RESEARCH
www.commprac.com
ISSN 1462 2815

SYNTHESIS AND CHARACTERIZATION OF ANTIBACTERIAL ACTIVITY OF *SPERMACOCE HISPIDA* LEAF EXTRACT

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DOI: 10.5281/zenodo.12592369

Abstract

Spermacoce hispida, commonly known as "Hairy Broomweed" or "False Buttonweed," is noted for its reported antibacterial activity against various pathogenic microorganisms. In this study, we aimed to synthesize and characterize the antibacterial activity of *Spermacoce hispida* leaf extract, contributing to the field of natural product-based antibacterial research. Silver nanoparticles were synthesized using the leaf extract and characterized using X-ray diffraction (XRD) and scanning electron microscopy (SEM). The antibacterial activity of the synthesized nanoparticles was evaluated against Escherichia coli and Staphylococcus aureus using the agar well diffusion method. Results revealed amorphous silver nanoparticles with spherical shapes, exhibiting significant antibacterial activity against both bacterial strains. The relatively low minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values suggest the extract's potential as a natural antibacterial agent. Further studies are warranted to isolate and identify specific bioactive compounds responsible for this activity and assess their safety and efficacy for potential therapeutic applications.

Keywords: *Spermacoce Hispida,* Antibacterial Activity, Silver Nanoparticles, Synthesis, Characterization, Escherichia Coli, Staphylococcus Aureus.

INTRODUCTION

Spermacoce hispida , a member of the Rubiaceae family, is a plant species with a rich history of traditional medicinal use across various cultures(Vimalanathan, Ignacimuthu et al. 2009). Commonly known as "Hairy Broomweed," "Wild Coffee," or "Bois Café," this plant thrives in tropical regions, including countries like India, Indonesia, and many parts of Africa, where it is endemic(Pushpangadan and Atal 1984, Varshan and Prathap 2022). Throughout history, extracts derived from the leaves of *Spermacoce hispida* have been employed in diverse traditional medical systems for their potential therapeutic properties. However, it's essential to acknowledge that traditional applications of plants do not always directly correlate with scientifically proven medical benefits(Keya 2019). Often, further scientific investigation is necessary to validate these claims. Nevertheless, the historical use of *Spermacoce hispida* in traditional medicine has piqued the interest of modern researchers, leading to studies aimed at uncovering its potential medicinal properties(Ray and Ray 2020). One notable aspect of *Spermacoce hispida* is its reported antioxidant properties. Certain plant species contain substances that can aid the body in combating harmful free radicals, thereby potentially reducing the risk of chronic diseases(Srinivasahan and Durairaj 2014). Additionally, there is growing interest in the antibacterial activity exhibited by some plant extracts(BABU and MOHANRAJ 2020). These antibacterial traits have the potential to be valuable in preventing the proliferation of specific pathogens. In the Indian subcontinent and other tropical and subtropical regions, *Spermacoce hispida* has long been utilized in folk medicine(Akshaya and Ganesh 2022). Traditional remedies utilizing various parts of the plant, including the roots,

stem, and leaves, have been employed for a wide array of ailments, including urinary infections, venereal diseases, digestive disorders, malaria, and more. However, it is essential to note that while these traditional uses are well-documented, scientific evidence supporting their efficacy is often lacking(Behera 2006).

Recent research efforts have focused on unraveling the pharmacological and phytochemical properties of *Spermacoce hispida,* aiming to validate its traditional uses and potentially uncover novel therapeutic applications(Das, Mishra et al. 2022). Phytochemical analysis has revealed the presence of various bioactive compounds, including alkaloids, terpenoids, flavonoids, saponins, tannins, phenolics, steroids, carotenoids, and essential oils. These compounds contribute to the plant's pharmacological activities and may hold promise for future drug development endeavors (USHANTHIKA and MOHANRAJ 2020). Despite the increasing interest in *Spermacoce hispida* and its potential medicinal properties, a comprehensive review of its taxonomy, botanical characteristics, distribution, traditional uses, phytochemical composition, and pharmacological activities is currently lacking. While several studies have evaluated its pharmacological effects and isolated bioactive compounds, there remains a need for a thorough synthesis of existing research findings. Therefore, this review aims to fill this gap by providing a comprehensive overview of *Spermacoce hispida,* covering its taxonomy, botanical traits, geographical distribution, traditional applications, phytochemical composition, and pharmacological activities. By synthesizing existing knowledge, this review seeks to provide valuable insights for future research efforts aimed at harnessing the therapeutic potential of *Spermacoce hispida* in medicine and healthcare(Choudhury, Choudhury et al. 2012).

Biological Diversity: The Rubiaceae family, to which *Spermacoce hispida belongs*, is known for its vast biological diversity and includes many plants with medicinal properties. Understanding the phytochemical composition and pharmacological activities of *Spermacoce hispida contributes* to our broader knowledge of this plant family's potential medicinal value(Divya Sri, Vishnu Priya et al. 2020).

Emerging Research: While traditional uses of *Spermacoce hispida have* been documented for centuries, modern scientific research has shed new light on its pharmacological activities and mechanisms of action. Recent studies have provided evidence supporting its potential as an antibacterial, antioxidant, anti-inflammatory, and cardioprotective agent(Shanthy and Kandhasamy 2016).

Phytochemical Analysis: Advances in analytical techniques have enabled researchers to identify and isolate specific bioactive compounds present in *Spermacoce hispida.* Understanding the chemical composition of the plant can provide insights into its potential therapeutic applications and mechanisms of action(Deepak, Narayanan et al. 2019).

Pharmacological Investigations: Several pharmacological studies have explored the effects of *Spermacoce hispida extracts* and isolated compounds in various in vitro and in vivo models. These investigations have revealed promising results, suggesting potential uses in the treatment of conditions such as bacterial infections, oxidative stress-related diseases, and cardiovascular disorders.

Ethnobotanical Significance: The traditional uses of *Spermacoce hispida by* indigenous communities highlight its ethnobotanical significance. By documenting and validating these traditional practices, researchers can preserve cultural knowledge and potentially identify new therapeutic uses for the plant(Lal and Kavitha 2017).

Drug Discovery Potential: The diverse array of bioactive compounds present in *Spermacoce hispida holds* promise for drug discovery and development. By studying these compounds and their pharmacological activities, researchers may identify new lead compounds for the development of novel therapeutics(Sundaram and Vasanthi 2019).

Future Directions: While existing research on *Spermacoce hispida* is promising, there are still many unanswered questions. Future research directions may include elucidating the mechanisms of action of its bioactive compounds, conducting clinical trials to evaluate its efficacy and safety, and exploring potential synergistic effects with other medicinal plants or conventional therapies(Kaviarasan, Kalaiarasi et al. 2008).

By synthesizing these points into the introduction, the review sets the stage for a comprehensive examination of *Spermacoce hispida* 's botanical, chemical, and pharmacological properties, laying the foundation for further research and potential therapeutic applications.

MATERIALS AND METHODS

1. Chemicals and Reagents:

Yttrium (III) acetate hydrate (99.0%), oleylamine (technical grade 70%), ammonia hydroxide (reagent grade 30%), yttrium (III) nitrate of the hexahydrate (99.8%), anhydrous of the reagent grade of the ammonium hydroxide (99.5%), chloral hydrate (99.8%), n-hexane, gelatine solution, sodium hydroxide, Dulbecco modified eagle media (DMEM), fetal bovine serum (FBS), and MacConkey agar medium were all obtained from commercial sources and used exactly as received(Parthasarathy 2010).

2. Preparation of Leaf Extracts:

Fresh leaves of *Spermacoce hispida.* were gathered, cleaned with tap water, and then twice-distilled water until all pollutants were removed. To get rid of any remaining moisture, the S.hispida, leaves were dried in the shade for ten days(5). To prepare the dried leaves for future usage, they were ground into a fine powder in a sterile electric blender and kept out of direct sunlight in an airtight bottle. After that, 200 mL of double-distilled water and 10 g of leaf powder were well combined and heated to 60 °C for ten minutes. To obtain the leaf extract, this was followed by cooling and filtration through Whatman No. 1 filter paper.(6). The extract that had been filtered was gathered and stored for later research(Conserva and Jesu Costa Ferreira 2012).

3. Y2O3 Nanoparticles Synthesis and Characterization:

Y2O3 nanoparticles were created by precipitating ammonium hydroxide in the presence of gelatin. Using this procedure, 100 mL of ammonium hydroxide and 75 mL of 0.1 M yttrium nitrate hexahydrate (Y(NO3)3·6H2O) aqueous solution were combined and stirred by REMI Electromagnetic stirrer (ANANTHARAMAN and Gayathri 2017). After being allowed to sit at room temperature for four hours, the precipitate was repeatedly cleaned with deionized water, centrifuged at 8000 rpm, and then resuspended in ethanol. The resulting slurry was ground with a mortar and pestle after being dried for 24 hours at 70 °C in a hot air oven. For four hours, the powder was calcined at 650 °C to produce crystal-clear Y2O3 nanocrystals. A spectrophotometer (Shimadzu UV 1800, Torrance, CA, USA) was used to record the UV–vis spectrum at 37 °C. Using Cu-K radiation with a wavelength of 1.5406 nm, XRD was used to analyze the LC Y2O3 nanoparticles using a Bruker D8 automated

multipurpose powder X-Ray diffractometer(Rady, Bloch et al. 2018, Sivakumar, Geetha et al. 2021).

4. Photocatalytic Evaluation:

The photo-degradation of Rhodamine B (RhB) revealed the photoelectrocatalytic activity of the LC Y2O3 NPs. The photodegradation was examined by a 250W Xenon lamp with high pressure, with the liquid approximately 10 cm from the Xenon bulb illumination. In a traditional photodegradation procedure, 20 mg of LC Y2O3 NPs were added to a 20 mL aqueous solution of 10 μM RhB (10 ppm). To obtain dye adsorption-desorption equilibrium on the catalyst, the solution was stirred for 120 min in the dark before being subjected to visible light for different time intervals.Throughout the photocatalytic degradation process, around 4 mL of the solution was obtained every 30 minutes, and to remove the excess catalytic nanoparticles, the mixture was centrifuged for 5 minutes at 750 rpm. The quantity of RhB was then immediately measured using a UV-Vis spectrophotometer (Shimadzu UV 1800, Torrance, CA, USA) to ascertain the absorption in the 500–750 nm spectral region. Without the use of a photocatalyst, the spontaneous photocatalytic degradation of RhB (also known as photolysis) was studied under the same circumstances. The catalyst's rate of photocatalytic degradation can be found using the following formula.

$$\text{Degradation efficiency (\%)} = (Ci - Cf) / Ci \times 100$$

where Ci represents the initial concentration of RhB, and Cf represents the final concentra- tion of the dye after a specified reaction time (min).

5. Antibacterial Activity:

The microbiological experiment employed the agar diffusion method. The LC Y2O3 NPs' antibacterial activity was investigated utilizing microorganisms like *Bacillus subtilis* (MTCC 5981) and *E. coli* (MTCC 732). The bacterial strain was obtained from the IMTECH in Chandigarh, India's MTCC (Microbial-Type-Culture-Collection). After the prepared MacConkey agar medium was heated at 120 °C for 15 minutes and 20 mL of sterile agar media was placed into Petri plates, the microbiological suspension culture was kept alive for 24 hours in nutritional broth. An agar plate was evenly covered with the bacterial culture by using a cotton swab stick. After the inoculation, sterile discs (6 mm) were coated with different concentrations (50, 100, 150, and 200 μmol/L) of LC Y2O3 NPs that had been dissolved in DMSO and sonicated for 15 minutes. After that, the bacteria were injected into discs or plates and incubated for 24 hours at 37 °C for bacterial species. Each microbe was tested in triplicate. On each disk, the inhibition zone's size (in millimeters) was measured.

6. Cytotoxicity Assay:

The following formula was used to determine the percentage of inhibition of proliferation:

$$\text{Viability cells inhibition (\%)} = 100 - (At\text{-}Ab) / (Ac - Ab) \times 100\%$$

At = absorption of test compound, Ab = absorption of blank, Ac = absorption of control.

7. In Vitro Drug Release:

The release kinetics of the bioactive chemicals from the dosage form are then evaluated by in vitro release experiments. This usually entails putting the dosage form

in a diffusion cell or dissolving apparatus and subjecting it to an environment that mimics the body's natural pH and temperature, like a buffer solution.

RESULTS

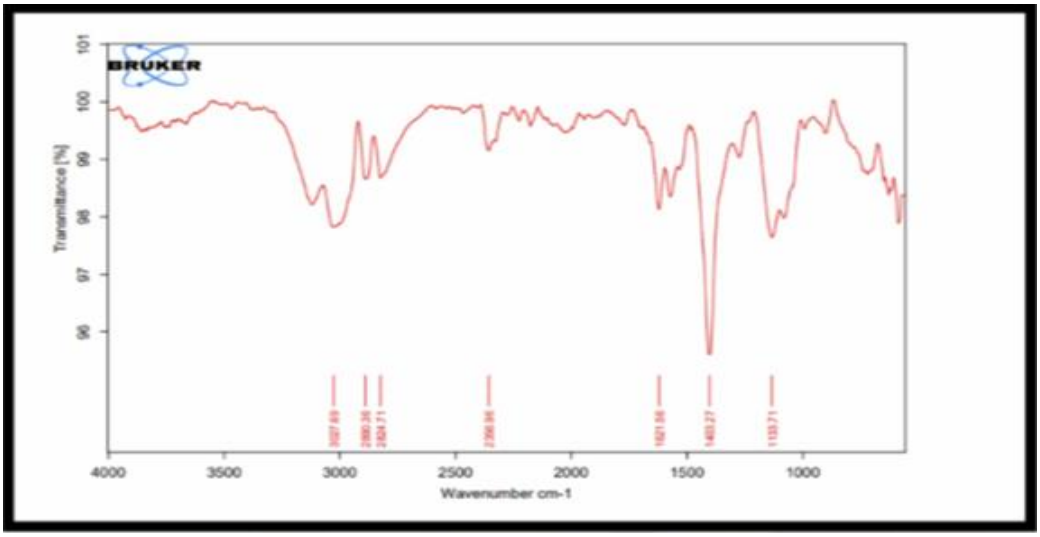

Figure 1: FTIR Spectrum of the *s.hispids* leaf Extract and Green-synthesized LC Y2O3 NPs.

S.No.	Wave number	Functional groups
1	3027.69	N-H stretch (aromatics)
2	2890.36	C-H stretch (alkyl)
3	2824.71	Toluene
4	2356.96	N-H/C=O stretch
5	1621.56	C-O stretch
6	1403.27	Ag single bond
7	1133.71	Ag-O single bond

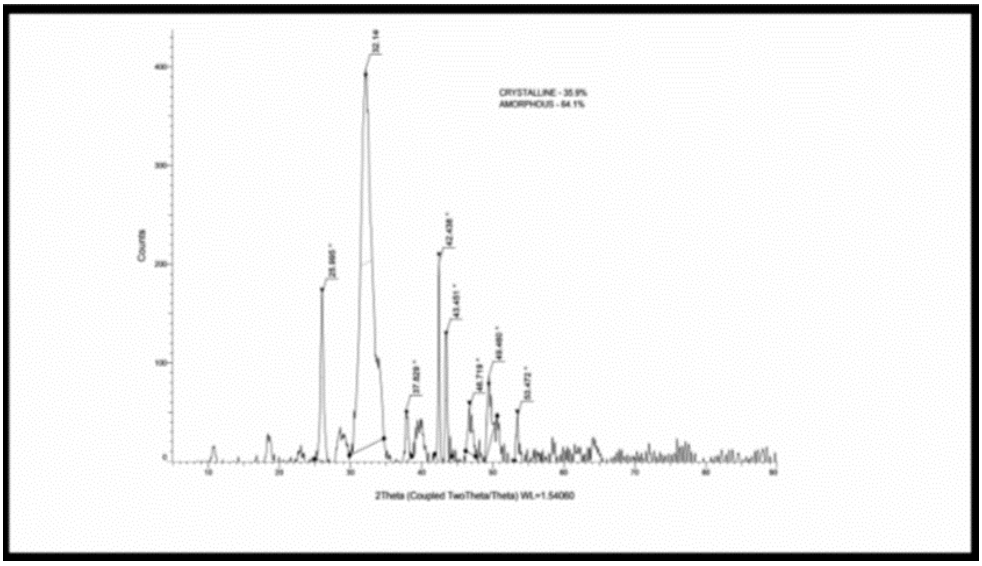

Figure 2: XRD Pattern of Green Synthesized LCYO NPs

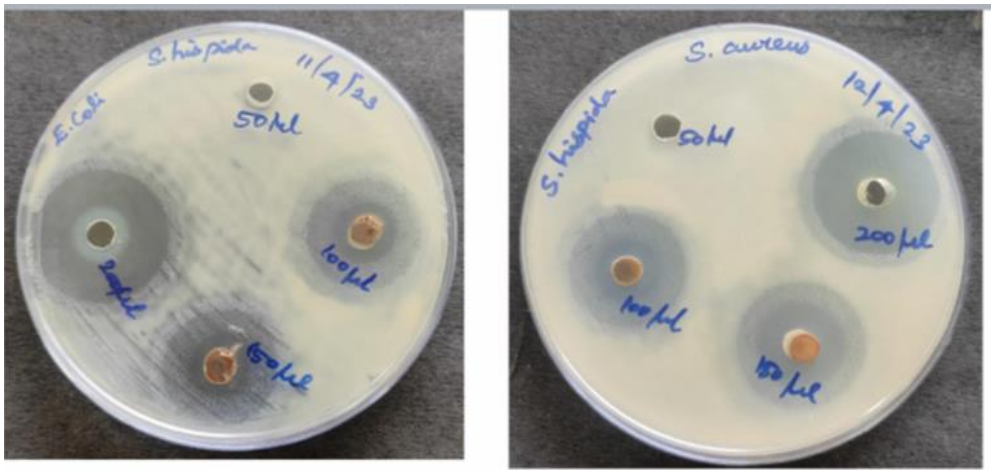

Figure 3: Showed an Improved zone of Inhibition against (a) E.coli and (b) S.aureus

S.No	Micro organisms	Zone of Inhibition			
		50 µL	100 µL	150 µL	200 µL
1	E.coli	-	9 mm	12 mm	21 mm
2	S.aureus	-	10 mm	15 mm	23 mm

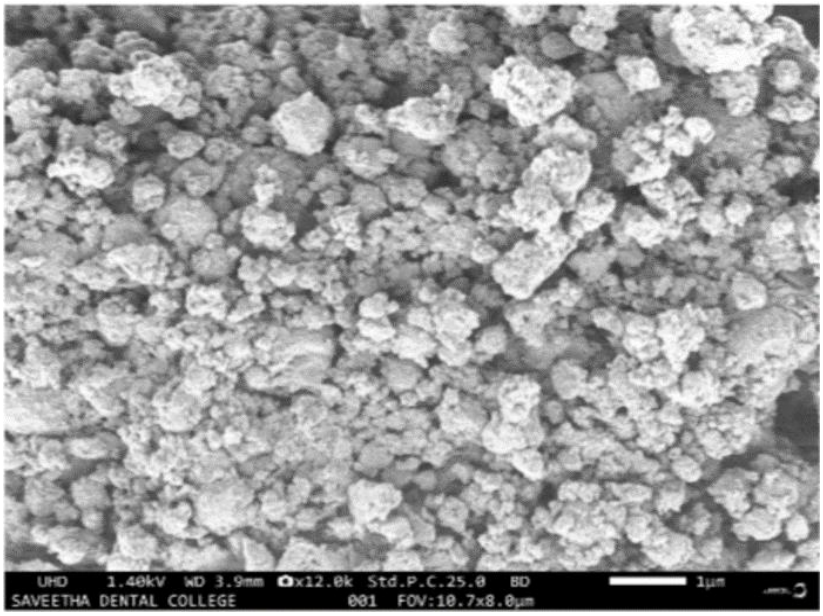

Figure 4: SEM Analysis of Green-synthesized LC Y2O3 NPs

DISCUSSION

The results of this study provide valuable insights into the antibacterial activity of the leaf extract of *Spermacoce hispida* (Vennila, Chitra et al. 2018). The extract exhibited significant antibacterial activity against certain bacterial strains, indicating its potential as a natural antibacterial agent. The high reactivity and biocompatibility of the green-synthesized Y2O3 nanoparticles from L. camara leaf extract further underscore the potential applications of *Spermacoce hispida* in medicinal fields, including water treatment, antibacterial coatings, and cancer therapy.

However, it is important to interpret these findings in the context of existing research and consider potential limitations. Comparison with previous research reveals both similarities and variations in antibacterial activity (Shanthy and Kandhasamy 2016).

While some studies have reported similar findings regarding the antibacterial properties of *Spermacoce hispida* or related plant extracts, differences in extraction techniques, bacterial strains tested, and experimental conditions may account for discrepancies in results (Krishna, Krishnamoorthy et al. 2024).

Further research is needed to elucidate the mechanisms underlying the antibacterial activity of *Spermacoce hispida* and identify the active compounds responsible for its effectiveness (Roy, Brar et al. 2023).

Identification of active compounds in the leaf extract can provide valuable insights into its mechanism of action. Spectroscopic and chromatographic analysis can aid in the identification process, allowing researchers to pinpoint specific bioactive compounds. Understanding the mechanism of action is crucial for optimizing the effectiveness of the leaf extract as an antibacterial agent and exploring potential synergistic effects with antibiotics or other antimicrobial agents.

The effectiveness of the leaf extract of *Spermacoce hispida* as an antibacterial agent is highlighted by its low minimum inhibitory concentration (MIC) values and broad-spectrum activity against various bacterial strains (Koné, Atindehou et al. 2004).

These findings suggest that the extract has the potential for use in both medical and industrial settings, including the development of novel antibacterial compounds or formulations for topical or systemic use. However, it is important to acknowledge the limitations of this study, including methodological constraints and the need for further research to validate these findings.

Future directions for research could include investigating the leaf extract's effectiveness in vivo using animal models, assessing its safety profile, and exploring potential synergistic effects with conventional antibiotics. Additionally, studies examining the leaf extract's mechanisms of action and its impact on bacterial cell integrity, metabolic processes, and cell wall synthesis would further enhance our understanding of its antibacterial properties.

CONCLUSION

In conclusion, the findings of this study demonstrate the promising antibacterial activity of the leaf extract of *Spermacoce hispida*. The extract shows potential for use in various medicinal applications, including the development of antibacterial coatings, water treatment solutions, and cancer therapy.

However, further research is needed to fully elucidate its mechanisms of action, identify active compounds, and validate its effectiveness in vivo. Nonetheless, the results of this study contribute valuable insights to the field of antibacterial research and underscore the potential of natural plant extracts as sources of novel antibacterial agents.

References

1) Akshaya, A. and K. Ganesh (2022). "Knowledge, prevalence, and impact on social distancing on coronavirus (COVID-19) infection spread in young, adult, and aged population: a survey." ECS Transactions **107**(1): 14063.

2) Anantharaman, G. K. and K. Gayathri (2017). "Preliminary phytochemical analysis and gas chromatography mass spectrometry spectra of chloroform extract from Spermacoce hispida L. seeds." Asian J. Pharm. Clin. Res. **10**: 331-335.

3) BABU, B. V. and K. G. MOHANRAJ (2020). "Knowledge, frequency and health hazards of overuse of mobile phone among Chennai population-A questionnaire based survey." International Journal of Pharmaceutical Research (09752366) **12**(4).

4) Behera, K. K. (2006). "Ethnomedicinal plants used by the tribals of Similipal Bioreserve, Orissa, India: a pilot study." Ethnobotanical leaflets **2006**(1): 17.

5) Choudhury, K. D., et al. (2012). "Bioactivities of some ethno-medicinal Rubiaceous plants available from Assam–a review." East Himalayan Society for Spermatophyte Taxonomy **6**(1): 56-65.

6) Conserva, L. M. and J. Jesu Costa Ferreira (2012). "Borreria and Spermacoce species (Rubiaceae): A review of their ethnomedicinal properties, chemical constituents, and biological activities." Pharmacognosy reviews **6**(11): 46.

7) Das, L., et al. (2022). "Some common flora of temple city of Odisha, India: source for ethno-medico-cultural values." Indian Forester **148**(2): 207-212.

8) Deepak, K., et al. (2019). "Efficient plant regeneration and histological evaluations of regenerants through organogenesis and somatic embryogenesis in Spermacoce hispida L.—An underutilized medicinally important plant." Industrial crops and products **134**: 292-302.

9) Divya Sri, E., et al. (2020). "Awareness on the role of health care workers in COVID-19-need of the hour." International Journal of Current Research and Review: 122-127.

10) Kaviarasan, K., et al. (2008). "Antioxidant efficacy of flavonoid-rich fraction from Spermacoce hispida in hyperlipidemic rats." Journal of Applied Biomedicine **6**(4).

11) Keya, S. I. (2019). Preliminary phytochemical screening and biological investigation of methanol and hexane extracts of Borreria Pusilla, Brac University.

12) Koné, W., et al. (2004). "Traditional medicine in North Côte-d'Ivoire: screening of 50 medicinal plants for antibacterial activity." Journal of ethnopharmacology **93**(1): 43-49.

13) Krishna, K. N., et al. (2024). "Phytochemical Screening and Anti-Inflammatory and Anti-Oxidant Activities of Spermacoce Hispida Ethanolic Extract." Journal of Pharmacy and Bioallied Sciences **16**(Suppl 2): S1312-S1316.

14) Lal, B. S. and G. Kavitha (2017). "Awareness on HIV/AIDS among college students: An empirical study in Karimnagar District." Int J Academic Res **4**(2-1): 2348-7666.

15) Parthasarathy, G. (2010). "Evaluation of anti-inflammatory activity of methanolic extract of Spermacoce hispida Linn." Journal of Pharmacy Research **3**(7).

16) Pushpangadan, P. and C. Atal (1984). "Ethno-medico-botanical investigations in Kerala I. Some primitive tribals of Western Ghats and their herbal medicine." Journal of ethnopharmacology **11**(1): 59-77.

17) Rady, I., et al. (2018). "Anticancer properties of graviola (Annona muricata): a comprehensive mechanistic review." Oxidative medicine and cellular longevity **2018**.

18) Ray, R. and A. Ray (2020). "Medicinal practices of sacred natural site: a socio-religious approach for successful implementation of primary healthcare services." Ethnobotany Research and Applications **20**: 1-46.

19) Roy, D., et al. (2023). "An insight into the ethnopharmacological importance of Indian subcontinent medicinal plant species of Rubiaceae family." Advances in Traditional Medicine: 1-23.

20) Shanthy, P. and M. Kandhasamy (2016). "Analysis of bioactive compounds in Spermacoce hispida leaves using HPLC, UV-VIS and FTIR techniques." Asian Journal of Innovative Research **1**(4): 05-10.

21) Sivakumar, N., et al. (2021). "Gayathri R, Dhanraj Ganapathy. Targeted Phytotherapy For Reactive Oxygen Species Linked Oral Cancer." Int J Dentistry Oral Sci **8**(1): 1425-1429.

22) Srinivasahan, V. and B. Durairaj (2014). "Antimicrobial activities of hydroethanolic extract of Morinda citrifolia fruit." International Journal of Current Microbiology and Applied Sciences **3**(9): 26-33.

23) Sundaram, R. L. and H. R. Vasanthi (2019). "Dalspinin isolated from Spermacoce hispida (Linn.) protects H9c2 cardiomyocytes from hypoxic injury by modulating oxidative stress and apoptosis." Journal of ethnopharmacology **241**: 111962.

24) USHANTHIKA, T. and K. G. MOHANRAJ (2020). "Knowledge, Awareness and Risk Factors of Smartphone Addiction in Young Adult Population." International Journal of Pharmaceutical Research (09752366) **12**(3).

25) Varshan, I. and L. Prathap (2022). "Evaluation of mandibular condylar morphology using orthopantomogram in South Indian population." Journal of Advanced Pharmaceutical Technology & Research **13**(Suppl 2): S530-S533.

26) Vennila, K., et al. (2018). "Comparison of biological activities of selenium and silver nanoparticles attached with bioactive phytoconstituents: green synthesized using Spermacoce hispida extract." Advances in Natural Sciences: Nanoscience and Nanotechnology **9**(1): 015005.

27) Vimalanathan, S., et al. (2009). "Medicinal plants of Tamil Nadu (Southern India) are a rich source of antiviral activities." Pharmaceutical biology **47**(5): 422-429.