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Cagayan's Endemic Medicinal Plants: Antimicrobial Activities and Phytochemical Profiles

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ABSTRACT
Individuals and communities are increasingly turning to medicinal plants for primary health care. Antimicrobial activity of two indigenous plants, Antidesma madagascariense, were studied in this study. Lam. and Erythroxylummacrocarpum. O. E. Schulz, which are included in the Cagayan pharmacopoeia. In vitro tests were performed on aqueous and methanol extracts of both plants' leaves and twigs. against three Gram-negative and five Gram-positive bacteria strains, as well as a resistant strain. They were also tested for antifungal resistance to Candida albicans. Aspergillusniger and ATCC 10231 ATCC 16404, a human pathogenic bacterium, and ATCC 16404, a plant pathogenic microbe, are both known to be effective markers of antifungal activity. In comparison to methanol extracts, which produced inadequate inhibition zones, aqueous extracts of both plants have broad-spectrum antibacterial activities against the test species. Furthermore, the antibacterial component found in these plants appeared to be most concentrated in the leaves and least concentrated in the twigs, with Gram-positive bacteria showing the strongest inhibitory activity (Staphylococcus aureus. ATCC 25923). Except for the methanol extract, neither plant's aqueous extracts had antifungal activity against A. niger. Phytochemical analysis of the plants revealed the presence of antimicorbial substances such as tannins, phenols, flavonoids, and alkaloids.
***Keywords**: medicinal plants, Antidesmamadagascariense,antimicrobial, inhibition zones*

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I. INTRODUCTION

Medicinal plants have been used as a source of medicine in practically all societies since time immemorial (Ang-Lee et al., 2001; Goldman, 2001). Herbal medicines are still widely used in India and China, and many of these ancient medicines have been rediscovered as inexpensive sources of complex bioactive molecules in Western countries. Oral dose forms of modern medications often contain single synthetic compounds with high clinical efficacy. Natural compounds from higher plants, on the other hand, are still used in pharmaceutical preparations, either as whole plants or as extracts (Gogtay et al., 2002).

Natural products remain one of the best reservoirs of new structural types, despite the availability of several methodologies for the development of medicinal drugs (Hostettmann, 1999). As a result, in the never-ending quest to improve the efficacy and ethics of modern medicine, researchers are increasingly looking to folk medicine as a source of new medications (Hoareau&Dasilva, 1999). The use of herbal medicine is a long-standing tradition among the people of Cagayan. Cagayan's indigenous and endemic plant species have long been employed in traditional medicine to cure a variety of human diseases (Baumer, 1979). According to available data, indigenous folk-medicinal plant preservation and research are critical since such plants are more adapted to local settings and conditions than foreign species. Phytochemicals and pharmacologically active substances identified from endemic and indigenous plants used in folk medicine have been the focus of research in recent decades.

Furthermore, it is critical to preserve and retain information about the therapeutic benefits of Cagayan's plant resources, which are distinguished by a diverse flora of alien, endemic, and indigenous plant species. Several types of extracts from exotic, endemic, and indigenous plants are currently sold as decoctions or "tisanes" to treat minor diseases in several markets across Cagayan. This native herbal folk medical practice is an important element of Cagayan's indigenous pharmacopoeia's legacy. Despite this, only a few medicinal plants have been proven to have medical properties. Furthermore, the biological activity of such medicinal plants against specific human pathogenic bacteria and fungus has been understudied, and a literature search revealed little information on the antimicorbial activity of Mauritian indigenous plants. The advent of microorganisms resistant to many regularly used antibiotics has prompted additional efforts to find novel antimicrobial agents to battle infections and overcome the challenges of resistance to already existing antimicrobial medicines (Bruce et. al. 1990). Plant-based antimicrobials and antibacterials are a hugely underutilized source of medications with significant therapeutic promise. They treat infections effectively while avoiding many of the adverse effects associated with synthetic antimicrobial and antibacterial drugs (Mathews et al., 1999; Bagghi, 2000). Therefore, the present day in vitro. antimicorbial examine became aimed toward validating the results of endemic medicinal flora of Cagayan, Antidesmamadagascariense. Lam. (Euphorbiaceae) and Erythroxylummacrocarpum. O. E. Schulz (Erythroxylaceae), for his or her antimicrobial pastime in opposition to 8 medical traces of microorganism and traces of fungi. In addition, we additionally evaluated their phytochemical profiles to correlate feasible organic sports of those flora.

II. Materials and Methods

Preparation of the medicinal plant extracts

Leaves and twigs of Antidesmamadagascariense. and Erythroxylummacrocarpum. used withinside the observe have been accrued from Maccabe forest. The Curator of the National Herbarium on the Cagayan Sugar Industry Research Institute (MSIRI) showed the identification of the plants. Voucher specimens have been deposited on the Herbarium series of the Department of Chemistry, Faculty of Science, University of Cagayan.

Extraction

Crude water excerpt of the industry material was secondhand in the current study. It was main to determine antimicrobial preparing of the raw water excerpts so that legalize the curative uses of these shops, as this is the hole or door in vessel that the original culture in the established drug of Cagayan uses the shops. In addition, intoxicating excerpts of the shops were secondhand for a relative base. more, leaves and outgrowths of two together shops were secluded for antimicrobial adapting, as the original community uses two together shop hallway as decoctions. new calm firm accoutrements were either air- drained or drained in a drying press at 50 °C for 5 – 7 days. Ten grams of the drained branch accoutrements (leaves and outgrowths) of the cooperative variety was alone smashed and base into fine maquillages utilizing a cuisine an instrument used to mix materials. Each stimulate branch material was destroyed to exhaustion accompanying water or intoxicating in a Soxhlet outfit for 5 h. The soap was refined off under weakened pressure to go raw industry excerpt. The adhesive was calm for test. Chance yield was deliberate, and the adhesive- specific water or flammable liquid anticipation was debased or dirty for further tests.Test

CULTURE

Bacillus cereus was employed as a test organism for antibacterial activity screening. Bacillus subtilis, ATCC 10702. Escherichia coli ATCC 6633 Staphylococcus aureus (ATCC 25922), methicillin-resistant Staphylococcus aureus (MRSA) Enterococcus faecalis, ATCC 43300. ATCC 29212, Pseudomonas aeruginosa. ATCC 27853, and Salmonella typhimurium. ATCC 14028. Stock cultures of the bacteria and fungi used were clinical isolates obtained from the Bacteriology Section of the Central Laboratory Candos, Victoria Hospital. Cultures were maintained as nutrient agar slants in screw-capped bottles and stored at 4°C. All cultures were checked for viability and purity by regular plating. Test cultures were prepared by transferring a loop full of bacteria from stock culture nutrient broth and incubated at 37°C for 24 h. Fungi were transferred into freshly prepared dextrose agar plates and incubated at 25°C for 3 days. The fungi used in the study were Aspergillusniger. and Candida albicans..

Antimicrobial bioassay procedure

Using the agar disk diffusion method, the antibacterial and antifungal activity were assessed (Mahasneh, 2002). The activities of the test extracts are expressed by measuring the diameter of the zone of inhibition, which is a highly effective method for quickly growing microorganisms. Filter paper disks (6 mm in diameter) were sterilized and impregnated with the necessary concentrations of plant extracts. Mahasneh mentioned allowing the disks (made from Whatman no. 1) to absorb the plant extracts (2002).Plates of Mueller-Hinton sensitivity agar (Oxoid, UK) were aseptically inoculated with broth cultures for the test organisms using sterile Pasteur pipette. The plates were allowed to dry. The disks containing the plant extract were transferred using flamed but cooled forceps onto the surface of the seeded agar plates. They were sufficiently spaced to prevent the resulting zones of clearing from overlapping. The extractive solvent (water) was used as a negative control. The plates with the organisms were incubated for 24 h. After incubation, the growth inhibition rings were quantified by measuring the diameter for the zone of inhibition to the nearest millimeter from the lower surface of the Petri dishes. Inhibition zone values were corrected, that is, disk diameter was subtracted from the value of the inhibition zone. As the diameter of the disk was 6 mm, inhibition zones of less than 7 mm were not evaluated (Hong et al., 2004) Negative control disks contained the solvent. Standard antibiotic (ampicillin) was used as positive control for comparison. The experiments were performed three-times to minimize errors.

Determination of minimum inhibitory concentration (MIC) values

A small modification of the tube dilution method was used to obtain the MIC of each plant extract (Omoregbe et al., 1996). The minimum inhibitory concentration (MIC) of an extract in a medium without apparent growth of the test organisms is known as the MIC. Sterile filter paper disks (6 mm in diameter) were impregnated with the different dilutions of the plant extract and aseptically transferred to the surface of the inoculated plates using flamed but cooled forceps. The disks were sufficiently spaced to avoid overlapping of zones of inhibition. The MIC of the different plant extracts that inhibited the growth of the test organism other than inhibition due to the diluent was taken as the MIC.

Phytochemical screening of *A. madagascariense*. and *E. macrocarpum*.

Both leaves and twigs were subjected to a thorough phytochemical screening using standard (Narod, 2002) protocols to detect the presence of the following secondary metabolites: alkaloids, coumarins, terpenes, anthraquinones, tannins, phenols, leucoanthocyanins, flavones, and saponins.

III. RESULTS

Comparative antimicrobial screening

Table 1 shows the results of antimicrobial tests performed on aqueous and methanol extracts of A. madagascariense and E. macrocarpum. The antimicrobial experiments revealed that the aqueous extracts of both plants had a broad spectrum of activity, being active against Gram-positive and Gram-negative organisms.The zones of inhibition ranged from 3 to 12 mm, and 3 to 9 mm for aqueous extract of leaves and twigs of A. madagascariense., respectively. For E. macrocarpum., the inhibitory zone ranged from 4 to 13 mm and 3 to 10 mm for leaves and twigs, respectively. Aqueous leaf extract of both endemic plants were found to exhibit more antibacterial activity (100%). The methanol extract of leaves and twigs of both plants gave poor inhibition zones (3–10 mm). The leaf extract was also found to be most active against Gram-positive bacteria (inhibition zone 6–12 mm) compared to Gram-negative strains (inhibition zones 5–8 mm). On the other hand, 87% for twigs of A. madagascariense. and 50% for twigs of E. macrocarpum., showed antimicrobial activities against the test organisms. Comparative inhibitory zones to standard antibiotic ampicillin was observed for aqueous leaf extract of E. macrocarpum. against S. aureus. and twigs of A. madagascariense. against E. faecalis.. It was also found that aqueous leaf extract of both endemic plants showed moderate antibacterial activities against a resistant strain of S. aureus. (MRSA). Neither aqueous leaf nor twigs extract of the two plants possess antifungal activities against A. niger. and C. albicans.. However, a methanol extract of A. madagascariense. showed antifungal activities against A. niger..

Table 1 Antimicrobial activities of *A. madagascariense*. and *E. macrocarpum*. against the test microorganisms.

			Diameter of zone of inhibiting(mm)b.			
			A.madagascariense.		E.macrocarpum.	
Test microorganisms	Gram stain+/−	Standard antibiotic (Ampicillin)[a]	Leaves	Twigs	Leaves	Twigs
Bacillus cereus.	G+	14	10	9	7	3
			(5)	(3)	(−)	(−)
Bacillus subtilis.	G+	12	7	8	8	4
			(3)	(4)	(−)	(−)
Escherichia coli.	G−	10	8	7	6	−
			(8)	(5)	(−)	(−)
Staphylococcus aureus.	G+	13	12	4	13	10
			(10)	(4)	(10)	(6)
MRSA	G+	0	3	−	5	−
			(−)	(−)	(−)	(−)
Enterococcus faecalis.	G+	7	6	7	4	−
			(3)	(−)	(−)	(−)
Pseudomonas aeruginosa.	G−	9	5	6	6	6
			(2)	(4)	(−)	(−)
Salmonella typhimurium.	G−	10	7	9	8	−
			(5)	(5)	(3)	(5)
Aspergillusniger.	F	0	−	−	−	−
			(5)	(7)	(−)	(−)

Minimum inhibitory concentration

The MICs of the extracts that showed antibacterial activity using the disk diffusion method were determined using the broth dilution assay. Table 2 shows the results of the MICs of the various plant extracts.The MIC value for aqueous extract of leaves and twigs of A. madagascariense. ranged from 2 to 32 mg/ml, respectively; 0.5 to 32 mg/ml for leaves and twigs of E. macrocarpum., respectively. The lowest MIC value (0.5 mg/ml) was recorded for E. macrocarpum. against the Gram-positive strain of S. aureus.. However, for the MRSA strains, high concentrations of the leaves extracts of both endemic plants were needed to inhibit growth of the bacteria. On the other hand, the methanol extract of both plants showed high MIC values except for leaves of E. macrocarpum. against S. aureus. (MIC of 1 mg/ml).

Table 2. Minimum inhibitory concentrations (mg/ml) of A. madagascariense. and E. macrocarpum. leaves and twigs for the 10 strains of microorganisms.

		Minimum inhibitory concentration (mg/ml)			
		A. madagascariense.		E. macrocarpum.	
Test microorganisms	Gram stain+/−	Leaves	Twigs	Leaves	Twigs
Bacillus cereus.	G+	2	8	16	16
		(4)	(8)	(−)	(−)
Bacillus subtilis.	G+	4	16	8	8
		(8)	(16)	(−)	(−)
Escherichia coli.	G−	8	16	10	−
		(8)	(4)	(−)	(−)
Staphylococcus aureus.	G+	2	4	0.5	0.5
		(4)	(8)	(1)	(4)
MRSA	G+	32	−	16	−
		(−)	(−)	(−)	(−)
Enterococcus faecalis.	G+	4	10	8	−
		(8)	(−)	(−)	(−)
Pseudomonas aeruginosa.	G−	8	8	4	8
		(8)	(8)	(−)	(−)
Salmonella typhimurium.	G−	8	8	4	−
		(8)	(8)	(8)	(8)
Aspergillusniger.	F	−	−	−	−
		(16)	(16)	(−)	(−)
Candida albicans.	F	−	−	−	−
		(−)	(−)	(−)	(−)

Phytochemical screening

Table 3 summarizes the phytochemical components of both plants. Tannins, phenols, flavonoids, and alkaloids were found in both E. macrocarpum and A. madagascariense leaves and twigs.

Table 3. Phytochemical components of A. madagascariense. and E. macrocarpum. leaves and twigs (+, presence; −, absence).

Plants	Part used	Alkaloids	Flavonoid	Tannins	Leucoanthocyanins	Terpenes	Phenols	Saponins
A. madagascariense.	Leaf	+	+	+	+	−	+	+
	Twigs	+	+	+	+	−	+	+
E.macrocarpum.	Leaf	+	−	+	+	−	+	−
	Twigs	+	+	+	+	+	+	−

IV. DISCUSSION

Intrigued in higher plant extricates showing antimicrobial movement has expanded in later a long time, and a few reports on this subject have been distributed De Dicastillo et. At. 2020). Both endemic plants considered within the current work appeared antibacterial exercises against the test microorganisms. In any case, the plant parts contrast altogether in their action against the test microorganisms. The contrasts watched within the antimicrobial exercises tests propose the defenselessness of the test microorganisms to different auxiliary metabolites show in these endemic plants. The composition of these auxiliary metabolites in turn changes from species to species, climatic conditions, and the physiological state of improvements of the endemic plants (Hussain&Deeni, 1991).Additionally, the antibacterial substance inside these plants within the current consider appeared to be most conspicuous within the leaves and slightest within the twigs, and the leading inhibitory action was watched for Gram-positive microbes (S. aureus.). This may be attributed to the reality that cell divider in Gram-positive microscopic organisms comprises of a single layer, though Gram-negative cell divider may be a multilayered structure bounded by an external cell film (Yao &Moellering, 1995). On the other hand, both plants were not successful against the test organisms but for methanol extricate of A. madagascariense..

Such comes about demonstrate that antibacterial operators are more common within the plants considered than antifungal operators. This may be attributed to basic contrasts between prokaryotic microbes and eukaryotic parasitic operators; antimicrobial operators ought to tie to sterols in eukaryotic film so as to show their activity, though this step isn't required for bacterial cells (Nishimura, &Matsumori (2020). Comes about from the current ponder too appear that methanol extricates of both plants gave destitute restraint zones. Undoubtedly, detailed that there are extraordinary contrasts between the movement of fluid and natural extricates in their anti-HIV screening: around 34% of the plant was dynamic in watery extricate and as it were 4% in natural extricate.

Contrasts in antimicrobial movement of restorative plants are clearly related to contrasts in their substance of dynamic compounds. Accessible reports tend to appear that alkaloids and flavonoids are the mindful compounds for the antimicrobial exercises in higher plants (Cordell et al., 2001). In addition, it is additionally claimed that auxiliary metabolites such as tannins and other compounds of phenolic nature are classified as dynamic antimicrobial compounds. Interests, phytochemicals screening of the current examination has uncovered that extricates from both endemic plants have at slightest four of the taking after classes of auxiliary metabolites: tannins, phenols, flavonoids, and alkaloids. In this manner, the nearness of these phytochemicals may to a few degree legitimize the observed antimicrobial exercises within the current consider. Furthermore, since the current antimicrobial think almost was done utilizing the same liquid course of action as embraced by the routine healers, these comes approximately would back the way individuals utilize these home grown cures. To this impact, it is conceivable that these two endemic therapeutic plant extricates can be utilized as antibacterial specialists in nourishment or other fixings. In any case, the precise component of antibacterial impacts from A. madagascariense. and E. macrocarpum. ought to be advance inspected for potential employments. In conclusion, the current think about advocates the require for proceeding screening for antimicrobial specialists from nearby endemic plants of Cagayan.

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