Journal of Community Pharmacy Practice
ISSN: 2799-1199
Vol: 03, No. 04, June – July 2023
http://journal.hmjournals.com/index.php/JCPP
DOI: https://doi.org/10.55529/jcpp.34.27.35

Exploring Nature's Antibacterial Potential: Validating the Effectiveness of Chosen Plant Species and Unraveling Molecular Mechanisms to Advance Drug Development Strategies

T. Kumaran[1*], S. Sujithra[2], G. Sindhu Kumari[3], M. Jenifer Tamizharasi[4], R. Rajila[5]

[1]Assistant Professor, Department of Zoology, Muslim Arts College, Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli, Tamilnadu, India.*
[2,3,4,5]Research Scholar, Department of Zoology, Muslim Arts College, Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli, Tamilnadu, India.

Corresponding Email: [1]kumaranaqua@yahoo.com*

Received: 12 March 2023 **Accepted:** 02 June 2023 **Published:** 17 July 2023

Abstract: ***This study seeks to validate the antibacterial efficacy of chosen plant species and identify the specific phytochemical groups responsible for this activity. Molecular-level identification of these plants and initial phytochemical analyses are conducted to reveal potential antibacterial phytochemical groups, bolstering the study's credibility. The exploration of plant molecular structures aims to offer insights into the underlying mechanisms of their antibacterial properties. Additionally, the research underscores the ongoing pursuit of new lead compounds from natural sources as alternatives for developing antifungal and anticancer drugs. Emphasizing the importance of compounds that are not only more effective but also less toxic, the study aligns with the broader goal of advancing drug development strategies. The investigation into novel therapeutic agents from natural origins holds promise in contributing to efforts addressing antibiotic resistance and discovering safer and more potent treatments for infectious diseases.***

Keywords: Antibacterial, Diseases, Natural Sources, Phytochemical.

1. INTRODUCTION

Chronic diseases constitute the predominant cause of global mortality, accounting for over 60% of annual deaths. Among the 57 million deaths reported in 2008, 36 million were attributed to chronic conditions, notably cardiovascular diseases, diabetes, chronic lung diseases, and cancers (Alwan et al., 2010). The escalating burden of chronic diseases is

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particularly pronounced in lower-income countries and populations, with a projected substantial increase over the next two decades (Ezzati, Lopez, Rodgers, & Murray, 2005)
Addressing this global health challenge necessitates comprehensive efforts, including preventive measures, the promotion of healthier lifestyles, and enhancements to healthcare infrastructure. Public health initiatives play a key role in raising awareness about risk factors, advocating for regular medical check-ups and supporting policies that create healthier environments. Collaborative efforts between governments, healthcare professionals, and communities are essential for developing effective strategies. Education programs, early detection initiatives, and improved access to healthcare services for diverse populations are crucial components of these strategies. As the prevalence of chronic diseases continues to rise, a concerted global focus on prevention, early intervention, and the enhancement of healthcare systems is imperative. This approach aims to mitigate the impact of chronic diseases on public health and foster a healthier global population.

Presently, antibiotic resistance poses a significant global challenge, particularly among medically important bacteria. The widespread and indiscriminate use of commercial antimicrobial drugs has led to the emergence of multiple drug-resistant strains. Additionally, antibiotics can induce adverse effects in hosts, including allergies, hypersensitivity, and immune suppression. Consequently, there is a critical need to explore alternative antimicrobial drugs for addressing infectious diseases. Antimicrobials derived from plant sources present a promising avenue with substantial therapeutic potential. These natural compounds have proven effectiveness in treating infectious diseases, offering a dual benefit of efficacy and a reduced incidence of side effects commonly associated with synthetic antimicrobials (Cunha, 2001). As the global medical community grapples with the escalating concern of antibiotic resistance, the exploration and development of plant-based antimicrobials emerge as a valuable strategy to combat infectious diseases more sustainably and with fewer associated complications.

2. MATERIALS AND METHODS

Collection of Medicinal Plants

Medicinal plants present all over the world have numerous antibacterial activities. Plants are normal dietary components and there is practically no risk of toxic substances and side effects, the extracts can be given orally. Plants are the better source of pharmacologically active compounds. Hence the medicinal plants were selected for the present study. Medicinal plants such as Adhatoda vasica, Annona muricata, Hemidesmus indicus, Phyllanthus emblica were selected. The samples were collected around the Western ghats area for the research work.

Preparation of Extracts

Ten grams dried powder of plant leaves were taken in 100ml distilled water and boiled at above 100°C with two hours, after filtered the extracts, supernatant was collected and residues were discarded. The collected supernatant was condensed in water bath and condensate was extracted again with methanol. The methanolic extract was concentrated in

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rotary evaporated under reduced pressure at room temperature 45-50°C, in order to avoid evaporation of plant metabolism. Aqueous extract was concentrated using lyophilizer and stored at 4°C. Phytochemical screening tests carried out by the following standard protocols (Kumaran & Citarasu, 2015)

Antioxidant Activity

The DPPH free radical scavenging assay, as per Brand-Williams et al. (1995), involved the preparation of a DPPH solution in methanol. This solution was then diluted multiple times with methanol until an absorbance of 0.9 at 516 nm was achieved using a spectrophotometer. Subsequently, 1 mL of the DPPH solution was combined with 100 µL of clove extract solution. The mixture underwent shaking in a vortex and was left undisturbed for 2 hours in a dark environment. Following this incubation period, the mixture was transferred to a microplate plastic, and the absorbance of the DPPH solution, post-sample addition, was measured at 516 nm using a spectrophotometer. The alteration in absorption for each sample was determined by computing the difference between the blank and sample readings. The percentage of DPPH scavenging activity was then calculated using the formula:

Radical scavenging (%) = [(A0 – A1/A0) × 100]

Where A0 is the absorbance of the control and A1 is the absorbance of the sample extracts.

Thin-Layer Chromatography

A Thin Layer Chromatography (TLC) technique was utilized to identify the active components. The process involved applying the extract onto a silica gel plate and developing it using an ethyl acetate: methanol (9:1) eluent system tailored for Annona muricata. After application, the TLC plates were thoroughly dried to eliminate any residual solvents. Subsequently, the silica plates were inspected under a UV illuminator or iodine crystals to visualize the separated bands indicative of different components.

$$\text{Rf value} = \frac{\text{Distance from origin to component spot}}{\text{Distance from origin to solvent front}}$$

Fourier-Transform Infrared (FTIR) Analysis

The identification of functional groups in the active components was conducted using Fourier Transform Infrared Spectroscopy (FTIR), which relies on peak values within the infrared radiation spectrum. In this study, qualitative analysis of selected hot water extracts was carried out for active compounds using the FTIR method as outlined by Kemp (1991). The spectra were acquired utilizing an OMNI-sampler attenuated total reflectance (ATR) accessory on a FTIR spectrophotometer. For the analysis, a small quantity of powdered leaves was directly placed on the germanium piece of the infrared spectrometer, maintaining a constant pressure. Infrared absorbance data were then collected over a wave number range from 4000 cm –1 to 675 cm –1 and subsequently computerized for further analyses. This FTIR method allowed for the examination and identification of specific functional groups present in the active components of the selected extracts.

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Antibacterial activity

The fractions obtained from the purified selected plant extracts were evaluated using the agar well plate method, as described by Bauer and Kirby in 1966. In this procedure, an agar plate was thoroughly and evenly inoculated (forming a lawn) with a vigorously growing culture of the target organisms. The chosen medium for this process was Muller Hinton agar. The effectiveness of the antimicrobial agent was assessed by the diffusion of substances from well to agar, establishing a concentration gradient, and allowing for the growth of organisms over a critical 16 to 24-hour period to ensure reliable results. the bacterial plates were then incubated at 37°C for 24 hours. Following the incubation period, the zone of inhibition (measured in millimeters) was carefully recorded. This method provides valuable insights into the antimicrobial activity of the fractions, with the size of the inhibition zone serving as an indicator of the effectiveness of the tested plant extracts against the cultured microorganisms.

3. RESULTS

Table 1. Indigenous plants from Western Ghats associated area and selection for the study

Sl. No.	Botanical name (Family)	Part used	Native	Traditional applications	Applications reported in literature
1	Adhatoda vasica (Acanthaceae)	Leaves	Sub Himalayan Region	Cold, cough, whooping cough, chronic bronchitis, asthma	Antimicrobial, antiviral, antioxidant, anti-inflammatory,
2	Hemidesmus indicus (Periploceceae)	Root	Gangetic India and West Bengal	Wound healing, hepatoprotective	Anticancer, antiarthritic, antimicrobial, antiulcer,
3	Phyllanthus emblica (Phyllanthaceae (Eu phorbiaceae)	Leaves	India	Diarrhea, jaundice, Inflammation	Anti-aging, anti-cancer, anti-diabetic, anti-pyretic, dermo protective, anti-anaemic
4	Annona muricata (Annonaceae)	Leaves	Tropical and subtropical regions	Fever, pain, respiratory and skin illness, hypertension, cancer, inflammation, diabetes, bacterial infections, wound healing	Anti-microbial, anti- inflammatory, anti-oxidant, anti-stress, anti-tumoral, anti- ulceric, hepato-protective

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Phytochemical Analysis

The phytochemical analysis is preliminary characterization of plant extracts which have bioactive compounds. The phytochemical screening showed that all the experimental plants contain phenols. H. indicus extract contained alkaloids, saponins, steroid, tannins, flavonoids, glycosides and phenols. P. emblica showed phytoconstituents like alkaloids, saponins, tannins, anthraquinones, flavonoids, glycosides and phenols. The phytochemicals such as terpenoid and steroid were absent in the extract. A. muricata extract showed all phytochemicals in the list except saponin and glycosides. The presence of phytochemicals showed that the selected plants were suitable for this study.

Table 2. Phytochemical analysis of extract of selected herbs by standard protocols

S. No.	Phytochemical constituents	Hemidesmus indicus	Adhatoda vasica	Phyllanthus emblica	Annona muricata
1	Alkaloid	+	+	+	+
2	Saponin	+	+	+	-
3	Steroids	+	+	-	+
4	Tannin	+	+	+	+
5	Terpenoids	-	-	-	+
6	Anthraquinones	-	+	+	+
7	Flavonoids	+	-	+	+
8	Glycosides	+	+	+	-
9	Phenols	+	+	+	+

+: Present; - : Absent

Antioxidant Activity

The results exhibited that the *A. vasica* and *H. indicus* extracts showed the lowest activity 38±0.12% and 47±0.84%. All of the crude extracts were found to have antioxidant properties, but the antioxidant activity varied depending on the solvent (Fig. 4.2). *A. muricata* had more antioxidant activity in an aqueous extract (75.51±0.14%), *P. emblica* had the high antioxidant activity in an extract (73.13±0.36%). The existence of antioxidant qualities supported the use of herbals as medical applications.

Active Compound Characterization of Herbals by Thin Layer Chromatography

TLC is also used to support the identity of a compound in a mixture when the Rf of a compound is compared with the Rf of a known. A. vasica revealed Rf values as 0.41, 0.65 and 0.59 in methanol and hot water extracts respectively. The Rf values of the fractions are 0.41 and 0.46 observed in A. muricata methanol and hot water extracts. The R_f value of the fractions are 0.46 in the A. muricata hot water extract respectively. Rf values of the H. indicus showed two active compounds as 0.57 and 0.63 in methanol and hot water extracts.

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There were five active compounds in P. emblica such as Rf values 0.41,0.89 in methanol extract and hot water extract had 0.36,0.58 and 0.83 fractions. There were no active compounds in methanol extract (Table 4.4, Figure 4.3).

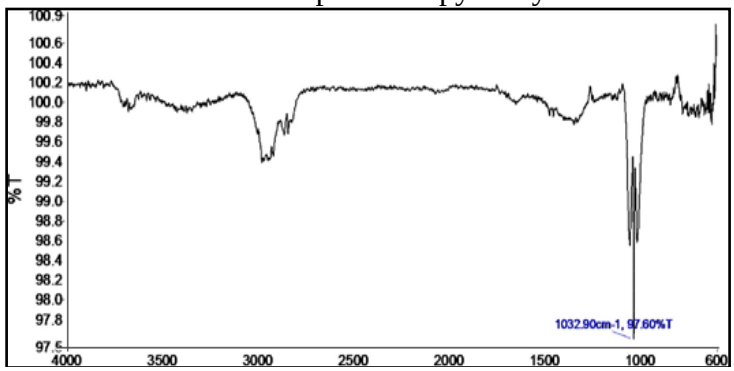
Table 3. Lists of eluted compounds present in the different plant extracts active fractions

Functional Group Analysis of A. Vasica:

Sl. No	Plant extracts	Eluted compounds with R_f values		
		Compounds	Methanol	Hot water
1.	Adhatoda vasica	3	0.41, 0.65	0.59
2.	Annona muricata	2	0.41	0.46
3.	Hemidesmus indicus	2	0.57	0.63
4.	Phyllanthus emblica	5	0.41, 0.89	0.36,0.58, 0.83

Fourier Transform Infrared Spectroscopy analysis of A. vasica extract active fractions is given in the figure 4.4.a. The possible functional groups of active principles were analyzed between in wave number 500- 4000 /cm.

Figure 1. Fourier transform infrared spectroscopy analysis for the *A. vasica* extract



The peak value 1032.90 contains C-O, C-X and C-N (bond type), C-O, C-F and C-N (specific context) (Table 4.5.a). In leaves of A. vasica, the higher ranges of C-H bending aromatic and C-H stretching alkane compounds overtone 3285.14. In the leaves of A. vasica, OAH is bending phenol 3915.14, CN extending imine/oxime or CO stretching conjugated ketone or alkenes, and CAN stretching amine 3935.14. These findings demonstrate that a quantitative analysis of significant bioactive chemicals extracted from A. vasica leaf sections was carried out utilising the FTIR technique. Vasicine, a quinazoline, is the main alkaloid present in Adhatoda leaves. The alkaloids 1-vasicine, maintone, vasicinolone, and vasicinol, which are also present in the leaves and roots and are responsible for Adathoda's bronchodilator activity, are also present. Vasicinone has an antiproliferative effect on cancer cells when it taken in safe doses from the A. vasica plant extract.

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In Vitro Antibacterial Activity for Different Active Fractions

The antibacterial activities of the selected herbal extracts against the selected strains (zone of inhibition of mm diameter) were given in the table 4.7 and plate 4.3. In the present study four different extracts such as A. vasica, A. muricata, H. indicus, P. emblica were used. The higher level of antibacterial activity occurred in A. vasica against E.coli, A.hydrophila and S.aureus. The lowest antibacterial activity observed against E.coli in H.indicus.

Table 4. Invitro antibacterial studies of the selected herbal extracts

Sl. No.	List of plants	Zone of inhibition (mm) of different active fraction of the herbal extracts		
		E. coli	A.hydrophila	S.aureus
1	Adhatoda vasica	13. 25[a] ± 0.94	13.15[a] ± 0.4	15. 47[a] ± 0.10
2	Annona muricata	14.15[d] ± 0.94	14.12[e] ± 1.02	15.65[b] ± 0.24
5	Hemidesmus indicus	5.30[b] ± 0.16	14.42[b] ± 0.61	10.30[b] ± 0.14
6	Phyllanthus emblica	9.10[e] ± 0.52	10.85[e] ± 1.13	12.10[c] ± 1.05

4. DISCUSSION

In this study, eight plants were carefully chosen for their recognized potential as traditional medicines. The selected plants include Adhatoda vasica, Annona muricata, Hemidesmus indicus, Phillanthus emblica. Various parts of these plants, such as leaves, roots, and flowers, were utilized for the investigation. Annona muricata, for instance, has noteworthy phytochemicals present in its leaves. These phytochemicals can be extracted by crushing the leaves along with the raw fruit from the plant and blending them with olive oil. The resulting oil has been identified for its potential in treating skin disorders such as rashes, boils, and sores (Kumaran, 2021). Moreover, the antioxidant properties of these plants, including their ability to quench reactive oxygen species like singlet molecular oxygen and peroxyl radicals, are associated with their antioxidant actions (Jenifer Tamizharasi et al., 2022). Annona muricata, in particular, has been noted for its extracts from leaves, stems, roots, and seeds, possessing antibacterial properties effective against various diseases (Sundarrao et al., 1993). This study aims to explore and document the medicinal potential of these selected plants and their parts, contributing to the understanding of their therapeutic applications in traditional medicine. In the current investigation, the herbal samples were subjected to analysis for active compounds using thin-layer chromatographic (TLC) methods. In Phyllanthus emblica, five active compounds were identified with Rf values of 0.41 and 0.89 in the methanol extract, and 0.36, 0.58, and 0.83 in the hot water extract. Adhatoda vasica exhibited three

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active compounds in both methanol and hot water extracts. Annona muricata and Hemidesmus indicus displayed two active compounds each in both methanol and hot water extracts. All selected herbs exhibited two or more active components. According to Kumaran's TLC study (2018), four distinct alkaloids codeine, morphine, piperine, and concessine along with some unidentified derivatives, were identified in the butanolic extracts of these plants. These findings suggest that the identified properties of the plants, including antioxidant, antimicrobial capabilities hold promise for their utilization in herbal remedies against oxidative stress and microbial infections. This supports their traditional use in conventional medicine. The growing utilization of plant extracts in medicine and the healthcare sector underscores the need for increased attention to these multifaceted aspects of the subject.

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