BIOACTIVITY SCREENING OF SELECTED TRADITIONAL MEDICINAL PLANTS OF KERALA

ALBY ALPHONS BABY1,2,*, REGI RAPHAEL K1,2
1Research and Development Centre, Bharathiar University, Coimbatore, Tamil Nadu, India. 2Department of Botany, St. Mary’s College, Thrissur, Kerala, India. Email: albyalphons@gmail.com

Received: 23 June 2018, Revised and Accepted: 07 August 2018

INTRODUCTION

Ayurvedic, tribal medicine, and folk medicines are the three plants based on healing systems in Kerala. Ayurvedic system possesses a very good written documents and history. Tribal medicine is the medicinal knowledge of forest-dwelling tribals, which is not having any documentation and procedure. Folk medicine is the orally transmitting knowledge on medicinal plants for the primary health care [1]. The wide acceptance of traditional medicine as an alternative form of health care and the alarming increase in the incidence of new and reemerging infectious diseases bring about the necessity to investigate these medicinal plants. Medicinal values of various plants are due to the presence of various bioactive compounds such as phenolic compounds, alkaloids, flavonoids, and tannins that produce definite physiological action in the human body [2].

Stereospermum suaveolens DC is a medicinal tree species native to India, Bangladesh, and Myanmar. The Bignoniaceae family having about 100 genera with 800 species is known for their antimicrobial and antiprotozoal properties [3].

Naravelia zeylanica DC belonging to the family Ranunculaceae is a woody climber in habit with tuberous roots, opposite and cordate leaflets, and flowers are small and arranged in panicles. Red-colored achenes with long feathery styles occur in the hot to warm regions in India [4]. The plant is traditionally used by the healers to treat pitta, vitiated vata, inflammations, skin diseases, headache, arthritis, colic, wounds, and ulcers [5]. Leaf paste is consumed to treat chest pain. The vines when crushed give a pungent odor which is inhaled to cure a cold and all types of headaches including migraine [6].

Keywords: Stereospermum suaveolens, Hygrophila spinosa, Naravelia zeylanica, Antimicrobial, Anthelmintic, Antioxidant, Traditional medicine.

METHODS

Collection of plant material and preparation of the extract

The fresh leaves of S. suaveolens and H. spinosa and aerial parts of N. zeylanica were collected in March 2014 from Mannamangalam village of Thrissur District and shade dried for several days. The coarse powder of the dried plant materials was prepared, and 50 g of the powder was soaked in 95% ethanol (1:5) for 72 h. Using a rotary evaporator, the solvents were removed. For the future studies, the concentrated extracts were refrigerated [11].

Antimicrobial assay

Organisms and culture media

The microorganisms for the antimicrobial screening were collected from the Microbiology Laboratory, St. Mary’s College, Thrissur. The pathogenic organisms used were Bacillus cereus, Staphylococcus aureus, Streptococcus pyogenes, Klebsiella pneumoniae, Salmonella
typhi, Pseudomonas aeruginosa, Candida albicans, Aspergillus niger, and Penicillium notatum. Nutrient agar (NA) was used to maintain bacterial cultures, and fungal cultures were maintained on Sabouraud Dextrose Agar (SDA).

**Antibacterial and antifungal activity of the plant extract**

Well diffusion assay [12] on NA and SDA plates was used to determine the antibacterial and antifungal properties, respectively. To prepare the microbial suspension, bacteria inoculated into nutrient broth (NB) and fungus into Sabouraud dextrose broth (SDB) and incubated at 37°C for 6 h. The transmittance of the resulting suspension was diluted using NB and SDB for getting the value 74.3% (absorbance of 0.132) at 600 nm. This percentage is comparable to 0.5 McFarland turbidity standards. This level of turbidity is equivalent to approximately 1.5×10^8 CFU/mL [13]. On the surface of NA plates, bacterial cultures were inoculated and fungal cultures were inoculated on SDA plates. Subsequently, wells with a diameter of 6 mm were prepared on NA and SDA plates using sterile cork borer, and 25 µL of sample in different concentrations (100 µg/mL, 250 µg/mL, and 500 µg/mL) was loaded in each well. Antibiotics were used as positive control (chloramphenicol for bacteria and fluconazole for fungus) [14]. The tests were carried out in triplicates. The plates were incubated at 37°C for 24 h. Zone of clearing was measured at the end of the incubation period using a transparent ruler. Zones of inhibition >6 mm were taken as susceptible to the extracts.

**Antimicrobial screening**

The results of the study showed that the ethanolic extracts of S. suaveolens leaves, H. spinosa leaves, and aerial parts of N. zeylanica had prominent antimicrobial activity against the human pathogenic bacteria and fungi studied [Tables 1-6]. All the medicinal plants screened were effective against all the bacterial and fungal species studied, while S. suaveolens shows more prominent antifungal activity with maximum zone of growth inhibition against Aspergillus flavus and C. albicans (23.3±0.57 mm and 23.6±0.57 mm at 500 µg/mL concentration). C. albicans was resistant to fluconazole, but it showed promising activity with plant extracts, and the maximum zone of inhibition against C. albicans was observed in N. zeylanica (24±1 mm at 500 µg/mL).

**RESULTS AND DISCUSSION**

**Antibacterial and antifungal activity of S. suaveolens leaves**

| Organism        | Chloramphenicol | Fluconazole |
|-----------------|-----------------|-------------|
|                 | 100µg | 250µg | 500µg | 100µg | 250µg | 500µg |
| K. pneumoniae   | 25±1  | 7.3±0.15 | 9.6±0.57 | 11.3±1.15 |
| S. typhi        | 23±0.57 | 9.6±0.57 | 13±1 | 16.6±0.57 |
| P. aeruginosa   | 11.6±0.57 | 6.6±0.57 | 8.6±0.57 | 10.6±1.15 |
| B. cereus       | 20.6±0.57 | 7.3±1.15 | 11±1 | 12.6±1.15 |
| S. pyogenes     | 18.6±1.15 | 6.6±1.15 | 9.6±0.57 | 12.3±0.57 |
| S. aureus       | 23.3±0.57 | 7.6±0.57 | 10±1 | 12.6±1.15 |

S. suaveolens: Stereospermum suaveolens, K. pneumoniae: Klebsiella pneumoniae, S. typhi: Salmonella typhi, P. aeruginosa: Pseudomonas aeruginosa, B. cereus: Bacillus cereus, S. pyogenes: Streptococcus pyogenes, S. aureus: Staphylococcus aureus.

**Antioxidant property screening**

**2,2-Diphenyl 1-picrylhydrazyl (DPPH) radical scavenging assay**

Free radical scavenging activity of the plant extracts was assessed on the basis of the radical scavenging effect of the stable DPPH, by a modified method [16]. The diluted working solutions of the test extracts (10–1000 µg/mL concentration) and 6.34 µM solution of DPPH were prepared in methanol, and 100 µL of drug to be tested, 100 µL DPPH solution, and 800 µL of methanol were taken in a test tube and mixed well. These solution mixtures were incubated in the dark for 20 min. Optical density was measured after incubation at 517 nm using Cecil-elect spectrophotometer. Methanol (900 µL) with DPPH solution (6.34 µM, 100 µL) was taken as control and methanol as blank. The optical density was recorded, and to calculate the percentage of inhibition, the following formula was used:

\[
\text{Percent (%) inhibition of DPPH activity} = \frac{A-B}{A} \times 100
\]

Where A = optical density of the control and B = optical density of the sample.

**Super oxide radical scavenging assay**

In vitro superoxide radical scavenging activity was measured by NBT reduction method [17]. In the presence of light, riboflavin undergoes auto-oxidation and forms superoxide radicals, and it reduces NBT to a blue-colored formazan which can be measured at 590 nm.

200 µL EDTA, 100 µL riboflavin solution, 200 µL ethanol, and 100 µL NBT solution were mixed in a test tube and made up to 3 mL using phosphate buffer. The solution was incubated in light for 15 min, and the absorbance of the resulting solution was measured at 590 nm using phosphate buffer as blank. This was taken as control reading. For screening of test sample, along with the above solutions, added 100 µL sample of varying concentrations (10–1000 µg/mL), and finally, the volume was made up to 3 mL using phosphate buffer and the reading was taken after 15 min of illumination. The formula given below was used to find out the percentage of inhibition:

\[
\text{Percent (%) inhibition} = \frac{A-B}{A} \times 100
\]

Where A = optical density of the control and B = optical density of the sample.
Table 4: Antifungal property of *H. spinosa* leaves

| Organism  | Zone of inhibition (mm) |
|-----------|-------------------------|
|           | Fluconazole (15 µg)     | 100 µg | 250 µg | 500 µg |
| A. niger  | 7.6±0.57                | 8.3±0.57 | 11.7±1.15 | 15.3±0.57 |
| A. flavus | 7.6±0.57                | 9.6±0.57 | 12.6±1.15 | 14.3±0.57 |
| P. notatum| 7.6±0.57                | 8.3±0.57 | 9.6±0.57  | 12.6±1.15 |
| C. albicans| R                      | 9.7±1.15 | 14.3±0.58 | 16.6±0.57 |

Due to the reported development of resistance by bacteria and fungi to various commercially available antimicrobial agents, the plant extracts are potential sources of new compounds, which may be developed as effective drugs against the infectious microorganisms. Further, the use of these plants may offer a new source of antifungal agent against the pathogenic fungus such as *C. albicans* which inhibited by the crude drugs in dose-dependent manner.

Table 5: Antibacterial property of *N. zeylanica* aerial part

| Organism  | Zone of inhibition |
|-----------|--------------------|
|           | Chloramphenicol (25 µg) | 100 µg | 250 µg | 500 µg |
| K. pneumoniae | 18.7±1.2               | 7.6±1.2 | 9.6±0.57 | 11.7±1.2 |
| S. typhi   | 16.6±0.57             | 7.7±1.15 | 9.7±1.15 | 12.6±0.57 |
| P. aeruginosa| 9.3±1.2                | 7.6±0.57 | 10.6±0.57 | 13.4±0.92 |
| B. cereus  | 16.7±1.15             | 8.7±1.15 | 11.6±0.57 | 13.3±0.57 |
| S. pyogenes| 17.6±2.5              | 8.7±0.57 | 10.6±1.2  | 12.6±0.57 |
| S. aureus  | 14.6±0.72             | 9.6±0.57 | 12.6±0.57 | 14.2±0.57 |

Table 6: Antifungal property of *N. zeylanica* aerial part

| Organism  | Zone of inhibition (mm) |
|-----------|-------------------------|
|           | Fluconazole (15 µg)     | 100 µg | 250 µg | 500 µg |
| A. niger  | 12.5±0.76               | 16.6±2.5 | 23.3±1.15 | 23.4±1.15 |
| A. flavus | 14.3±0.64               | 11.1±1   | 12.6±1.15 | 18.6±1.15 |
| P. notatum| 8.6±0.57                | 14.6±1.15 | 17.6±1.15 | 19.3±0.57 |
| C. albicans| R                      | 17.6±0.57 | 12.6±0.57 | 24±1 |

Table 7: Anthelmintic property of *S. suaveolens* leaves

| Observation                  | Distilled water | Albendazole (25 mg/mL) | Drug (25 mg/mL) | Drug (50 mg/mL) | Drug (100 mg/mL) |
|-----------------------------|-----------------|------------------------|-----------------|-----------------|-----------------|
| Time taken for paralysis (min) | -               | 27±0.72               | 38±1.53         | 25.7±2.08       | 13±1.19         |
| Time taken for death (min)   | -               | -                     | 78.6±2.3       | 63.3±2.5        | 27.3±2.08       |

*S. suaveolens: Stereospermum suaveolens*

Table 8: Anthelmintic property of *H. spinosa* leaves

| Observation                  | Distilled water | Albendazole (25 mg/mL) | Drug (25 mg/mL) | Drug (50 mg/mL) | Drug (100 mg/mL) |
|-----------------------------|-----------------|------------------------|-----------------|-----------------|-----------------|
| Time taken for paralysis (min) | -               | 32.4±2                 | 56.3±0.57       | 32.7±3.21       | 24±1.73         |
| Time taken for death (min)   | -               | -                     | 119.7±3.5      | 53±2            | 43.7±1.53       |

*H. spinosa: Hygrophila spinosa*

Table 9: Anthelmintic property of *N. zeylanica* aerial part

| Observation                  | Distilled water | Albendazole (25 mg/mL) | Drug (25 mg/mL) | Drug (50 mg/mL) | Drug (100 mg/mL) |
|-----------------------------|-----------------|------------------------|-----------------|-----------------|-----------------|
| Time taken for paralysis (min) | -               | 29±2                   | 28±1.5          | 21±2            | 16±3            |
| Time taken for death (min)   | -               | -                      | 35±2.3          | 31±2.6          | 21±2            |

*N. zeylanica: Naravelia zeylanica*
The present study indicates that the studied medicinal plant extracts could inhibit the oxygen radicals as seen from scavenging superoxide and DPPH radicals, and it could reduce the oxygen radicals and subsequently reduce the harmful effects. The literature supports that phytoconstituents such as polyphenolic compounds in drugs are responsible for the antioxidant potential [22,23]. Further, phenolic compounds are effective hydrogen donors, which make them antioxidant [24]. Leaves of S. suaveolens and H. spinosa and aerial parts of N. zeylanica possess valuable secondary metabolites such as phenols, flavonoids, alkaloids, and tannins [3, 25, 26], the observed activity may be due to the presence of these phytoconstituents.

**CONCLUSION**

All the extracts showed varying degrees of antimicrobial, anthelmintic, and antioxidant properties. All these plants were more effective than the commercial antibiotics in combating the helminth studied. To combat pathogenic microorganisms, the folk medicines screened in this *in vitro* study can be used as effective agent due to the efficiency and less side effects. The luxuriant use of these medicinal plants in folk medicine proves that they represent a safe and economic alternative to treat various infectious diseases and oxidative stress damages.

**ACKNOWLEDGMENTS**

The authors are grateful to St. Mary’s College Thrissur, Kerala, India, for the provision of laboratory facilities and to the traditional healers for sharing their valuable knowledge.

**AUTHORS’ CONTRIBUTION**

Alby Alphons Baby has performed all the experiments in the laboratory. Regi Rahael K has provided the design, intellectual content to choose the plant, and act as a mentor for the works.

**CONFLICTS OF INTEREST**

The authors declare that they do not have any conflicts of interest.

**REFERENCES**

1. Beegam AR, Nayar TS. Plants used for natal health care in folk medicines of Kerala, India. Indian J Tradit Knowledge 2011;10:523–74.
2. Nastro A, Germano M, Marino DV, Cannatelli M. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett Appl Microbiol 2000;30:379–84.
3. Meena K, Yadav AK, Panda P, Preet K, Rao MM. Review on Stereospermum suaveolens (Roxb.) DC; a potential herb. Drug Invert Today 2010;2:238–39.
4. Gamble JS. Flora of the Presidency of Madras. Dehradun: Bishen Singh and Mahendra Pal Singh; 1915.
5. Udayan PS, George S, Tushar KV, Balachandran I. Medicinal plants used by malayali tribes of Sevarayan hills Yercad, Salem district, Tamilnadu, India. Zoo Print J 2006;21:2223–4.
6. Anonymous. The Useful Plants of India. New Delhi: NISCAIR; 1992. p. 392.
7. Asolkar LV, Kakkar KK, Chakre OJ. Second Supplement to Glossary of Indian Medicinal Plants with Active Principles. New Delhi: NISCAIR, CSIR; 2005.
8. Khare CP. Indian Medicinal Plants: An Illustrated Dictionary. India: Springer Publications; 2007.
9. Sharma PC, Yelne MB, Dennis TJ. Database on Medicinal Plants used by Ayurveda and Siddha; 2002.
10. Rastogi RP, Mehrotra BN. Compendium of Indian Medicinal Plants. Vol. 3. New Delhi: National Publication and Information Directorate, CSIR; 1993.
11. Baby AA, Raphael KR. First step towards unravelling the medicinal properties of an endemic traditional medicine Bauhinia pinnata Willd. J Pharm Sci 2015;7:403–5.
12. Rojas JJ, Ochoa VJ, Ocampo SA, Munoz JF. Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: A possible alternative in the treatment of nosocomial infections. BMC Complement Altern Med 2006;6:2.
13. du Toit EA, Rautenbach M. A sensitive standardized micro-gel well diffusion assay for the determination of antimicrobial activity. J Microbiol Methods 2000;42:159–65.
14. Vital PG, Velasco RN, Demigillo JM, Rivera WL. Antimicrobial activity, cytotoxicity and phytochemical screening of Ficus septica Burm and Sterculia foetida L. leaf extracts. J Med Plant Res 2010;4:58–63.
15. Baby AA, Raphael KR. Potential antimicrobial, anthelmintic and antioxidant properties of Areca catechu L root. Int J Pharm Pharm Sci 2014;6:486–89.
16. Braca A, Sortino C, Politi M. Anti-oxidant activity of flavonoids from...
Licania licaniaeflora. J Ethnopharmacol 2002;79:379-81.
17. Deb L, Dubey SK, Jain A, Jain AK, Pandian GS. Preventive effect of Thuya occidentalis (Linn) on gastric ulcer—a novel role of free radical scavenger. J Nat Remedies 2009;9:152-8.
18. Andlauer W, Farst P. Antioxidative power of phytochemicals with special reference to cereals. Cereal Foods World 1998;43:356-9.
19. Jayaprapashka GK, Selvi T, Sakariah KK. Antibacterial and antioxidant activities of grape (Vitis vinifera) seed extract. Food Res Int 2003;36:117-22.
20. Uttara B, Ajay VS, Zambi P, Mahajan RT. Oxidative stress and neurodegenerative disease: A review of upstream and downstream antioxidant therapeutic options. Curr Neuropharmacol 2009;7:65-74.
21. Koleva II, Van Beek TA, Linsee JP, de Groot A, Evstatieva LN. Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. Phytochem Anal 2002;13:8-17.
22. Khushad MM, Masuunas MA, Smith W, Eastmank K. Health promoting phytochemicals in vegetables. Hortic Rev 2003;28:125-85.
23. Goojer CR, Evade H, Zappay H. Liquid chromatography with atmospheric pressure chemical ionization and electrospray ionization mass spectrometry of flavonoids with triple quadrupole and ion trap instruments. J Chromatogr 1997;984:45-8.
24. Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB. The relative antioxidant activity of plant derived polyphenolic flavonoids. Free Radic Res 1995;22:375-83.
25. Sutharsingh R, Kavimani S, Jayakar B, Uvarani M, Thangathirupathi A. Quantitative phytochemical estimation and Antioxidant studies on aerial parts of Naravelia zeylanica DC. Int J Pharm Stud Res 2011;2:52-6.
26. Kshirsagar AD, Ingale KG, Vyawahare NS, Thorve VS. Hygrophila spinosa: A comprehensive review. Pharmacogn Rev 2010;4:167-71.