Advanced Studies in Biology, Vol. 15, 2023, no. 1, 1 – 8
HIKARI Ltd, www.m-hikari.com
https://doi.org/10.12988/asb.2023.91605

Phytochemical and Antimicrobial Evaluations of Selected Medicinal Plants Used in the Northern Part of Surigao del Sur, Philippines

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Abstract

Medicinal plants are widely used to cure and prevent numerous ailments. They usually contain phytochemicals which aid in developing innovative drugs that tackle the threat of resistant strains. This study investigated the ethanol extracts of four plant species commonly used in the northern part of Surigao del Sur, Philippines, for the treatment of various diseases through a qualitative phytochemical analysis. The investigation revealed the presence of Alkaloids and Steroid flavonoids in all four plant samples. The plant extracts of Chromolaena odorata, Celosia argentea, Cordia dichotoma, and Nepenthes bellii showed antibacterial activity to both Staphylococcus aureus (gram-positive) and Escherichia coli (gram-negative) bacteria, which were evident in the Zone of inhibition. The findings support using herbal plants in ethnomedicine to treat various infectious ailments.

Keywords: Phytochemical analysis, Antimicrobial activity, Medicinal plants, Leaf extract, Zone of inhibition

I. Introduction

The occurrence of various ailments causes individuals to seek remedies to alleviate them through the discovery of medications. However, when the expense of living rises, so does the cost of treating specific ailments. Herbal healing is a practical method to investigate an option that is less expensive and simpler to get. Human-plant interaction has long been recognized as one of the forces shaping human civilization, particularly in medical sectors [18]. The documentation of plant medicinal uses through ethnobotanical research allows for the creation modern medications and therapies and plant conservation [7].

Traditional medicine is the knowledge, skills, and practices based on indigenous to different cultures' theories, beliefs, and experiences that are utilized in the maintenance of health in the prevention, diagnosis, improvement, and treatment of physical and mental disorders [10].Traditional medicinal plants include a diverse spectrum of bioactive compounds, making them valuable sources of several forms of medicine, including antibacterial characteristics [12]. These are secondary metabolites such as alkaloids, flavonoids, steroids, tannins, and phenol chemicals.

Infections have grown dramatically in recent years, and antibiotic resistance has become a growing therapeutic issue [11] , Barbour et al., [4]. Higher plant natural products may have a reliable supply of antibacterial compounds with potentially unique modes of action [1]. They are helpful in the treatment of infectious diseases and reducing many of the adverse effects associated with synthetic antimicrobials [8]. As a result, screening these plants is of significant interest to authenticate their use in traditional medicine and uncover the active principle by separating and characterizing their elements. A systematic screening of them lead to discovery of new active molecules [16].

Four frequently used medicinal herbs in the northern section of Surigao del Sur were investigated in this study. The phytochemical content and antimicrobial activity of leaf extracts from Chromolaena odorata (Hagonoy), Celosia argentea (Kudyapa), Cordia dichotoma (Anonang), and Nepenthes bellii (Hara-hara) against Staphylococcus aureus (gram-positive) and Escherichia coli (gram-negative) bacteria were evaluated.

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II. Materials and Methods

Collection of Plant Material

Permits were obtained first from the Carrascal and Cantilan municipal governments before collecting the samples. Fresh leaves of Chromolaena odorata (Hagonoy), Celosia argentea (Kudyapa), Cordia dichotoma (Anonang), and Nepenthes bellii (Hara-hara) were gathered in the municipalities of Carrascal and Cantilan, Surigao del Sur, from April to May of 2020. A botanist verified the authenticity of these plant samples.

Preparation of Plant Extract

The four plant leaves samples were processed following the protocol [(12)] described: leaf samples were air-dried under the shade for three weeks at room temperature until crispy; powdered using a blender; and stored inside an airtight plastic container prior to their extraction. In preparing the ethanolic extract, 100 grams of the powdered of each leaf sample were soaked in 200 ml of pure ethanol for seven days. Mixtures were placed in a container covered with black cloth and foil and stored in a locker at room temperature. Residues were filtered and re-extracted with ethanol. The ethanol (solvent) was separated using Whatman No. 1 filter paper. After filtration, a rotary evaporator removed the solvent in a vacuo. The concentrated leaf ethanolic crude extract of the four herbal plants was obtained. The crude extracts were stored in a tight glass container and refrigerated at 7°C until used.

Phytochemical screening

Phytochemical screening of the ethanolic leaf extracts of Chromolaena odorata, Celosia argentea, Cordia dichotoma, and Nepenthes bellii was processed following the protocol [(14)] with modifications. Ethanolic leaf extracts of were ethanolic leaf extracts of Chromolaena odorata, Celosia argentea, Cordia dichotoma, and Nepenthes bellii were subjected to qualitative phytochemical screening for the determination of the presence of various classes of active chemical constituents such as alkaloids, Quaternary Bases and Amine oxide, steroids, flavonoids, saponins, and tannins.

Antimicrobial evaluation

Two test pathogens, Escherichia coli, and Staphylococcus aureus, used in this study were isolated from water samples by standard methods. The identity of the isolates was confirmed by morphological characteristics and conventional biochemical tests [(5)]. Determination of antibacterial activity Antimicrobial activity of five medicinal plant extracts were identified through the agar well diffusion method. Pure cultures were preserved at 4°C on nutrient agar. A 0.1 mL of a freshly grown culture of test organisms (106 CFU/mL) was aseptically introduced and spread on the surface of sterile Muller Hilton agar plates. Wells of 6 mm diameter were made in agar plate with the help of a sterile cork-borer. Fifty microliters of different plant extracts were filled in the wells with the help of a micropipette. Plates were left for some time at 4°C till the extract diffused in the medium with the lid closed and incubated at 37°C for 24 hr. The plates were observed for the Zone of inhibition. Antibacterial activity was evaluated by measuring the diameter of the Zone of inhibition against the tested bacterial pathogens. Each assay in this experiment was replicated three times (8).

III. Results and Discussion

Table 1 presents the phytochemical analysis of the four medicinal plants, indicating the presence of various secondary metabolites. Alkaloids, Quaternary Bases and Amines, Steroids, Flavonoids, and Saponins were revealed, while Tannins were not found in Chromolaena odorata extract. Alkaloids, Quaternary Bases and Amines, Steroids, Flavonoids, Tannins, and the absence of Saponins were revealed in the ethanolic extract of Celosia argentea. The Cordia dichotoma extract bares no presence of quarternary bases, amine oxide, and saponins. For Nepenthes bellii extract, the amount of Alkaloids, Steroids, Flavonoids, Saponins, and Tannins is present. At the same time, the absence of Quaternary Bases and Amine was learned.

Alkaloids, Steroids, and Flavonoids were all found present among these species of ethnomedicinal plants tested. Studies have shown that flavonoids have a wide range of biological actions, including antioxidant, anti-inflammatory, antibacterial, anti-anionic, anti-cancer, and anti-allergic properties (3). These secondary metabolites may have been previously associated with antimicrobial activity (13). Further, Steroids were also identified in the evaluation, which has been reported to have antibacterial properties (14). Saponin has an antimicrobial property which is due to its ability to cause leakage of proteins and certain enzymes from the cell (19). In general, the antimicrobial activity of Chromolaena odorata, Cordia dichotoma, Celosia argentea, and Nepenthes belii may be referred to as their rich Alkaloids, Steroids, and flavonoid content. As a result, the antibacterial activity of ethnomedicinal plant ethanolic extracts could be attributed to the bioactive components found in the various extracts.

The total absence of Quaternary Bases and Amine Oxide for Cordia dichotoma and Nepenthes Bellini; saponins in Celosia argentea and Cordia dichotoma; Tannins in Chromolaena odorata is not concluded in this section of the study. This study only used ethanol as the solvent for extraction, and the mentioned secondary metabolites can be detected through other methods of extraction and other solvents.

Table 2 shows the measured diameter of the inhibition zones of the different treatments against S. aureus and E.coli. The calculated means are reflected in the table, suggesting that the treatment effects are significant. The average Zone of inhibition values for each sample shows that the treatments brought active to very active antimicrobial activity with the lowest average Zone of inhibition value of

15mm, with the highest value of 29mm. The Leaf extract of Chromolaena odorata) exhibited the largest average diameter for the Zone of inhibition against Staphylococcus aureus (Gram-positive bacteria) with a diameter of 29mm. Chromolaena odorata Leaf extract also shows the largest diameter for the average Zone of inhibition for Escherichia coli (Gram-negative) of 19mm. This entails that the Leaf extract of Chromolaena odorata records the highest antimicrobial activity among the other herbal plant samples, followed by the antimicrobial activity of (Cordia dichotoma) Leaf extract, (Celosia argentea leaf extract), and (Nepenthes bellii leaf extract).

The results demonstrated that all treatments could inhibit the bacterial growth of S. aureus and E. coli. [(5)] stated that the demonstration of antimicrobial activity against the plants' gram-positive and gram-negative bacteria might indicate the presence of a broad spectrum of antibiotic compounds. Among the plant extracts tested, Chromolaena odorata leaf extract) possessed the most significant inhibitory property, while (Nepenthes bellii leaf extract) had the lowest inhibitory property. Regardless of their Zone of inhibition measurement, medicinal plants' antimicrobial action is attributable to several chemical agents classed as bioactive antimicrobial compounds [(2)], as shown in Table 1. Further, in this study, S. aureus (gram-positive) bacteria were sensitive to four plant extracts. The highest sensitivity of S. aureus may be due to its cell wall structure and outer membrane [(19).]

The current study's findings supported the validity of these medicinal plants' usage as traditional remedies. Also, they suggested that some plant extracts contain antimicrobial components that can be employed as antimicrobial agents. Herbal medication is less dangerous than manufactured pharmaceuticals. The study demonstrates plant components' role in producing a potent antibacterial agent. However, further research is desired to sufficiently evaluate the potential efficacy of crude extracts of the four herbal plants as antibacterial agents.

Table 1: Phytochemical analysis of Ethanol extracts of Selected Medicinal Plants

	Medicinal Plants			
Secondary Metabolites	*C.odorata*	*C. argentea*	*C.dichotoma*	*N. bellii*
Alkaloids	+	+	+	+
Test for Quaternary Bases and Amine oxide	+	+	-	-
Steroids				
-Keller-Killiani Test: 2-deoxysugars	+	+	+	+
-Liebermann-burchard Test: Unsaturated steroids	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	-	-	+
Tannins	-	+	+	+

(-) absence of secondary metabolites, (+) presence of secondary metabolites done in triplicates

Table 2: Zones of Inhibition (in millimeter) of Chromolaena odorata, Cordia dichotoma, Celosia argentea, Nephenthes belii against E. coli and S. aureus

Medicinal Plants	Average Zone of Inhibition in mm	
	S. aureus (Gram positive)	*Escherichia coli* (Gram -)
C.odorata	29 mm	19 mm
C. dichotoma	25 mm	16 mm
C. argentea	23 mm	15 mm
Nep bellii	19 mm	16 mm

Legend: <10mm-inactive, 10-13mm- partially active, 14-19mm- active, > 19mm- very active

Conclusion

The current study shows that all four medicinal plants exhibited antimicrobial activity. Further, Chromolaena odorata records the highest antimicrobial activity among the other herbal plant samples against two test organisms, S. aureus (gram-positive) and E. coli (gram-negative). test organisms S. aureus (gram positive) and E. coli (gram negative)

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Received: December 15, 2022; Published: January 5, 2023