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Full Paper

Comparative Study on the Antibacterial Activity of Selected Medicinal Plants against *Escherichia coli* and *Staphylococcus aureus* Clinical Strains

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Abstract

Antibiotic resistance is one of the most significant challenges of the 21st century, leading to increased global healthcare expenditure due to the necessity for more complex and expensive treatments. In response, researchers are investigating target product profiles as alternative antimicrobial sources capable of combating this prevalent issue. Medicinal plants used in traditional practices have emerged as promising candidates for developing new drug leads effective against antibiotic resistant bacteria. This study aims to evaluate the *in vitro* antibacterial activity of selected medicinal plants against common human pathogenic bacteria; *Escherichia coli* (ATCC® 25922™) and *Staphylococcus aureus* (ATCC® 29213™). The plant materials examined included *Abelmoschus moschatus* (leaves and stems), *Aporosa cardiosperma* (*Gaertn*.) *Merr*. (leaves and stems), *Celosia argentea* (leaves and flowers) and *Nauclea orientalis* (leaves and roots). A sequential extraction procedure using industrial solvents, methanol, ethyl acetate, and hexane, was performed, followed by qualitative phytochemical analysis to identify secondary metabolites present in the extracts. Antimicrobial susceptibility was assessed using the EUCAST disk diffusion assay. As the main findings of the research, the methanolic crude extracts of all plants exhibited inhibitory effects on the proliferation and growth of both test microorganisms. Among all plant extracts, 400 µg/mL concentrated crude methanolic extracts of *Nauclea orientalis* leaves exhibited the highest zone of inhibition against Gram-negative *E. coli* (12.67±0.58 mm) and Gram-positive *S. aureus* (10.00±2.65 mm). Preliminary phytochemical screening of plant extracts was conducted using standard qualitative methods; Hager's test, foam test, alkaline reagent test, and Ferric chloride test revealed the presence of alkaloids, saponins, flavonoids, phenols, and tannins, respectively. The antibacterial activity was observed in the plant extracts may be caused by the presence of these secondary metabolites. Hence, this study emphasizes the potential of the selected medicinal plants as sources of novel antibacterial agents that can be further improved and developed for pharmaceutical applications against antibiotic resistant bacteria.

Keywords: antimicrobial, ethnopharmacology, natural products, medicinal properties, phytoconstituents

Introduction

Antibacterial resistance is a rapidly escalating global problem. The overuse of antibiotics and inadequate regulation, particularly in developing countries, have led to the widespread emergence of multidrug-resistant bacteria. According to the United States Centers for Disease Control and Prevention (CDC) [1]

antimicrobial resistance is an urgent global public health threat that is responsible for approximately 1.27 million deaths worldwide and associated with nearly 5 million deaths in 2019 alone. In response to this crisis, there is a growing global interest in discovering novel antibacterial treatments. Historically, plants have been a cornerstone of traditional medicine, used to treat various ailments, promote health, combat infections, and support the immune system. Consequently, plants have become a consistent source of new antibacterial drugs in modern medicine. However, given the limited research on the antibacterial properties of many plants used in traditional medicine, investigating antibacterial activities of selected Sri Lankan conventional medicine plants could contribute significantly to developing new treatments for bacterial infections.

Abelmoschuis moschatus, *Aporosa cardiosperma (Gaertn.) Merr*., *Celosia argentea* and *Nauclea orientalis* are four medicinal plants native to Sri Lanka and the Indian subcontinent, which are widely used in traditional Ayurvedic medicine. *Abelmoschus moschatus*, commonly referred to as "Kapukinissa" in Sinhala, is an aromatic medicinal shrub used in both traditional medicine and the perfume industry [2]. Historically, it has been used to treat a range of ailments, including asthma, colds and influenza, snake bites, digestive disorders, and even certain types of cancers [3, 4]. Several research indicate the presence of significant bioactive compounds in *Abelmoschus moschatus*, including flavonoids, ambrettolide, farnesol, and β-caryophyllene, which have anti-inflammatory, antibacterial, and antioxidant properties that are consistent with the traditional therapeutic applications. Notably, the antibacterial and antifungal properties of ambrettolide, a macrocyclic lactone, have garnered interest [5, 6].

Ghorpade *et al*., (2017) stated that the *Celosia argentea*, commonly known as "Kiri Handa" in Sinhala, is a tropical herb found in parts of Africa and the Indian Ocean islands. It has been used to treat conditions such as fatigue, leucorrhea, atherosclerosis, osteoporosis, and more. The plant has demonstrated antimicrobial effects attributed to compounds such as celosianin II, phenolic acids, and flavonoids, which are known to inhibit microbial growth through oxidative stress induction and cell wall disruption [7]. *Aporosa cardiosperma*, known as "Kebella" (Sinhala), is a leafy vegetable consumed in Sri Lanka. In indigenous medicine, it is utilized as a diuretic and as a treatment for headaches, fever, diabetes, liver diseases, and other ailments [8]. Preliminary investigations have demonstrated that its crude extracts can inhibit the growth of microbes, likely due to the presence of flavonoids, phenolics, and glycosides in plant parts [9].

Nauclea orientalis, which refers to "Bak-Mee" in Sinhala, is also an important medicinal plant used in Ayurveda and various folk medicine systems. Previous studies indicate that the presence of various phytochemicals in *N. orientalis* inhibits crucial cellular mechanisms necessary for the survival of microorganisms. Furthermore, bioactive compounds extracted from the plant have demonstrated potential to enhance the efficacy of formulated ointments and significantly impact phagocytosis and helper T cell production [10]. In traditional medicine, parts of this plant are applied to treat conditions such as boils, gastric ulcers, and tumors.

Therefore, this study explores four understudied, native Sri Lankan medicinal plants that are widely used

in ayurvedic medicine to treat various infectious conditions, but where there is limited existing literature and a lack of comparative studies. The study was conducted using sequential extraction and qualitative phytochemical analysis, followed by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) antimicrobial susceptibility test to evaluate the *in vitro* antimicrobial activity of selected plant parts against common human pathogenic bacteria, *E. coli* and *S. aureus*.

The current study serves as foundational research, with its outcomes providing the necessary basis for an extended, future study focused on the development of antimicrobial agents with minimal negative impacts. Thus, investigating the bioactivities of selected medicinal plants offers a wide range of benefits and has the potential to address deficiencies and overcome barriers in various fields, particularly in the medical sector.

Materials and Methods

Plant Collection

Matured and healthy plant parts of *Abelmoschuis moschatus* (leaves and stems), *Aporosa cardiosperma (Gaertn.) Merr.* (leaves and stems), *Celosia argentea* (leaves and flowers) and *Nauclea orientalis* (leaves and roots) were collected from home gardens of Western and Southern provinces of Sri Lanka in fresh condition during the day after studying the morphological and organoleptic properties carefully.

Formulation of Powder and Solvent Extraction

The collected plant parts were washed, shade-dried, and ground into powder using a mechanical grinder to increase the surface area for extraction. Sequential extraction was performed to obtain the extracts from the plant parts using Hexane, Ethyl acetate, Methanol as industrial solvents. Three hundred grams (300 g) of plant material was macerated in 350 mL of Hexane for 24 h at room temperature (25 °C). The process was repeated 3 times to ensure complete extraction. The defatted plant material was then sequentially extracted using solvents with increasing polarity (Ethyl acetate, Methanol). The solvents from each extract were then removed using a rotary evaporator under reduced pressure at 40°C. The remaining dried extracts were then weighed and stored at -20 °C before use.

Determination of Extraction Yield

Extracted filtrates were allowed to evaporate until completely dry, with no trace of moisture or solvents remaining. Then, the weight of the dried pulps was measured separately. According to Truong *et al*., (2019), the extraction yield (%) was calculated using the formula as follows [11]:

$$\text{Extraction yield (\%)} = \frac{\text{Weight of crude extract after solvent evaporation}}{\text{Dry weight}} \times 100\%$$

Qualitative Phytochemical Screening

The crude methanolic extracts of the selected plants were used for phytochemical screening. The plant parts were screened for alkaloids, phenols, saponins, flavonoids, and tannins by following standard procedures to identify the chemical constituents of plant extracts [12, 13]. Formations of precipitates and the intensity of the color change were used as a measure of the analyses, where the following notations were used to interpret the results based on visual observations:

(+) = presence in trace or low amount (faint coloration or minimal precipitate)

(++) = presence in moderate amount (moderate intensity of color or precipitate)

(+++) = presence in high amount (strong intensity of color or dense precipitate)

(-) = absence of phytoconstituents

Antimicrobial Assay

The EUCAST disk diffusion assay was used to investigate the susceptibility of bacterial cultures to the crude plant extracts, as stated in the European Society of Clinical Microbiology and Infectious Diseases [14]. Test organisms were *Escherichia coli* ATCC® 25922™ and *Staphylococcus aureus* ATCC® 29213™. According to the McFarland standard, the turbidity of the inoculum was adjusted to 0.5. About 400 μL of the inoculum was swabbed onto the surface of a Mueller-Hinton agar plate. Sterilized filter paper discs (5 mm) impregnated with 20 μL volume of 400 μg mL^{-1} concentrated crude extracts were transferred onto the agar plates, and a positive control was set up using Gentamycin (20 μg mL^{-1}), and methanol-soaked filter paper disks were used as the negative control. Agar plates were incubated overnight at 37 °C ± 2 °C in an inverted position. The diameters of the inhibitory zones were calculated to the nearest millimeter. With the triplicate data, mean diameters were calculated and recorded as mean ± standard deviation.

Statistical Analysis

The results are presented as mean ± SD (Standard Deviation) was calculated by descriptive statistical analysis using the statistical package, Minitab version 20.4, by using one-way ANOVA. Statistical significance was determined at $p < 0.05$.

Results and Discussion

Extraction Yield

As shown in Table 1, the highest overall yield was achieved with methanol as the solvent, indicating that extraction efficiency favors highly polar solvents, and the yield of each extract varied with the plant material. Also, it can be indicated that most compounds consisting in plant parts show polar characteristics rather than non-polar characteristics, respectively. This supports previous findings indicating that methanol, due to its high polarity, is an effective solvent in extracting polar secondary metabolites such as

phenols, flavonoids, tannins, and alkaloids [15]. In contrast, hexane-which is a non-polar solvent and the least polar of the three solvents used in this study showed lower yields and was ineffective for *Nauclea orientalis* (0.05%), suggesting that most of the extractable phytochemicals in these plant parts are polar or moderately polar.

Table 1. Percentage extraction yield of crude plant extracts

Solvents	Crude Plant Extract	Percentage Yield (%)
Hexane	*Abelmoschus moschatus* leaves	7.21
	Abelmoschus moschatus stems	6.04
	Aporosa cardiosperma (Gaertn.) Merr. leaves	7.65
	Aporosa cardiosperma (Gaertn.) Merr. stem	5.07
	Celosia argentea leaves	7.37
	Celosia argentea flowers	5.32
	Nauclea orientalis leaves	0.05
	Nauclea orientalis roots	-
Ethyl Acetate	*Abelmoschus moschatus* leaves	4.91
	Abelmoschus moschatus stems	9.39
	Aporosa cardiosperma (Gaertn.) Merr. leaves	9.61
	Aporosa cardiosperma (Gaertn.) Merr. stem	5.67
	Celosia argentea leaves	8.71
	Celosia argentea flowers	6.61
	Nauclea orientalis leaves	2.13
	Nauclea orientalis roots	1.98
Methanol	*Abelmoschus moschatus* leaves	6.15
	Abelmoschus moschatus stems	7.45
	Aporosa cardiosperma (Gaertn.) Merr. leaves	6.04
	Aporosa cardiosperma (Gaertn.) Merr. stem	9.51
	Celosia argentea leaves	6.50
	Celosia argentea flowers	11.30
	Nauclea orientalis leaves	2.54
	Nauclea orientalis roots	3.19

Qualitative Phytochemical Analysis

As shown in Table 2, all plant extracts contained phytoconstituents, but their abundance varied with the solvent type. Phenol, flavonoid, tannins, and alkaloids were all found in methanolic extracts. This is consistent with their known polarities in the literature. As these phytochemicals are polar, the polar methanol is effective at extracting these compounds. Saponin is an amphiphilic compound with both polar and non-polar moieties, which are also well extracted by Methanol. Therefore, despite following a sequential extraction procedure, where methanolic extraction was preceded by Hexane and Ethyl acetate extraction, Methanol remained an effective solvent to recover the broadest range of target phytochemicals tested [16].

Nauclea orientalis (leaves and roots) showed a high abundance of alkaloids, phenols, tannins and saponins. The methanolic extracts of *Celosia argentea* exhibited a strong presence of phenolics and flavonoids, consistent with the existing literature [17] [18], which has documented their antioxidant and antibacterial activities. Ethyl acetate was moderately effective, particularly for flavonoids and phenols in *A. moschatus* and *C. argentea.*

Therefore, methanolic extracts were found to contain a higher abundance of phytochemicals than extracts obtained with other solvents. Hence, methanolic extracts of the plant parts are the most appropriate for phytoconstituent extraction, as all five phytochemicals are present at high abundance relative to Ethyl acetate and Hexane extracts. Thus, as per the results of both percentage extraction yield and preliminary phytochemical test, Methanol crude extracts of plant parts were selected for further investigation of antimicrobial susceptibility test.

Table 2. Results for the phytochemical analysis of crude extracts of selected plant parts

Solvents	Crude plant extract	Phytoconstituents[a]				
		Alkaloids	**Phenols**	**Saponins**	**Flavonoids**	**Tannins**
Methanol	*Abelmoschus moschatus* leaves	++	+++	-	-	++
	Abelmoschus moschatus stems	+	+	-	+	-
	*Aporosa cardiosperma (Gaertn.) Merr. l*eaves	+	+++	+++	+++	-
	*Aporosa cardiosperma (Gaertn.) Merr. s*tems	+	+++	+++	-	++
	Celosia argentea leaves	++	+++	+++	+++	+
	Celosia argentea flowers	+++	+++	+++	+++	-
	Nauclea orientalis leaves	++	+++	+	++	+++
	Nauclea orientalis roots	+++	+++	+++	++	+++

Ethyl acetate	*Abelmoschus moschatus* leaves	+++	++	+	+	+++
	Abelmoschus moschatus stems	+++	+	-	-	+
	Aporosa cardiosperma (Gaertn.) Merr. leaves	-	++	++	++	+
	Aporosa cardiosperma (Gaertn.) Merr. stems	++	+	+	-	-
	Celosia argentea leaves	+	++	-	+++	-
	Celosia argentea flowers	-	-	+	+	-
	Nauclea orientalis leaves	-	++	+	-	-
	Nauclea orientalis roots	-	-	+	-	+++
Hexane	*Abelmoschus moschatus* leaves	+	-	++	-	-
	Abelmoschus moschatus stems	+	-	++	-	-
	Aporosa cardiosperma (Gaertn.) Merr. leaves	++	-	++	++	-
	Aporosa cardiosperma (Gaertn.) Merr. stems	++	-	+++	++	-
	Celosia argentea leaves	+	++	+	++	+
	Celosia argentea flowers	+	-	-	+	-
	Nauclea orientalis leaves	-	-	+	-	-
	Nauclea orientalis roots	-	-	+	-	-

[a] Key: (+) = Presence in trace or low amount, (++) = Present in moderate amounts, (+++) = Present in high amounts, and (−) = Absence of active constituents

Antimicrobial Assay

The results of the study, as shown in Table 3, indicate that all plant extracts possess an antimicrobial spectrum against selected disease-causing pathogens. Among all the extracts, methanolic extracts of *Nauclea orientalis* leaves exhibited the highest zone of inhibition against Gram-negative *E. coli* (12.67 ± 0.58 mm), and Gram-positive *S. aureus* (10.00 ± 2.65 mm). Furthermore, the inhibition potential varies by plant species, with *Nauclea orientalis* exhibiting the highest levels of inhibition, followed by *Aporosa cardiosperma (Gaertn.) Merr*., *Celosia argentea*, and *Abelmoschus moschatus* against *E. coli* and *S. aureus.* The reported

antimicrobial potential of *N. orientalis* is consistent with previous studies, which reported slightly similar inhibition ranges [18]. Further, *A. moschatus* has been documented to show moderate activity against these pathogens [19]. The inhibition zones observed for *C. argentea* also align with published values [20]. There is limited literature available on the antimicrobial activity of *A. cardiosperma* and *C. argentea*, making this study one of the few to report such findings. This suggests a potential novelty in the antimicrobial profiling of these species, particularly in the context of their comparative efficacy against both Gram-positive and Gram-negative bacteria. However, the inhibition zones produced by all methanolic plant extracts were significantly lower compared to the positive control Gentamycin (approximately 23 mm) for both *E. coli* and *S. aureus*, indicating that although the plant extracts exhibited antimicrobial activity, their efficacy in crude form is considerably less than the standard antibiotic.

Table 3. Zone of inhibition diameter (mm) of the selected plant parts

Sample	Inhibition zone diameter (mm)[a]	
	E. coli	***S. aureus***
Gentamycin	23.33 ± 1.16	21.67 ± 1.56
Abelmoschus moschatus leaves	8.00 ± 2.65	7.00 ± 0
A. moschatus stems	7.67 ± 0.58	7.67 ± 0.58
Aporosa cardiosperma (Gaertn.) Merr. Leaves	10.66 ± 0.1	10 ± 0
A. cardiosperma (Gaertn.) Merr. Stem	9.6 ± 0.2	8 ± 0.1
Celosia argentea leaves	10 ± 3	7.33 ± 6.3
C. argentea flowers	8.3 ± 0.88	7.3 ± 9.3
Naulcea orientalis leaves	12.67 ± 0.58	10.00 ± 2.65
N. orientalis roots	7.00 ± 1.00	9.00 ± 1.00

[a] Results are given as mean values ± standard deviation of triplicate data.

Conclusion

All methanolic extracts of the four tested medicinal plant species exhibited mild to moderate antibacterial activity against *E. coli* and *S. aureus*. Although the activity was lower than that of the positive control Gentamycin, the results suggest the presence of bioactive compounds with potential antimicrobial effects. Hence, the findings indicate that the extracts of the plants contained various biologically active compounds responsible for the antibiotic properties, which have great importance as therapeutic agents for the infectious diseases caused by selected pathogens, and to improve the health status of the consumers. These preliminary findings support the traditional use of these plants and highlight their relevance for further investigation. Future studies should explore the synergistic effects of these extracts with standard antibiotics against resistant strains and expand the antimicrobial screening to a broader spectrum of pathogens. Hence, the findings from this study can be further developed through modifications and may lead to the isolation of potential drug leads with antioxidant and antimicrobial activity in the future.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this research.

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