Morphology, Phytochemical and Antimicrobial Activities of *Globba arracanensis* Kurz.

Saw Ohnmar[1], Yee Yee Thu[2], Hnin Wit Mhone[3], Ngwe Soe[4]

Abstract

Globba arracanensis Kurz. belongs to the family Zingiberaceae. The plants were collected from Ponnagyun, Rakhine State. The morphological characters of this plant have been studied and identified by the available literatures. The plant is deciduous and rhizomatous herb. Leaves are elliptic, tapering to a thread-like tip. The flowers are lilac. Bracts and bracteoles are persistent. Calyx is tubular with three lobes. The petals are three, white to light lilac. Lateral staminodes are white while the labellum is bifid, purple with yellow tip. Fertile stamen is one. The ovary is oblong, unilocular, parietal placentation. The presence of carbohydrates, α-amino acids, steroids, alkaloids, flavonoids, glycosides, saponins, tannins, terpenoids, coumarins, phenols, starchs, reducing sugars, proteins and phlobatannins was found in phytochemical investigation. According to physicochemical properties, the powdered leaves were most soluble in chloroform and least soluble in pet ether and methanol. In antimicrobial activity, leaves extracts were used to evaluate their activities on eight test organisms: *Agrobacterium* sp., *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococcus aureus*. These extracts indicated good activities in some test organisms *in vitro*.

Keywords: *Globba arracanensis* Kurz., morphological study, phytochemical investigation, antimicrobial activity

Introduction

Globba arracanensis Kurz. belongs to the genus *Globba* and family Zingiberaceae. *Globba* is the third largest genus of the Zingiberaceae with 100-110 species in the world (Williams et al., 2004). They are used traditionally in number of ailments such as postpartum, mouth ulcer, post natal care of mother and child, conjunctivitis, eye abrasians, asthma, leucoderma, cough, food poisoning, analgesic, antipyretic, heart pain and stomach pain (Muhammad Shahzad Aslam, et al.,2017).

The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents. Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases (Wadood et al., 2013).

Phytochemical analysis of *Globba* species has shown the biological compound like carbohydrates, amino acids, steroids, alkaloids, flavonoids, glycosides, saponins, tannins, terpenoids, phlobatannins, coumarins and phenols (NarasingaRao, 2014).The objectives of this research work are to identify and confirm the morphological characters, to examine the solubility test and phytochemical constituents and to evaluate the antimicrobial activity of the leaves of *Globba arracanensis* Kurz.

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Materials and Methods

Morphological Studies

Collection and Identification

The specimens of *Globba arracanensis* Kurz. were collected from Ponnagyun, Rakhine State during the flowering period (June to November). After the collection, the plants were identified with the help of literatures Baker (1968), Bordelon and John Kress (2009), Hooker (1894), Hundley and Chit Ko Ko (1987) and Kress *et al.* (2003).

Phytochemical investigation

The preliminary phytochemical investigation on powdered leaves was carried out at the department of Botany, University of Yangon to determine the presence or absence of chemical constituents such as alkaloids, phenols, flavonoids, steroids, terpenoids, starchs, reducing sugars, glycosides, saponins, tannins, amino acids, proteins, coumarins, phlobatinnins and carbohydrates according to method of British Pharmacopoeia (1968), Marini Bettolo *et al.*(1981) and Trease and Evans (2002).

Solubility test on leaves and roots of *Globba arracanensis* Kurz.

The solubility of powdered leaves was investigated to determine amount of total solids soluble in various solvents. The solubility characters such as extractive values for the various solvents were determined according to British Pharmacopoeia, 1968.

Preparation of the crude extracts

Distilled water, methanol, ethanol, acetone, ethylacetate, chloroform and petroleum ether soluble matter contents were determined by the methods given in British Pharmacopoeia (1968). Three grams of powdered leaves were extracted with 100 ml of each solvent respectively. The samples were soaked for three days. The soluble contents were filtered and then the filtrates were taken in the petri dishes and evaporated on water bath at 50˚C. The different in weight give the soluble matter contents in each solvent.

Antimicrobial activities

Various crude extracts of powdered leaves such as petroleum ether, chloroform, ethyl acetate, acetone, ethanol, methanol and distilled water extracts were used for antimicrobial study. Antimicrobial activities of different solvent extracts were tested on eight microorganisms by agar well diffusion method at Microbiology Lab, University of Yangon.

Test organisms utilized to the antimicrobial activities

Test organisms (*Agrobacterium* sp., *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococcus aureus*) utilized in this study were incubated in fresh medium for a day. After 24 hrs, 100 µL of each test organism was added to 100 mL of assay medium (sucrose 1.0g, malt extract 0.5g, agar 1.8g, distilled water 100ml), then poured into test plates.

Antimicrobial activities of various extracts were tested on eleven test organisms. Screening was done by the use of paper discs (6 mm) that were sterilized by autoclaving.

Table 1. Test organisms utilized to the antimicrobial activities

No.	Test organisms	Source	Diseases
1.	*Agrobacterium* sp.	-	Plant pathogenic bacterium, crown gall disease
2.	*Bacillus subtilis*	JAP-0225215	Pathogenic group, anthrax in animals
3.	*Candida albicans*	IFO-1060	Skin infection, vaginal candidiasis alimentary tract infection
4.	*Escherichia coli*	ATCC-25922	Cholrea, diarrhea and vomiting, urinary tract infection
5.	*Micrococcus luteus*	-	Pneumonia, meningitis, septic arthritis, bacteremia, peritonitis
6.	*Pseudomonas aeruginosa*	IFO-3080	Infection in lungs (pneumonia)
7.	*Salmonella typhi*	-	Typhoid
8.	*Staphylococcus aureus*	ATCC-12277	Skin infection, pneumonia, heart valve infection, bone infection

(Ref.: Cooper et al., 2003; Dunne *et al.*, 1993; Hulse *et al.*, 1993; Humphrey, 2004; Kamal and Modi, 2005; Ryan and Ray, 2004; Reid, et al., 2001; Scheidegger and Payne, 2003).

Paper disc diffusion assay

Paper discs were impregnated with concentrated extracts (50 µL per disc) and then they were allowed to dry at room temperature. Dry paper discs impregnated with extracted samples were applied on various test plates. Then, these plates were incubated for 24-36 hours at 30°C. After 24-36 hours, clear zones (inhibitory zones) surrounding the test discs indicate the presence of the bioactive compounds in the extracts that inhibit the growth of test organisms selectively. The diameters of clear zones including 6 mm disc were measured. At the same time the controlled experiments were prepared with only solvents for the comparison with plant extracts (Davis and Stout,, 1971).

Results

Morphological studies

Scientific Name : *Globba arracanensis* Kurz.
Myanmar Name : Waso-pan
Family : Zingiberaceae
Flowering and Fruiting Period : June to November

Globba arracanensis Kurz. are deciduous, rhizomatous herbs. Leaves are green up to 5 cm wide and 26 cm long, elliptic, tapering to a thread-like tip. The inflorescence is terminal on the leafy shoots, up to 20 cm long, peduncle curved. The flowers are lilac. Bracts are persistent, white-lilac.Bracteoles are persistent and light lilac. Calyx is lilac, tubular with three lobes. The petals are 3, white to lilac, the corolla tube is white. Petal-like lateral staminodes of the flowers are white. The labellum is bifid, yellow and purple with yellow tips, one crossing over the other. The fertile stamen is one, the filament is white, long, curved in upper part, the style often becoming separated from it and forming a bow-string across the curvature and the anther light lilac with a darker purple tip. The pollen is white. The ovary is oblong, unilocular, parietal placentation. The style is filiform inserted within the grooved filament of the fertile stamen.

Figure 1. Morphological characters of *Globba arracanensis* Kurz.

Phytochemical investigation

The preliminary phytochemical test of powdered leaves of *Globba arracanensis* Kurz. indicated that the presence of carbohydrates, amino acids, steroids, alkaloids, flavonoids, glycosides, saponins, tannins, terpenoids, coumarins, phenols and phlobatanins. The results were shown in Table 2.

Table 2. Phytochemical test on the leaves of Globba *arracanensis* Kurz.

No.	Constituents	Extract	Test reagents	Obeservation	Results
1.	Alkaloid	MeOH	Mayer's reagent Hager reagent Wagner reagent	White ppt. Yellow ppt. Brown ppt.	+
2.	Glycoside	MeOH	1 ml H_2O+NaOH	Yellow color	+
3.	Phenolic compound	MeOH	2ml H_2O +10%$FeCl_3$	Yellowish green color	+
4.	Flavonoid	MeOH	Mg turning+HCl	Pink color	+
5.	Steroid	MeOH	$CHCl_3$+H_2SO_4 (conc)	Reddish brown	+
6.	α amino acid	H_2O	Ninhydrin reagent	Pink spot	+
7.	Terpenoid	MeOH	$CHCl_3$+H_2SO_4 (conc)	Reddish brown	+
8.	Starch	H_2O	Iodine	Blue-black ppt	+
9.	Reducing sugar	H_2O	Benedict solution	Brick red ppt	+
10.	Saponin	H_2O	2 ml H_2O	Frothing	+
11.	Tannin	H_2O	5%$FeCl_3$+ H_2SO_4 (dil)	Yellowish brown	+
12.	Phlobatannin	H_2O	HCl (dil)	Red ppt	+
13.	coumarin	EtOH	10%NaOH + Chloroform	Yellow color	+
14.	Carbohydrate	H_2O	Fehling sol: A+B	Red ppt	+
15.	Protein	H_2O	NaOH+3% $CuSO_4$	Red or violet color	+

Solubility test on leaves of *Globba arracanensis* Kurz.

The powdered leaves were most soluble in chloroform (0.6 g), medium soluble in distilled water, ethanol, acetone (0.3 g in each) and ethyl acetate (0.2) least soluble in petroleum ether and methanol (0.1 g in each).

Antimicrobial Activity of Different Leaves Extracts

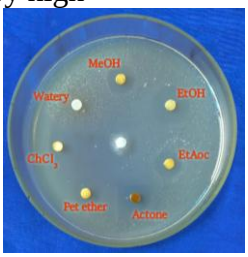
For screening of antimicrobial activities of various solvent extracts from *Globba arracanensis* Kurz. Leaves on eight pathogenic microorganisms, the results of the diameters of inhibitory zones were given in Table 4 and Figure 2.

In this experiment, methanol, ethanol and ethyl acetate extracts from the leaves of *Globba arracanensis* Kurz. showed very highly antimicrobial activity on eight pathogenic microorganisms (*Agrobacterium* sp., *Bacillus subtilis, Candida albicans, Escherichia coli Micrococcus luteus, Pseudomonas aeruginosa, Salmonella typhi* and *Staphylococcos aureus*). Acetone and petroleum ether extracts from the leaves exhibited highly antimicrobial activity on eight test organisms. Chloroform extract from the leaves indicated antimicrobial activity against on eight test organisms whereas watery extract from the leaves exhibited no activity.

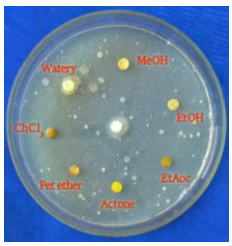
Table 4. Antimicrobial activities of various solvents extracts of leaves from *Globba arracanensis* Kurz. (Inhibitory zone = mm)

Test organisms	Solvents used for leaves extraction						
	Methanol	Ethanol	Ethyl acetate	Acetone	Pet-ether	Chloroform	Watery
Agrobacterium sp.	19	21	20	16	18	13	-
Bacillus subtilis	25	20	22	18	19	14	-
Candida albicans	22	20	22	14	14	12	-
Eschirichea coli	24	23	23	17	15	15	-
Micrococcus luteus	25	18	16	17	16	15	-
Pseudomonas aeruginosa	26	25	20	18	14	14	-
Salmonella typhi	22	21	17	15	15	13	-
Staphylococcos aureus	20	20	18	17	16	14	-

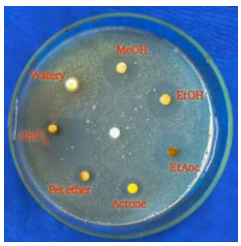
- = no activity, 10 -12 mm = weak activity, 13–17 mm = high activity, >18 mm = very high



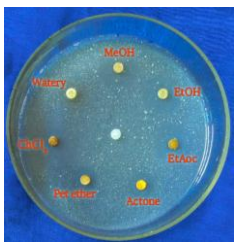
Inhibitory zone against *Agrobacterium* sp.



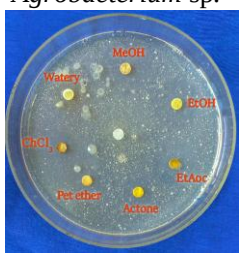
Inhibitory zone against *Bacillus subtilis*



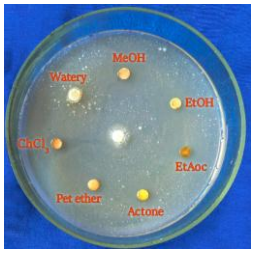
Inhibitory zone against *Candida albicans*



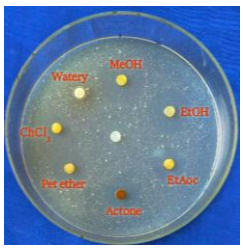
Inhibitory zone against *Eschirichea coli*



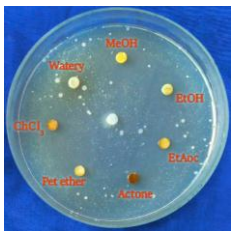
Inhibitory zone against *Micrococcus luteus*



Inhibitory zone against *Pseudomonas aeruginosa*



Inhibitory zone against *Salmonella typhi*



Inhibitory zone against *Staphylococcos aureus*

Figure 2. Antimicrobial tests of various solvents extracts of *Globba arracanensis* Kurz. leaves

Discussion and Conclusion

In this research, morphological characters, preliminary phytochemical tests and antimicrobial activities on the powdered leaves had been undertaken.

The plant is deciduous and rhizomatous herb. Leaves are elliptic, tapering to a thread-like tip. The flowers are lilac. Bracts and bracteoles are persistent. Calyx is

tubular with three lobes. The petals are three, white to light lilac. Lateral staminodes are white while the labellum is bifid, purple with yellow tip. Fertile stamen is one. The ovary is oblong, unilocular, parietal placentation. These characters are in agreement with those mentioned by Backer, 1968; Hooker, 1875 and Dassanayake, 1999.

Carbohydrates, α-amino acids, steroids, alkaloids, flavonoids, glycosides, saponins, tannins, terpenoids, coumarins, phenols, starchs, reducing sugars, proteins and phlobatannins were found in phytochemical tests.

In antimicrobial activities, methanol, ethanol, ethyl acetate, acetone and petroleum ether extracts from the leaves of *Globba arracanensis* Kurz. showed good antimicrobial activity on eight pathogenic microorganisms (*Agrobacterium* sp., *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococcos aureus*). However, chloroform extract from the leaves indicated antimicrobial activity against on eight test organisms while watery extract from the leaves exhibited no activity.

Muhammad Shahzad Aslam, *et al*.,2017 have stated that *Globba* spp. are used in traditionally to treat postpartum, mouth ulcer, post natal care of mother and child, conjunctivitis, eye abrasians, asthma, leucoderma, cough, food poisoning, analgesic, antipyretic, heart pain and stomach pain. However, there was no previous record for antimicrobial activities of *Globba arracanensis* Kurz.

In conclusion, it is very important to search for new antimicrobial drugs since life-threatening bacterial and fungal diseases are strongly increasing nowadays. In this research, methanol, ethanol, ethyl acetate, acetone and petroleum ether extracts from *Globba arracanensis* Kurz. have selectively shown good antimicrobial activities on eight pathogenic microorganisms such as *A.* sp., *B. subtilis*, *C. albicans*, *E. coli*, *M. luteus*, *P. aeruginosa S. typhi*, *S. aureus.* Among them, methanol, ethanol, ethyl acetate extracts indicated very strong activities on *B. subtilis*, *M. luteus*, *E. coli* and *P. aeruginosa* which can cause cholrea, diarrhea and vomiting, urinary tract infection, meningitis, septic arthritis, bacteremia, peritonitis and pneumonea. Therefore, these bioactive extracts could be applied in traditional medicine and can possess beneficial effects for health of human beings.

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