Phytochemical Screening And Antimicrobial Study Of The Different Leaf Extracts Of *Alocasia sanderiana* Bull., An Endemic Philippine Plant

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Abstract: The objective of this study is to investigate the phytochemical contents and evaluate the antimicrobial property of *Alocasia sanderiana* Bull. against a large number of pathogens. To do this, *Alocasia sanderiana* Bull. was screened for qualitative phytochemical tests including thin layer chromatography. Aside from the crude extract from the Rotary evaporator, three fractions from the plant were prepared using methanol, dichloromethane (DCM) and hexane. The 4 solvent extracts were then evaluated for antimicrobial activity using disc diffusion method on 18 strains of organisms. About this study, it was found out that triterpenes, tannins and saponins are present during phytochemical screening. Zones of inhibitions during the antimicrobial tests were observed but did not reach the desired zone for antimicrobial activity. The DCM fraction produced 4 mm zone against *Proteus mirabilis*, 3 mm for *Pseudomonas aeruginosa*, 1 mm for *Pectobacterium carotovorum* and 1 mm for *Candida albicans*. The methanol fraction also produced a 1 mm zone against *Pseudomonas aeruginosa*. The results show that *Alocasia sanderiana* Bull. leaf extracts contain polyphenolic compounds but this study shows that it exhibits non-active antimicrobial activity against the 18 strains that it was tested and may not be utilized as a potential antimicrobial drug for the said strains.

Index Terms: *Alocasia sanderiana* Bull. , Antimicrobial, Phytochemical screening

1 INTRODUCTION

Alocasia sanderiana Bull. is an endemic Philippine plant, although there has been studies on other *Alocasia* spp., there hasn't been any for the subject plant. *Alocasia* spp. has been used for economic purposes mainly as food[1] from its leaf to the whole plant depending on the specie but for *Alocasia sanderiana* Bull., it serves as an ornamental in Filipino houses. This study would like to find out if it may be used as a potential antimicrobial since previous literatures show indirect antibacterial[2,3] and antifungal[4] properties for other Alocasia spp. There are evidently less studies undertaken for this genus and none for *Alocasia sanderiana* Bull. but should a medicinal or pharmaceutical use be discovered for this plant, it may become more than just an ornamental in the Filipino houses. Medicinal studies on *Alocasia* spp. include the hepatoprotective effect[5,6] of Alocasia indica Linn. which may be due to an antioxidant property that may be present as in *Alocasia macrorrhiza* Linn.[7]

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A. macrorrhiza has also been recorded for its folkloric use in Malaysia for cough and toothache[8] as well as in parasitic infestations[9]. On the other hand, *Alocasia odora* (Lindl.) K. Koch burned leaves have also been used in folkloric medicine as a liniment against small pox.[10] Another interesting medicinal use of the *Alocasia* spp. particularly *Alocasia Cucullata* (Lour) Schott. is its use against snake bites[11] where it is believed to be an anti-venom[12,13,14] but there are no scientific studies to prove this medicinal property. *Alocasia* spp. is not all about medicinal properties; in fact, there are numerous literatures that classify it as poison[15,16] with one literature citing it as part of the 55 cases of herb-induced poisoning in a hospital based study from 1995 to 2007[17] that may cause neurotoxicity[18,19] which may be due to its calcium oxalate content[20] and other chemical irritants that also cause local pain, swelling, blistering of skin and mucous membranes[21] as well as dermatitis[22,23]. It may also cause excessive salivation and oral irritation[24] contrary to its use as food. It is also recorded that *Alocasia* spp. causes toxic reaction in pets usually birds[25]. This conflicting literature calls for a need to further study *Alocasia* spp. including *Alocasia sanderiana* Bull. because there is no clear information for its safety, there is no available scientific study for its antimicrobial property and there is even no study for the specie itself. *Alocasia sanderiana* Bull. may cause irritation and even poisoning owing to its calcium oxalate content that was observed microscopically by the researcher upon histological examination of the plant leaf but calcium oxalates are easily removed after heating[26] which may enable clinical trials for antibacterial property after this pre-clinical study. The purpose of this study is to confirm its indirect use as antimicrobial which is still not backed by scientific data up to the present day which may be due to its phytochemical constituents particularly the polyphenols after removal of the calcium oxalate as part of the plant extract concentration through the use of a rotary evaporator.

2 METHODOLOGY

2.1 Plant Materials

The plant material was collected in December 2012 at Salay, Misamis Oriental where it was asexually cultivated for research purposes in a loamy soil. The samples were washed, air-dried for 30 days in a clean well-ventilated area and packed in a polyethylene bag for transport in Manila. The botanical identification of species was carried out by the scientists at the Botany Division of the Philippine National Museum.

2.2 Materials

Methanol, Hexane and DCM were all obtained from RCI Labscan Limited through their local distributor, Belman Laboratories. Mueller Hinton agar and Sabouraud Dextrose agar were obtained from Sigma-Aldrich Chemicals through the same local distributor as well as the 6mm Antibiotic Assay Disk from GE.

TABLE 1

PHYTOCHEMICAL SCREENING OF *ALOCASIA SANDERIANA* BULL. LEAF EXTRACT

Phytoconstituents	Test Tube Reaction		Thin Layer Chromatography	
	Method	Result	Method	Result
Glycosides		Positive		
Tannins		Positive (non-hydrolysable)	Ferricyanide-ferric chloride	Positive
Alkaloids		Negative	Dragendorff's	Negative
Organic Acids		Negative		
Sterols and Triterpenes		Positive	Vanillin-sulfuric acid	Positive
Saponins		Positive		
Flavonoids		Negative		
Starch		Positive		
Albuminoids		Positive		
Sugars			α-Naphthol-sulfuric acid	Positive
	Molisch's	Positive		
	Iodine	Positive		
	Anthrone	Positive	Borntrager	Positive
	Barfoed's	Negative		
	Seliwanoff;s	Negative		
	Benedicts's	Positive		

2.3 Extraction of the Plant Material

The leaves of *A. sanderiana* Bull were cut to pieces and milled to fine powder then percolated with methanol at 1:10 ratio[27] for 48 hours. The percolated material was then filtered and the filtrate underwent concentration through Rotary Evaporator. The concentrated sample was subjected to fractionation using Vacuum Liquid Chromatography using 3 solvents added in the following order: methanol>DCM>hexane. The fractions were then stored at 4°C when not in use. The antimicrobial assay was performed using the fractions while the crude drug product from the Rotary Evaporator was used for the Phytochemical analysis.

2.4 Phytochemical Screening

Phytochemical screening for major phytoconstituents of the plant extracts was undertaken using standard qualitative methods as described by various authors[28,29]. The plant extracts were screened of biologically active compounds like glycosides, tannins, alkaloids, organic acids, sterol and triterpenes, saponins, flavonoids, starch, albuminoids, sugars. Thin layer chromatography was also used to confirm the previous test tube methods where it was found out that 1 part hexane and 9 parts ethyl acetate may be used as the solvent mixture but pure ethyl acetate was eventually used due to the better separation of compounds.

TABLE 2

ANTIMICROBIAL ACTIVITY OF THE DIFFERENT LEAF EXTRACTS OF *ALOCASIA SANDERIANA* BULL.

Microorganisms	Zone of Inhibition (mm)			
	Crude Extract	Methanol Fraction	DCM Fraction	Hexane Fraction
Gram +	0	0	0	0
E. faecalis	0	0	0	0
S. pyogenes	0	0	0	0
S. pneumoniae	0	0	0	0
B. subtilis	0	0	0	0
S. aureus	0	0	0	0
MRSA	0	0	0	0
S. epidermidis	0	0	0	0
B. cereus	0	0	0	0
Gram -	0	0	0	0
P. mirabilis	0	0	4 mm	0
E. coli	0	0	0	0
S. marcescens	0	0	0	0
E. aerogenes	0	0	0	0
P. carotovorum	0	0	1 mm	0
S. typhimurium	0	0	0	0
P. aeruginosa	0	1 mm	3 mm	0
S. cerevisae	0	0	0	0
Fungus	0	0	0	0
C. albicans	0	0	1 mm	0
S. cerevisiae	0	0	0	0
A. niger	0	0	0	0

2.5 Antimicrobial Assay

2.5.1 Test Organisms

The test microorganisms used in this study included 18 strains all obtained from the University of Santo Tomas – Tomas Aquinas Research Center (TARC). The Gram positive organisms were *Enterococcus faecalis*, *Streptocuccus pyogenes* (ATCC 19615), *Streptococcus pneumoniae* (ATCC 49619), *Bacillus subtilis* (UST CMS 1011), *Staphylococcus aureus* (ATCC 29213), *Staphylococcus aureus* MRSA (ATCC 43300), *Staphylococcus epidermidis* (ATCC 12228), *Bacillus cereus* (UST CMS 1009). The Gram negative organisms were *Proteus mirabilis* (UST CMS 1070), *Escherichia coli* (ATCC 25922), *Serratia marcescens* (UST CMS 1095), *Enterobacter aerogenes* (UST CMS 1021) *Pectobacterium carotovorum*, *Salmonella typhimurum*, *Pseudomonas aeruginosa* (ATCC 27853). The fungal specimens were *Candida albicans* (UST CMS 1201), *Saccharomyces cerevisiae* (UST CMS 1211), *Aspergillus niger*.

2.5.2 Inoculum Preparation

The microbial suspensions were standardized from previously conserved strains at TARC and inoculated at Mueller-Hinton broth for bacteria and Sabouraud Dextrose broth for fungi at 37°C. After 24 hours of incubation, suspensions were diluted. Inocula were set to 0.5 McFarland equivalent to an optical density from 0,08 to 0.13 at 625 nm wavelength, which corresponds to 108 CFU/mL[30,31].

2.5.3 Disc Diffusion Method

Disposable Petri dish (9 cm) were prepared with 20 mL of a base layer of molten Mueller Hinton agar for bacteria and Sabouraud Dextrose agar for fungi. Each Petri dish was inoculated with 15 uL of bacterial suspension or fungal suspension equivalent to 10^6 CFU/mL[32]. After drying in a hood,

6mm diameter discs were each added separately with 20 uL *Alocasia sanderiana* Bull. fractions an crude extract using a micropipette. The plates were then incubated for 24 hours at 37°C for bacteria[33] and 48 hours at 37°C for fungi. The diameters of the zones were evaluated in millimeters and fractions inducing inhibition zones at least 8 mm[34] around the disc were considered antimicrobial. All tests were performed in triplicate[35].

3 RESULTS

3.1 Phytochemical Screening

The phytochemical screening of the crude extracts of *Alocasia sanderiana* Bull. using test tube methods showed that glycosides, non-hydrolysable tannins, sterols and triterpenes, saponins starch, albuminoids and most sugars are present while colorimetric method using TLC futher confirmed the presence of tannins, triterpenes and sterols and some sugars, it also showed the presence of anthrone specifically (Table 1).

3.2 Antimicrobial Activity

The antibacterial activity of *Alocasia sanderiana* Bull. leaves is non-active using the criteria of at least 8 mm zone of inhibition for antimicrobial activity. There are zones though that may be seen below the 8 mm criteria, the DCM fraction showed 4 mm zones for *P. mirabilis*, 3 mm zones for *P. aeruginosa* and 1 mm zones for *P. carotovorum* and *C. albicans*. The methanol fraction also showed 1 mm zone of inhibition against *P. aeruginosa* (Table 2).

4 DISCUSSION

Although *Alocasia sanderiana* Bull. leaf extracts contain polyphenolic compounds this study shows that it has no antimicrobial activity against the 18 microorganisms that it was tested and may not be utilized as a potential antimicrobial drug. There are small zones of inhibitions though that were observed in some microorganisms but it was not enough for the minimum of 8 mm zone applied to this study, this zone however may be due to the presence of protease inhibitors discovered in other *Alocasia* spp.[36] that may be present in *Alocasia sanderiana* Bull. but there may not be enough present in the plant to cause an antimicrobial property. There are also other compounds like trypsin inhibitors[37,38] and lectins[39,40] that are present in other *Alocasia* spp. where its role is not yet well studied in *Alocasia* spp. that may affect its antimicrobial property. Therefore more bioactive components need be discovered and may be possible through the use of instrumentation like Gas Chromatography[41] like in the recent case of *Alocasia indica* (Lour.) Spach. It is also important to note that there are different interpretations in the zone of inhibitions, this study focused on the more commonly used minimum zone of inhibition for an antibacterial effect but some studies are utilizing at least 3 mm as weak antibacterial activity. Future studies may focus on its toxicity data to be able to further study its medicinal potential like in coagulation due to the mentioned trypsin inhibitors present in other species and in viral studies owing to the protease inhibitors in its other species as well. One of the limitations of this study is that it only focused on the leaves, other plant organs like roots (tubers) may also be studied in the future as there are folkloric utilizations of the organ.

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