinal plants of the Nilgiri Bio Reserve were chosen for this study. The plants were purchased in dry form from the local Nilgiri Bio Reserve market. The medicinal plant species and components used in this experiment are stated (Table 1). Two solvents—ethanol and water—were examined to determine how they affected the extracted

© ICAS 2022

yield and were found to have no effect. Conventional extraction and ultrasonic procedures are also employed as extraction techniques. For a standard extraction, 20 gm of powder from the examined plants' components were soaked in 180 ml of distilled water, heated for 30 min at 90°C, and then incubated over night at 37°C and 150 rpm in a shaking incubator. (2) In a manner similar to this, round bottom flasks containing 10 gm of each examined plant material were separately mixed with 9:1 ethanol and then incubated at 37°C at 150 rpm for whole night. After filtering the resultant liquid extracts through a Whatman No. 1 filter to eliminate the solid residue, they were concentrated using a shaking incubator. For ultrasound tests, 180 mili litre of distilled water or 9:1 ethanol were added to 10 gm of the powdered plant material from each species that was examined separately in beakers. Thirty minutes at a frequency of 53 Kilo Hertz were spent submerging each beaker in the ultrasonic bath. The two beakers were then moved to an incubator shaker and left there for the duration of the night at a temperature of room temperature and a rotational speed of 150 rotation per minute. (3)

Similar to the normal extraction process, the dried extracts of a few selected plants were extracted from the supernatant. For ethanolic extracts, dried extracts were dissolved in 10 percent DMSO and for water extracts, in water. For each extract, the final w/v concentration was 20%. The raw extract was then stored for future research at -20 degrees Celsius. The following equation has been used to determine the extraction yield for the selected plants:

$$\text{Yield}(\%)=(X_1*100)/X_0$$

X_0 represents the dry weight of the plant powder prior to extraction and X_1 represents the weight of the extract following the evaporation of the solvent.

Making the inoculum

Gram-positive and Gram-negative bacteria were used to test the potency of plant extracts as an antibacterial agent. The bacteria were pre-cultured in Mueller Hinton broth for an overnight period at room temperature in a rotary shaker. (4) Then, at a concentration of 108 cells/mili litre, each strain was corrected using the 0.5 McFarland standard. A 48 hours culture of fungi isolated in potato dextrose broth was used to create the fungus inoculum.

Screening of Antimicrobial Properties

The effectiveness of various solvent extracts as an antibacterial agent was assessed using the agar well diffusion method. A sterile Petri dish's centre was pipetted with 1 mili litre of a fresh bacterial culture. After the holes had set, 6 mili meter diameter sterile cork borer holes were drilled into agar plates containing inoculums. Then, 100 mili litre of each extract (20 percent w/v) were injected into the pertinent wells.

© ICAS 2022

We chose the extract concentration (20 percent w/v) based on our pre-experiments and previous research. The plates were cooled for 30 minutes to allow the extracts to fully diffuse into the agar. The plates were then kept at room temperature for an additional 18 hours of incubation. After the incubation period, the zone of inhibition, which covered the wells' diameter, was measured in order to identify antimicrobial activity. 10 percent DMSO concentration was used as a bad control. (5)

Establishment of Minimum Inhibitory Concentrations

At a 20 percent (w/v) concentration, each of the investigated extracts exhibited antibacterial activity. This concentration was altered in order to ascertain their minimum inhibitory concentrations using the agar well diffusion method and assess how well they inhibited diseases and germs. By serially diluting two times, various concentrations of 1.25, 2.5, 5 and 10 percent were created. Molten agar was added after each prepared inoculum was well combined with 1 mili litre of each one pipetted into sterile Petri dishes. Then, 100 l of each extract's 10, 5, 2.5, and 1.25% were added to the appropriate wells on each of the four plates. The plates were frozen for 30 minutes before an 18-hour incubation period at 37°C. The lowest concentration that stopped the related bacterium from multiplying was referred to as the MIC. Three separate assays were performed for each. For water extracts, control was used by distilled water, while DMSO was used for ethanolic extracts.

Assessment of Cytoplasmic pH

In order to assess how plant extracts deal with bacteria, the two strains (SA and AH) have been used as examples of Gram-positive and Gram-negative bacteria, respectively. The pH of the cytoplasm of bacterias was examined as a potential sign of the antibacterial action of plant extracts pH. Carboxyfluorescein diacetate succinimidyl ester was the fluorescent probe used in this study. Modifications were made to the cytoplasmic pH assay. AH and SA bacterial cells were first cultivated in nutritious broth for 24 hours at room temperature and 128 rotation per minute followed by a 3 hours subculture at the same conditions. (6) Centrifugation at 11200 g-force for five minutes at 4 °Celcsius was used to collect the cells. The cells were washed twice and resuspended in potassium phosphate buffer (50 Mm with pH 7). A 0.5 McFarland standard was used to increase the cell density to 108 cells/mili litre. Then, One M CFDA SE dye was applied, and the bacteria were allowed to grow for 30 minutes at 37 °Celcsius and 128 rotation per minute. The cells were then resuspended in potassium phosphate buffer with pH 7 and addition of 10 mM glucose solution and centrifuged at 11200 g-force for 5 minutes before being incubated for a further 30 minutes at room temperature. The cells were washed once, and then centrifuged for five minutes at 11200 g-force before being resuspended in potassium phosphate buffer. The cell-free filtrate, treatment, and control groups each received an equal number of the tagged cells. To one mili litre of bacteria or cell free filtrate, plant extracts were added at a final concentration of 20 percent (w/v). After 10 minutes, the fluorescence intensity was measured with a fluorescence

© ICAS 2022

spectrophotometer using 520 nano meter for emission and 490 nano meter for excitation. The emission slit width is 10 nano meter, whereas the excitation slit width is 5 nano meter. One mili litre of potassium phosphate buffer (50 mM with pH 7) was added to 1 mili litre of bacteria for the control samples and the filtrate devoid of bacteria. By deducting the fluorescence of the corresponding cell-free filtrate from the treatment or control groups, the fluorescence for the bacterial cells was derived.

Determination of Membrane Potential Disruption

The Bis-(1,3-Dibutylbarbituric Acid)Trimethine Oxonol allowed for the detection of the cell membrane rupture. Simply put, bacteria from the AH and SA strains were grown in nutritious broth for 3 hours at 37°C and 128 rpm. Centrifuging cells at a speed of 12000 g for 5 minutes allowed for the collection of the cells. The cells were resuspended at a concentration of approximately 108 cells per ml using 1/2 McFarland standard after being washed in potassium phosphate buffer (50 mM with pH 7) following cell harvesting. After which, the mixture was incubated for 30 minutes with the membrane potential sensitive fluorescent probe (Bis-(1,3-Dibutylbarbituric Acid)Trimethine Oxonol). [(7)]

Aliquots of labelled cells were divided to form the groups for cell-free extract, control and treatment. The addition of 1 mili litre of bacteria or cell-free filtrate after adding one mili litre of 20 percent plant extract. One mili litre of potassium phosphate buffer was added to one mili litre of bacterial control groups. The fluorescence intensity was measured at 492 ano meter for excitation and 518 ano meter for emission using a fluorescence spectrophotometer. At room temperature, the excitation and emission slit widths were 5 nano meter and 10 nano meter, respectively. It was possible to see the background fluorescence that the extracts added to the media caused.

Analytical Statistics

The data display the standard deviation and the three replicates' average. Using SPSS version 20.0, a multiway analysis of variance was utilised to compare the outcomes, and a multiple range test was employed to compare the means.

Results Obtained

Table 1 provides the yield of each medicinal plant tested from the Nilgiri Bio Reserve after being treated conventionally or ultrasonically with water and ethanol. The conventional method's extraction yield exhibited a poor percentage yield when compared to those using the ultrasound approach (Table 1). The yield of water extracts made using the ultrasound approach was considerably higher (p 0.05) than that of ethanolic extracts for all of the plants that were studied (Table 1). Water extracts of different plants often offer considerably better yields compared to ethanolic extracts of the same plants. Both the enhanced polarity of the water and the extraction's

© ICAS 2022

30-minute use of a high temperature could be to blame. Additionally, the use of vibrations to break up plant cell walls caused chemicals and molecules to be released into the solvent. Use of ultrasound is highly recommended for compound extraction from a variety of sources and for use in a variety of applications. (8) In this study, antibacterial properties of 20% ethanolic and aqueous extracts of medicinal plants from the Nilgiri Bio Reserve were assessed against Aeromonas hydrophilla, Salmonella typhi, and Staphylococcus aureus species. The results demonstrated that specific plants' ethanolic and water extracts can successfully stop the growth of bacteria and diseases of varied severity. Both Table 2 and Figure 1 demonstrate this. The findings revealed that the examined bacteria's smallest number of colonies were present. It has been determined that thymol, which can adhere to membrane proteins and change their permeability by forming hydrogen and hydrophobic interactions with them, is what gives thyme its ability to prevent the growth of germs. Our research has revealed that the antibacterial properties of the medicinal plant extracts from the Nilgiri Bio Reserve are effective against the strains of Aeromonas hydrophilla, Salmonella typhi, and Staphylococcus aureus. (9) The current study confirms past findings in the literature that the antibacterial activities are directly correlated with increasing extract concentration (percentage). The considerable antimicrobial properties of the medicinal plants from the Nilgiri Bio Reserve against the test microorganisms are displayed in Table 3 as MIC values. The Nilgiri Bio Reserve's therapeutic plant extracts vary in their MIC, according to data. The ethanolic extracts of medicinal plants from the Nilgiri Bio Reserve have also been shown to include a wide range of distinct chemical components, including phenolic compounds and their derivative, fatty acid, esters of weak acid and terpene. These chemical elements can therefore affect a variety of target areas in bacterial cells. Similar results for MIC values were observed in other studies, with a few minor exceptions. (52) The development of efficient antibiotic tactics depends on knowledge of how plant crude extracts influence pathogenic microorganisms. It was therefore determined how plant extracts influenced membrane potential and pH (cytoplasmic) of Gram +ve and Gram -ve bacteria. Substantial decrease in cytoplasmic pH was brought of plant extracts (Figure 2). Variations in pH indicate damage to the bacterial cell membrane.

These findings are consistent with prior study in which we found that oregano essential oil, thymol, and carvacrol treatment of SA induces a drop in internal pHint. Overall, the pH (cytoplasmic) of the cells has an impact on how brightly the dye fluoresces; a low pH corresponds to a brightly fluorescing dye. But utilising DiBAC4, it was feasible to determine how SA and AH membrane potential had changed after being exposed to extracts of plant to Bis-(1,3-Dibutylbarbituric Acid)Trimethine Oxonol fluoresce dye. Our findings revealed a reduction in the fluorescence of labelled cells, indicating cell membrane hyperpolarization (Figure 3). The most important signs of membrane damage in bacterial cells has been proposed to be hyperpolarization. In cells with polarised membrane potential, $DiBAC_4(3)$ has increased permeability and accumulation since it is an anionic oxonol dye. This is because the cytoplasmic membrane contains more +ve charges than

© ICAS 2022

normal. Hyperpolarization causes inadequate absorption, which reduces the dye's concentration inside the bacterial cytoplasmic membrane and resulting in low fluorescence intensity. (10)

Conclusion

The findings show that ultrasound has a significant beneficial effect on improving extraction yield. Generally speaking, water and ethanolic extracts from specific plants have an antibacterial impact since they can stop the development of known pathogens and harmful germs. Cells treated with plant extracts showed a drop in cytoplasmic pH and rupturing of the cell wall, which could be a sign of an antibiotic's mode of action.

The study's findings suggest that the plant extracts could be utilised as all-natural antibacterial remedies to either inhibit the growth of dangerous pathogens or to eradicate them. (11) The Nilgiri plants' antibacterial properties, as well as their minimum inhibitory concentrations have been studied using the agar well diffusion method (Aeromonas hydrophilla, Salmonella typhi). In tests involving several microbes, extracts demonstrated antibacterial properties.

REFERENCE

[1] Khan UA, Rahman H, Niaz Z, Qasim M, Khan J, Tayyaba, Rehman B. Antibacterial activity of some medicinal plants against selected human pathogenic bacteria. Eur J Microbiol Immunol (Bp). 2013 Dec;3(4):272-4. doi: 10.1556/EuJMI.3.2013.4.6. Epub 2013 Nov 21. PMID: 24294497; PMCID: PMC3838543.

[2] Xu GH, Chen JC, Liu DH, Zhang YH, Jiang P, Ye XQ. Minerals, phenolic compounds, and antioxidant capacity of citrus peel extract by hot water. J Food Sci. 2008 Jan;73(1):C11-8. doi: 10.1111/j.1750-3841.2007.00546.x. PMID: 18211343.

[3] Buxani, Neelam & Gupta, Madhu & Shrivastava, Rakesh & Mehta, Darshana & Mehta, Bhupendra. (2016). Effect of extraction methods on yield, phytochemical constituents, antibacterial and antifungal activity of Capsicum frutescens L. Indian Journal of Natural Products and Resources. 7. 32-39.

[4] Felhi, Samir & Daoud, Amal & Hajlaoui, Hafedh & Mnafgui, Kais & Gharsallah, Neji & Kadri, Adel. (2017). Solvent extraction effects on phytochemical constituents profiles, antioxidant and antimicrobial activities and functional group analysis of Ecballium elaterium seeds and peels fruits. Food Science and Technology (Campinas). 37. 10.1590/1678-457x.23516.

[5] Nisha, M.C. & Subramanian, M.S. & Prathyusha, P. & Santhanakrishnan, R.. (2010). Comparative studies on antimicrobial activity of Artemisia sieversiana Ehrhart. Ex. Willd. and Origanum vuigare L. International Journal of PharmTech Research. 2. 1124-1127.

© ICAS 2022

[6] A. Mostafa, Ashraf & Al-Askar, Abdulaziz & Almaary, Khalid & Dawoud, Turki & Sholkamy, Dr. Essam & Bakri, Marwah. (2017). Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. Saudi Journal of Biological Sciences. 25. 10.1016/j.sjbs.2017.02.004.

[7] Sánchez E, García S, Heredia N. Extracts of edible and medicinal plants damage membranes of Vibrio cholerae. Appl Environ Microbiol. 2010 Oct;76(20):6888-94. doi: 10.1128/AEM.03052-09. Epub 2010 Aug 27. PMID: 20802077; PMCID: PMC2953017.

[8] Clementi, E. A. , Marks, L. R. , Roche - Hakansson, H. , and Hakansson, A. P. (2014). Clementi EA, Marks LR, Roche-Håkansson H, Håkansson AP. Monitoring changes in membrane polarity, membrane integrity, and intracellular ion concentrations in Streptococcus pneumoniae using fluorescent dyes. J Vis Exp. 2014 Feb 17;(84):e51008. doi: 10.3791/51008. PMID: 24637356; PMCID: PMC4124894.

[9] Liu Q, Meng X, Li Y, Zhao CN, Tang GY, Li HB. Antibacterial and Antifungal Activities of Spices. Int J Mol Sci. 2017 Jun 16;18(6):1283. doi: 10.3390/ijms18061283. PMID: 28621716; PMCID: PMC5486105.

[10] Thuille N, Fille M, Nagl M. Bactericidal activity of herbal extracts. Int J Hyg Environ Health. 2003 Jun;206(3):217-21. doi: 10.1078/1438-4639-00217. PMID: 12872531.

[11] Suzuki H, Wang ZY, Yamakoshi M, Kobayashi M, Nozawa T. Probing the transmembrane potential of bacterial cells by voltage-sensitive dyes. Anal Sci. 2003 Sep;19(9):1239-42. doi: 10.2116/analsci.19.1239. PMID: 14516073.

© ICAS 2022

Ann. For. Res. 65(1): 826-844, 2022
DOI: 10.5281/zenodo.7256398

Figure 1: The inhibitory zone (mili meter) of 20% (w/v) aqueous (water) and ethanolic extracts of (1) Achyranthes aspera, (2) Andrographis lobelioides, (3) Arisaema auriculatum, (4) Arisaema pulchrum and (5) Rhinacanthus nasutus on bacterial species of (Ah) Aeromonas hydrophilla, and (St) Salmonella typhi, and (Sa) Staphylococcus aureus.

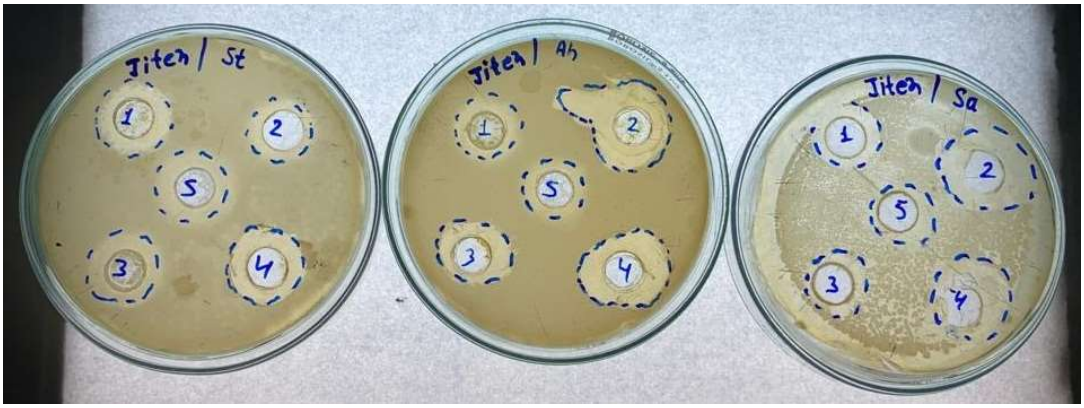
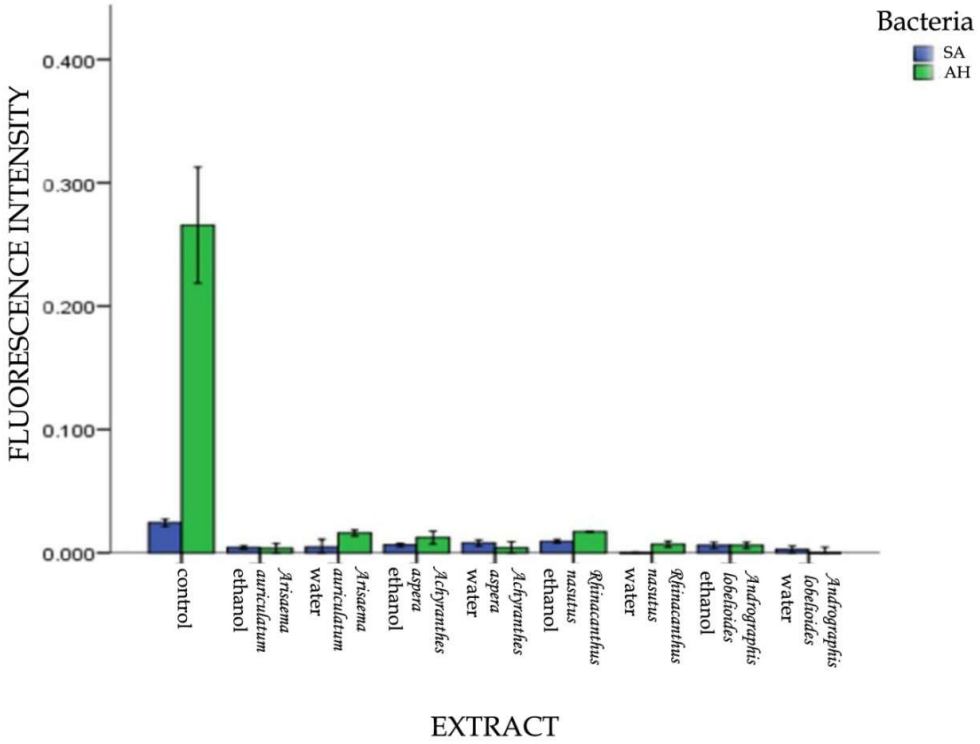


Figure 2: Effects of Rhinacanthus nasutus, Achyranthes aspera, and Arisaema auriculatum aqueous and ethanolic extracts on the pH (cytoplasmic) of Aeromonas hydrophilla and Staphylococcus aureus. Values are measurements means made in triplicate. Standard deviation is shown in bars.



© ICAS 2022

Figure 3: Membrane potentials effects of Aeromonas hydrophilla and Staphylococcus aureus of aqueous and ethanolic extracts of Andrographis lobelioides, Rhinacanthus nasutus, Achyranthes aspera, and Arisaema auriculatum. The values correspond to the average of three triplicate measurements. The standard deviation is represented by bars.

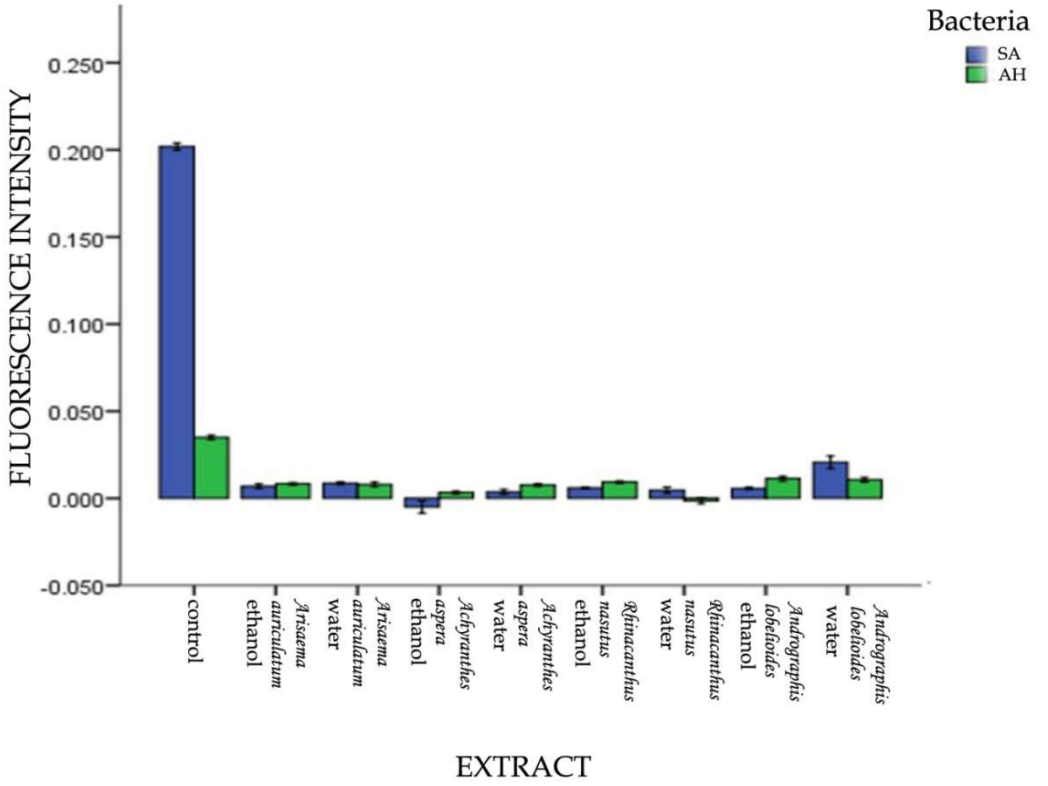


Table 1: Each species of medicinal plant from the Nilgiri Bio Reserve employed in this study was analysed, as well as the traditional and ultrasound methods' extraction yield percentages. (Values are triplate determination averages with (n-3) standard deviations.)

Botanical Name	Plant Part Used	Extraction Yield Method (%)			
		Water		Ethanol	
		Conventional	Ultrasound	Conventional	Ultrasound
Andrographis lobelioides	Leaves	21.11±1.36	21.76±0.95	20.67±1.22	11.14 ± 0.67
Rhinacanthus nasutus	Leaves	15.32±1.52	12.48±0.47	23.47±1.41	12.18 ± 0.40
Achyranthes aspera	Leaves	17.35±0.84	15.97±0.33	13.89±1.19	12.12±0.73
Arisaema auriculatum	Leaves	13.74±1.36	12.30±0.27	17.09±0.52	21.12±1.43

© ICAS 2022

Arisaema pulchrum	Leaves	21.59±0.92	12.14 ± 0.43	13.02±1.34	15.37±1.25
Caralluma nilagiriana	Leaves	12.36±0.17	15.85 ± 0.34	20.70±1.24	17.04±0.68
Helichrysum wightii	Flower	15.79±0.31	22.22 ± 0.48	23.94±1.54	13.55±1.67
Impatiens denisonii	Flower	12.28±0.27	20.00 ± 0.55	17.08±0.56	21.66±0.89
Impatiens laticornis	Flower	21.91±1.33	19.17 ± 1.76	23.74±1.74	12.74±0.79
Impatiens munronii	Flower	15.71±1.42	19.25 ± 1.12	13.96±1.98	15.78±0.03
Impatiens neo-barnesii	Flower	17.20±0.85	23.04±1.41	32.16 ± 0.26	12.93±0.70
Eriocaulon christopheri	Flower	13.53±1.66	13.99±1.19	62.36 ± 0.27	12.14 ± 0.43
Dalechampia velutina	Flower	21.46±0.97	17.80±0.52	17.50±0.85	15.85 ± 0.34
Swertia lawii	Flower	12.45±0.79	23.47±1.34	13.24±1.49	22.22 ± 0.48
Anisochilus dysophylloides	Flower	15.67±0.83	13.69±1.94	20.37±1.02	20.00 ± 0.55
Leucas pubescens	Flower	12.37±0.70	32.16 ± 0.26	23.42±1.40	19.17 ± 1.10
Litsea stocksii	Flower	12.14 ± 0.43	62.36 ± 0.27	13.19±1.99	19.25 ± 1.01
Dendrophthoe neelgherrensis	Flower	15.85 ± 0.34	17.05±0.55	17.10±0.58	23.40±1.94
Loranthus recurvus	Flower	22.22 ± 0.48	13.42±1.46	13.21±1.74	13.09±1.98
Memecylon lawsonii	Flower	20.00 ± 0.55	20.73±1.72	20.27±1.27	17.09±0.75
Sonerila versicolor	Flower	19.17 ± 1.10	23.42±1.49	23.43±1.64	23.84±1.46
Bulbophyllum aureum	Flower	19.25 ± 1.11	13.19±1.09	13.49±1.95	13.97±1.59
Bulbophyllum fusco-purpureum	Flower	23.14±1.94	17.01±0.50	17.05±0.45	17.60±0.54

© ICAS 2022

Bulbophyllum kaitiense	Flower	13.92±1.98	13.22±1.94	13.62±1.43	13.25±1.34
Coelogyne odoratissima	Flower	17.30±0.75	20.73±1.28	20.77±1.22	20.57±1.22
Corymborkis veratifolia	Flower	23.44±1.46	23.44±1.74	23.84±1.41	23.34±1.14
Eria nana	Flower	13.59±1.59	13.95±1.96	13.99±1.19	13.92±1.91
Eria polystachya	Flower	17.06±0.55	17.60±0.55	21.01±1.32	17.10±0.25
Habenaria cephalotes	Flower	13.72±1.44	13.27±1.46	15.30±1.23	13.21±1.43
Liparis biloba	Flower	20.78±1.23	20.87±1.52	17.95±0.48	20.27±1.42
Spiranthes sinensis	Flower	23.94±1.24	23.49±1.44	13.78±1.65	23.43±1.45
Symplocos microphylla	Flower	13.90±1.91	13.09±1.39	21.79±0.69	13.49±1.69
Viola hamiltoniana	Leaves	17.00±0.15	21.10±1.32	12.63±0.77	17.05±0.57
Arisaema translucens	Leaves	13.92±1.42	15.93±1.12	15.95±0.83	13.62±1.84
Myriophyllum tuberculatum	Leaves	20.78±1.32	17.58±0.81	12.42±0.79	20.77±1.29
Hedyotis leschenaultiana	Leaves	23.74±1.44	13.77±1.26	21.13±1.03	23.84±1.04
Grewia bracteata	Leaves	13.96±1.59	21.96±0.93	15.37±1.20	13.99±1.90
Impatiens gardneriana	Leaves	17.50±0.56	12.53±0.37	21.82±1.93	21.01±1.93
Berberis nilghiriensis	Leaves	13.24±1.74	15.94±0.34	15.12±1.28	15.30±1.28
Aeschynanthus perrottetii	Leaves	20.37±1.28	12.32±0.57	17.91±0.78	17.95±0.78
Lilium wallichianum	Leaves	23.42±1.94	21.12±1.36	13.21±1.66	13.78±1.66
Magnolia nilagirica	Leaves	13.19±1.90	15.27±1.72	21.43±0.59	21.79±0.59
Medinilla beddomei	Leaves	21.11±1.03	21.81±1.38	12.24±0.74	12.36±0.74

© ICAS 2022

Ligustrum robustum	Leaves	15.23±1.29	15.12±1.92	15.50±0.33	21.58±1.33
Bulbophyllum sterile	Leaves	17.53±0.88	17.92±0.80	12.48±0.72	15.24±1.22
Coelogyne mossiae	Leaves	13.47±1.67	13.31±1.06	12.65±0.17	17.39±0.18
Disperis neilgherrensis	Leaves	21.95±0.69	21.44±0.19	15.64±0.31	13.12±1.61
Eria pauciflora	Leaves	12.63±0.75	12.25±0.72	13.27±1.24	21.14±0.29
Gastrochilus acaulis	Leaves	15.97±0.43	15.60±0.33	20.87±1.23	12.21±0.72
Actephila excelsa	Leaves	12.82±0.73	12.87±0.74	23.49±1.44	15.20±0.33
Fragaria nilgerrensis	Leaves	21.19±1.23	12.86±0.57	13.09±1.95	12.83±0.47
Rosa leschenaultiana	Leaves	15.07±1.21	15.49±0.36	17.00±0.65	12.36±0.75
Vepris bilocularis	Leaves	13.14 ± 0.40	12.02±0.77	13.92±1.47	15.44±0.63
Boesenbergia tiliifolia	Leaves	20.00 ± 0.76	21.40±0.97	20.78±1.92	12.52±0.77
Curcuma neilgherrensis	Leaves	12.14 ± 0.34	12.92±0.78	23.94±1.48	15.85 ± 0.46

Table 2: Plant extracts' antimicrobial properties were tested against each medicinal plant from the Nilgiri Bio Reserve using test bacteria. (Values are the mean and (n-3) standard deviations of the triplate determination. N, meaning there was no zone of inhibition.)

Biological Name	**Zone of Inhibition (mm)a**	**Bacteria (Gram +ve)**	**Bacteria (Gram –ve)**	
		Staphylococcis aureus	***Aeromonas hydrophilla***	***Salmonella typhi***
Andrographis lobelioides	Ethanol	21.1+1.3	20.1+1.8	23.4+1.4
	Water	15.3+1.2	15.9+1.7	13.9+1.9
Rhinacanthus nasutus	Ethanol	17.5+0.8	14.2+2.0	17.0+0.5
	Water	13.7+1.6	13.8+1.8	13.2+1.4
Achyranthes aspera	Ethanol	21.9+0.9	N	20.7+1.2
	Water	12.3+0.7	N	N

© ICAS 2022

Arisaema auriculatum	Ethanol	15.9+0.3	13.3+1.3	N
	Water	12.2+0.7	14.7+0.1	N
Arisaema pulchrum	Ethanol	21.1+1.3	20.4+1.2	23.4+1.4
	Water	15.7+1.2	N	13.9+1.9
Caralluma nilagiriana	Ethanol	17.0+0.8	N	17.0+0.5
	Water	13.5+1.6	N	23.4+1.4
Helichrysum wightii	Ethanol	21.6+0.9	21.7+1.3	13.9+1.9
	Water	12.4+0.7	15.6+1.2	17.0+0.5
Impatiens denisonii	Ethanol	15.7+0.3	17.5+0.8	13.2+1.4
	Water	12.3+0.7	13.9+1.6	20.7+1.2
Impatiens laticornis	Ethanol	N	21.1+0.9	23.4+1.4
	Water	21.8+1.3	12.2+0.7	13.9+1.9
Impatiens munronii	Ethanol	15.2+1.2	15.8+0.3	17.0+0.5
	Water	17.9+0.8	12.2+0.7	13.2+1.4
Impatiens neo-barnesii	Ethanol	13.1+1.6	N	20.7+1.2
	Water	21.4+0.9	N	23.4+1.4
Eriocaulon christopheri	Ethanol	12.2+0.7	N	13.9+1.9
	Water	15.0+0.3	21.1+1.3	17.0+0.5
Dalechampia velutina	Ethanol	12.8+0.7	15.9+1.2	13.2+1.4
	Water	12.6+0.7	17.6+0.8	20.7+1.2
Swertia lawii	Ethanol	15.4+0.3	13.2+1.6	23.4+1.4
	Water	12.2+0.7	21.8+0.9	13.9+1.9
Anisochilus dysophylloides	Ethanol	20.1+1.8	23.4+1.4	21.1+1.3
	Water	15.9+1.7	13.9+1.9	15.3+1.2
Leucas pubescens	Ethanol	14.2+2.0	17.0+0.5	17.5+0.8
	Water	13.8+1.8	13.2+1.4	13.7+1.6
Litsea stocksii	Ethanol	N	20.7+1.2	21.9+0.9
	Water	N	N	12.3+0.7
Dendrophthoe neelgherrensis	Ethanol	13.3+1.3	N	15.9+0.3
	Water	14.7+0.1	N	12.2+0.7
Loranthus recurvus	Ethanol	20.4+1.2	23.4+1.4	21.1+1.3
	Water	N	13.9+1.9	15.7+1.2
Memecylon lawsonii	Ethanol	N	17.0+0.5	17.0+0.8
	Water	N	23.4+1.4	13.5+1.6
Sonerila versicolor	Ethanol	21.7+1.3	13.9+1.9	21.6+0.9
	Water	15.6+1.2	17.0+0.5	12.4+0.7
	Ethanol	17.5+0.8	13.2+1.4	15.7+0.3

© ICAS 2022

Bulbophyllum aureum	Water	13.9+1.6	20.7+1.2	12.3+0.7
Bulbophyllum fusco-purpureum	Ethanol	21.1+0.9	23.4+1.4	N
	Water	12.2+0.7	13.9+1.9	21.8+1.3
Bulbophyllum kaitiense	Ethanol	15.8+0.3	17.0+0.5	15.2+1.2
	Water	12.2+0.7	13.2+1.4	17.9+0.8
Coelogyne odoratissima	Ethanol	N	20.7+1.2	13.1+1.6
	Water	N	23.4+1.4	21.4+0.9
Corymborkis veratifolia	Ethanol	N	13.9+1.9	12.2+0.7
	Water	21.1+1.3	17.0+0.5	15.0+0.3
Eria nana	Ethanol	15.9+1.2	13.2+1.4	12.8+0.7
	Water	17.6+0.8	20.7+1.2	12.6+0.7
Eria polystachya	Ethanol	13.2+1.6	23.4+1.4	15.4+0.3
	Water	21.8+0.9	13.9+1.9	12.2+0.7
Habenaria cephalotes	Ethanol	15.9+0.3	N	N
	Water	12.2+0.7	21.1+1.3	N
Liparis biloba	Ethanol	21.1+1.3	20.1+1.8	23.4+1.4
	Water	15.3+1.2	15.9+1.7	13.9+1.9
Spiranthes sinensis	Ethanol	17.5+0.8	14.2+2.0	17.0+0.5
	Water	13.7+1.6	13.8+1.8	13.2+1.4
Symplocos microphylla	Ethanol	21.9+0.9	N	20.7+1.2
	Water	12.3+0.7	N	N
Viola hamiltoniana	Ethanol	15.9+0.3	13.3+1.3	N
	Water	12.2+0.7	14.7+0.1	N
Arisaema translucens	Ethanol	21.1+1.3	20.4+1.2	23.4+1.4
	Water	15.7+1.2	N	13.9+1.9
Myriophyllum tuberculatum	Ethanol	17.0+0.8	N	17.0+0.5
	Water	13.5+1.6	N	23.4+1.4
Hedyotis leschenaultiana	Ethanol	21.6+0.9	21.7+1.3	13.9+1.9
	Water	12.4+0.7	15.6+1.2	17.0+0.5
Grewia bracteata	Ethanol	15.7+0.3	17.5+0.8	13.2+1.4
	Water	12.3+0.7	13.9+1.6	20.7+1.2
Impatiens gardneriana	Ethanol	N	21.1+0.9	23.4+1.4
	Water	21.8+1.3	12.2+0.7	13.9+1.9
Berberis nilghiriensis	Ethanol	15.2+1.2	15.8+0.3	17.0+0.5
	Water	17.9+0.8	12.2+0.7	13.2+1.4

© ICAS 2022

Aeschynanthus perrottetii	Ethanol	13.1+1.6	N	20.7+1.2
	Water	21.4+0.9	N	23.4+1.4
Lilium wallichianum	Ethanol	12.2+0.7	N	13.9+1.9
	Water	15.0+0.3	21.1+1.3	17.0+0.5
Magnolia nilagirica	Ethanol	12.8+0.7	15.9+1.2	13.2+1.4
	Water	12.6+0.7	17.6+0.8	20.7+1.2
Medinilla beddomei	Ethanol	15.4+0.3	13.2+1.6	23.4+1.4
	Water	12.2+0.7	21.8+0.9	13.9+1.9
Ligustrum robustum	Ethanol	N	15.9+0.3	N
	Water	N	12.2+0.7	21.1+1.3
Bulbophyllum sterile	Ethanol	17.0+0.5	17.0+0.8	N
	Water	23.4+1.4	13.5+1.6	N
Coelogyne mossiae	Ethanol	13.9+1.9	21.6+0.9	21.7+1.3
	Water	17.0+0.5	12.4+0.7	15.6+1.2
Disperis neilgherrensis	Ethanol	13.2+1.4	15.7+0.3	17.5+0.8
	Water	20.7+1.2	12.3+0.7	13.9+1.6
Eria pauciflora	Ethanol	23.4+1.4	N	21.1+0.9
	Water	13.9+1.9	21.8+1.3	12.2+0.7
Gastrochilus acaulis	Ethanol	17.0+0.5	15.2+1.2	15.8+0.3
	Water	13.2+1.4	17.9+0.8	12.2+0.7
Actephila excelsa	Ethanol	20.7+1.2	13.1+1.6	N
	Water	23.4+1.4	21.4+0.9	N
Fragaria nilgerrensis	Ethanol	13.9+1.9	12.2+0.7	N
	Water	17.0+0.5	15.0+0.3	21.1+1.3
Rosa leschenaultiana	Ethanol	13.2+1.4	12.8+0.7	15.9+1.2
	Water	20.7+1.2	12.6+0.7	17.6+0.8
Vepris bilocularis	Ethanol	23.4+1.4	15.4+0.3	13.2+1.6
	Water	13.9+1.9	12.2+0.7	21.8+0.9
Boesenbergia tiliifolia	Ethanol	N	N	15.9+0.3
	Water	21.1+1.3	N	12.2+0.7
Curcuma neilgherrensis	Ethanol	20.1+1.8	23.4+1.4	21.1+1.3
	Water	15.9+1.7	13.9+1.9	15.3+1.2

© ICAS 2022

Table 3: The Nilgiri Bio Reserve's extracts of each medicinal plant were tested for test microorganisms using their Minimum Inhibitory Concentration. (Values are the mean and standard deviations of the triplate determination. N, meaning there was no zone of inhibition.)

Botanical Name	(% w/v) minimum inhibitory concentration	Bacteria (Gram +ve)	Bacteria (Gram –ve)	
		Staphylococcis aureus	***Aeromonas hydrophilla***	***Salmonella typhi***
Andrographis lobelioides	Ethanol	5	2.5	2.5
	Water	5	5	5
Rhinacanthus nasutus	Ethanol	2.5	0.625	5
	Water	5	2.5	10
Achyranthes aspera	Ethanol	5	N	N
	Water	20	N	N
Arisaema auriculatum	Ethanol	10	2.5	N
	Water	5	10	N
Arisaema pulchrum	Ethanol	5	5	2.5
	Water	N	5	5
Caralluma nilagiriana	Ethanol	N	1.25	20
	Water	2.5	5	1.25
Helichrysum wightii	Ethanol	5	0.313	5
	Water	5	2.5	2.5
Impatiens denisonii	Ethanol	10	0.625	2.5
	Water	5	5	2.5
Impatiens laticornis	Ethanol	2.5	5	5
	Water	N	10	5
Impatiens munronii	Ethanol	5	5	N
	Water	20	1.25	N
Impatiens neo-barnesii	Ethanol	N	N	20
	Water	1.25	5	2.5
Eriocaulon christopheri	Ethanol	5	N	N
	Water	5	2.5	5
Dalechampia velutina	Ethanol	10	2.5	5
	Water	2.5	2.5	5
Swertia lawii	Ethanol	5	0.625	2.5
	Water	2.5	0.625	10
Anisochilus dysophylloides	Ethanol	5	5	5
	Water	2.5	5	5

© ICAS 2022

Leucas pubescens	Ethanol	2.5	2.5	5
	Water	2	10	5
Litsea stocksii	Ethanol	2.5	2.5	10
	Water	5	N	20
Dendrophthoe neelgherrensis	Ethanol	N	5	5
	Water	10	2.5	5
Loranthus recurvus	Ethanol	5	0.625	2.5
	Water	5	5	5
Memecylon lawsonii	Ethanol	2.5	2.5	5
	Water	2.5	5	2.5
Sonerila versicolor	Ethanol	5	5	5
	Water	5	2.5	0.625
Bulbophyllum aureum	Ethanol	10	5	2.5
	Water	2.5	5	5
Bulbophyllum fusco-purpureum	Ethanol	N	20	N
	Water	5	10	2.5
Bulbophyllum kaitiense	Ethanol	N	5	10
	Water	2.5	5	5
Coelogyne odoratissima	Ethanol	5	N	5
	Water	20	N	1.25
Corymborkis veratifolia	Ethanol	1.25	2.5	5
	Water	5	5	1.5
Eria nana	Ethanol	2.5	5	2.5
	Water	2.5	10	0.625
Eria polystachya	Ethanol	2.5	5	5
	Water	5	2.5	5
Habenaria cephalotes	Ethanol	5	N	10
	Water	N	5	5
Liparis biloba	Ethanol	N	20	1.25
	Water	20	N	N
Spiranthes sinensis	Ethanol	2.5	1.25	5
	Water	N	5	N
Symplocos microphylla	Ethanol	5	5	2.5
	Water	5	10	2.5
Viola hamiltoniana	Ethanol	5	2.5	2.5
	Water	2.5	5	0.625
Arisaema translucens	Ethanol	10	2.5	0.625
	Water	5	5	5

© ICAS 2022

Myriophyllum tuberculatum	Ethanol	5	2.5	5
	Water	5	2.5	2.5
Hedyotis leschenaultiana	Ethanol	5	N	10
	Water	10	N	2.5
Grewia bracteata	Ethanol	20	N	N
	Water	5	N	N
Impatiens gardneriana	Ethanol	5	10	2.5
	Water	2.5	5	0.625
Berberis nilghiriensis	Ethanol	5	5	5
	Water	5	2.5	2.5
Aeschynanthus perrottetii	Ethanol	5	5	N
	Water	1.25	20	N
Lilium wallichianum	Ethanol	5	1.25	2.5
	Water	1.5	5	5
Magnolia nilagirica	Ethanol	2.5	2.5	5
	Water	0.625	2.5	10
Medinilla beddomei	Ethanol	5	2.5	5
	Water	5	5	2.5
Ligustrum robustum	Ethanol	10	5	N
	Water	5	N	5
Bulbophyllum sterile	Ethanol	1.25	N	20
	Water	N	20	N
Coelogyne mossiae	Ethanol	5	2.5	1.25
	Water	N	N	5
Disperis neilgherrensis	Ethanol	2.5	5	5
	Water	2.5	5	10
Eria pauciflora	Ethanol	2.5	5	2.5
	Water	0.625	2.5	5
Gastrochilus acaulis	Ethanol	0.625	10	2.5
	Water	5	5	5
Actephila excelsa	Ethanol	5	5	2.5
	Water	2.5	5	2.5
Fragaria nilgerrensis	Ethanol	10	5	N
	Water	2.5	10	N
Rosa leschenaultiana	Ethanol	N	20	N
	Water	N	5	N
Vepris bilocularis	Ethanol	2.5	5	10
	Water	0.625	2.5	5

© ICAS 2022

Boesenbergia tiliifolia	Ethanol	5	5	5
	Water	2.5	5	2.5
Curcuma neilgherrensis	Ethanol	5	5	2.5
	Water	2.5	5	2.5

© ICAS 2022