

Morphological, Phytochemical and Antimicrobial activity from the rhizome of *Alpinia officinarum* Hance.

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Abstracts

Alpinia officinarum Hance is a highly valuable medicinal plant belonging to the family Zingiberaceae. It grows widely distributed in Myanmar. It was collected from Hlegu Township in Yangon region. The rhizome was used as traditional medicine for the treatment of aromatic stimulant, condiment, carminative, dyspepsia, vomiting and hemorrhoids. Morphological characters of vegetative and reproductive parts of this plant were studied for classification and identification. Preliminary phytochemical and physicochemical parameters studies of the powdered rhizomes are also carried out. Phytochemically, it exhibited saponins, tannins, glycosides, phenolic compounds, alkaloids, α -amino acid, reducing sugars, terpenoids and steroids, starch, flavonoids and carbohydrates are present. Total ash was approximately ten and two times more than water soluble ash respectively. Ethyl acetate extractive value were more soluble than other solvents. Antimicrobial activity of seven different extracts were performed by using paper disc diffusion method. Seven different solvents extract of rhizome showed the best effective antimicrobial activity against *Bacillus subtilis* and *Escherichia coli*.

Introduction

Alpinia officinarum Hance. Padagaw gale belongs to the family Zingiberaceae. Zingiberaceae family consists of about 49 genera and 1300 species. The plants of Zingiberaceae are perennial aromatic herbs with fleshy rhizomes and tuberous roots; flowers are arranged in racemes, head or cymes. Perianth 6-merous, the outer ones calyx-like, the inner corolla-like. Two of the stamens are modified as a petaloid labellum. Fruit, a loculicidal capsule. Seed with perisperm. Zingiberaceae includes following genera such as; *Curcuma* (5 spp.), *Alpinia* (250 spp.), *Zingiber* (80-90 spp), *Amomum* (150 spp.), *Elettaria* (7 spp.), *Aframomum* (50 spp.) and *Hedychium* (50 spp.). Zingiberaceae family contains tannins, phenolic acids, leucoanthocyanins, flavonoids, ketones, terpenoid and alkaloid. (Trease and Evans, 2002)

Lawrence, 1963; Datta, 1970, stated that the Zingiberaceae family consists of 47 genera and 1400 species. The members of this family are distributed throughout the eastern hemisphere, particularly in the Indo-Malayan area. *Alpinia officinarum* (China) yield which is used in medicine and spices. According to Hundley and Chit

Ko Ko (1987), the Zingiberaceae family has 15 genera and 124 species which belong to Myanmar.

The rhizome is pungent, bitter, heating, and stomachic; improves appetite, taste, and voice; useful in bronchitis and diseases of the heart (Ayurveda). The rhizome has a sharp odour and fairly good taste; stomachic, aphrodisiac, tonic, diuretic, expectorant, carminative; useful in headache, rheumatic pain, sore throat, sour eructation, stuttering, pain in the chest, diabetes, burning of the liver, tubercular glands, diseases of the kidney (Yunani) Kirtikar & Basu)

The aim of the present study is to investigate the morphological, phytochemical constituents and antimicrobial activity of *Alpinia officinarum* Hance. The objectives are to identify the vegetative and reproductive parts of the plant, the preliminary phytochemical tests for the evaluation of major constituents present in this plant and to screen the antimicrobial activity of different solvents extracts of rhizomes.

Materials and Methods

Botanical Studies

Collection, Identification and Preparation of *Alpinia officinarum* Hance.

The specimens of *Alpinia officinarum* Hance. used in this research were collected from Hlegu township in Yangon region. Flowering and fruiting periods July to December. Fresh specimens of the vegetative and floral parts were used for identification in the Department of Botany, Taungoo University with the help of reference literature of Hooker (1885); A Key to the families of flowering plants (1988); John Kress, (2003).

The collected samples were washed with water to remove impurities. After washing and cleaning, the sample was air dried and ground to get powdered and stored in air tight container.

Chemical study

Preliminary phytochemical examination

Preliminary phytochemical investigation of rhizome of *Alpinia officinarum* Hance. has been conducted with tested reagents in Botany Department of Taungoo University according to the methods of British Pharmacopoeia, 1965; Harbone, 1973; Marini-Bettelo, 1981; Trease & Evans, 2002. The tests indicated the presence or absence of saponins, tannins, glycosides, phenolic compounds, alkaloids,

α -amino acid, reducing sugars, terpenoids and steroids, starch, flavonoids and carbohydrates.

Physicochemical characters

Physicochemical properties including moisture, total ash, acid insoluble ash, water soluble ash and solubility of non polar and polar solvent such as petroleum ether (60-80°C), chloroform, acetone, ethyl acetate, 95% ethanol, 95% methanol and water contents were made according to the procedure of B.P., 1968 and W.H.O, 1998. The results of physicochemical properties were shown in Table (2).

Microbiological studies

Extraction and testing of antimicrobial activity

Preparation for rhizome extracts

Three grams of powdered samples (rhizomes) were dissolved in 30ml of acetone, chloroform, ethyl acetate, ethanol, methanol, and pet-ether and watery for ten days respectively. Then the extracts were filtered. The filtrates were concentrated on water bath. The dried extracts were dissolved in 2-3 drops of each solvent to get the required concentration before testing. Preparations of test organisms were obtained from Department of Botany, Yangon University. These test organisms were sub – culture inoculated in 5ml of nutrient broth and incubated for 5 hours of bacteria and 24 hours of fungi at room temperature.

Antimicrobial activity of rhizome extracts by paper disc diffusion method

The paper discs were applied and 20 μ l extracts were placed on petridishes and dry at room temperature. Forty ml of sterilized nutrient agar cooled at 50°C and mixed to dry 10 μ l test organisms and then shook and poured onto each petridish and allowed to solidify for 15 minutes. Paper discs with 20 μ l extract were gently placed on nutrient agar medium. Then the plates were incubated for 24-48 hours at room temperature. The size of paper disc having six millimeters in diameter was utilized for antimicrobial test. The clear zone (inhibitory zones) surrounding the paper disc were measured. (Cruickshank, 1975)

Results

Morphological Character of *Alpinia officinarum* Hance (Peda-gaw-gale)

Habit is rhizomatous herbs, aromatic, aerial stems elongated, leafy roots adventitious. Leaves 2-rank, simple, parallel, venation, sheathing petiolate, exstipulate. Inflorescences terminal panicle. Flowers bracteate, bracteolate,

pedicellate, complete, bisexual, irregular, zygomorphic, tri-merous, cyclic, epigynous. Sepals-3, synsepalous, bell-shaped, valvate, sepaloid, persistent, superior. Petals-3, synpetalous, posterior one is larger, petaloid (yellowish white), imbricate, superior. Androecium ($2^{\text{st}}+2^{\text{st}}+1^{\text{fertile}}$), apostemonous, petalostemonous, staminodes fused to form labellum; the filament short; the anther ditheous, introrse, dorsifixed, longitudinal, dehiscence, superior. Gynoecium tricarpellary, syncarpous, trilocular, axile placentation, 1 or 2-ovules in each locules, style filiform and lying in the groove of the fertile stamen; the stigma infundibuliform, inferior. Fruit a loculicidal, capsule, globose. Seed numerous, small, angled, arillate, aromatic, endospermic. (Fig. 1)

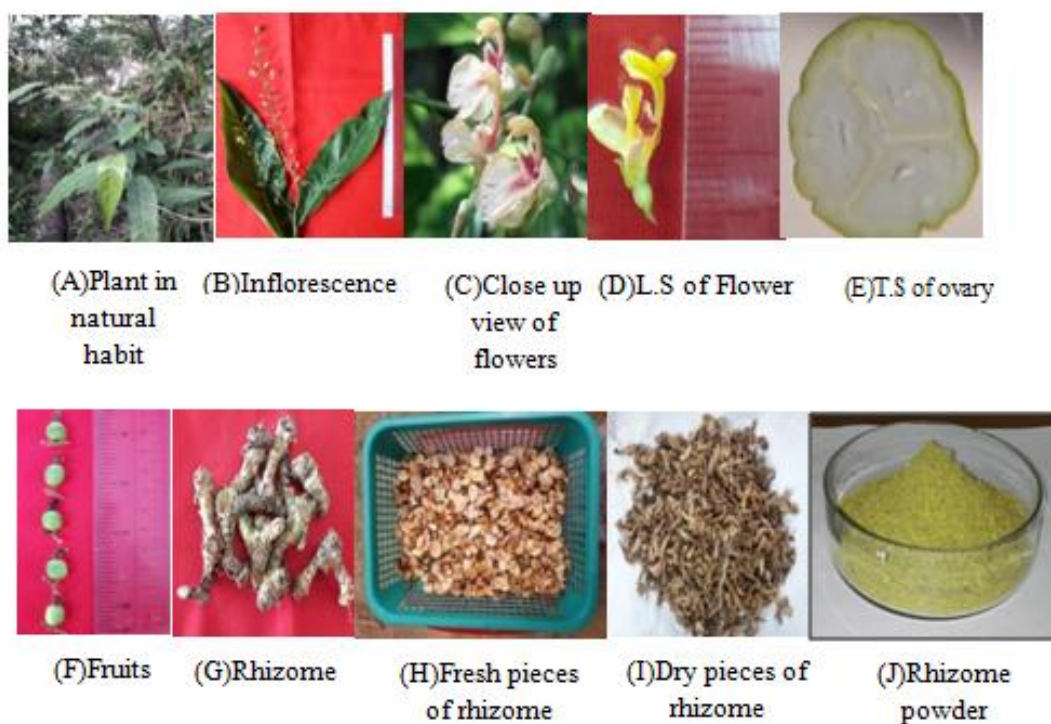


Figure1. Morphological Characters of *Alpinia officinarum* Hance.

Preliminary Phytochemical tests of *Alpinia officinarum* Hance.

The preliminary phytochemical tests of rhizomes were indicated the presence of saponins, tannins, glycosides, phenolic compounds and alkaloids, α -amino acid, reducing sugars, terpenoids and steroids, starch, flavonoids and carbohydrates. The preliminary phytochemical tests were shown in Table.1.

Table1. Preliminary phytochemical Tests of *Alpinia officinarum* Hance (Peda-gaw-gale)

No	Test	Extract	Test Reagent	Observation	Result
					Rhizome
1.	Saponins	H ₂ O Extract	Distilled Water	Frothing	+
2.	Tannins	H ₂ O Extract	1% FeCl ₃ and Dil H ₂ SO ₄	Yellowish brown ppt	+
3.	Glycosides	H ₂ O Extract	10% Lead acetate	White ppt	+
4.	Phenolic Compounds	H ₂ O Extract	K ₃ Fe (CN) ₆ and 1% FeCl ₃	Deep blue colour ppt	+
5.	Alkaloids	10% Acetic acid + EtOH	(i) Dragendroff's reagent (ii) Mayer's reagent	Orange ppt White ppt	 +
6.	α -Amino acid	H ₂ O Extract	Ninhydrin Reagent	Violet colour	+
7.	Reducing Sugars	Dil H ₂ SO ₄ + 5N NaOH	Benedict's Solution	Brick-red ppt	+
8.	Terpenoids and Steroids	Pet-ether Extract	Acetic anhydride + Conc. H ₂ SO ₄	Blue	+
9.	Starch	H ₂ O Extract	I ₂ Solution	Bluish-black ppt	+
10.	Flavonoids	Ethanol	HCl/Mg turning	Pink	+
11.	Carbohydrates	H ₂ O Extract	10% α -naphthol + Cons: H ₂ SO ₄	Red ring	+

Physicochemical characters of *Alpinia officinarum* Hance.

Physicochemical characters of air dried rhizome powdered of *Alpinia officinarum* Hance. were shown in Table (2). In this table the moisture content is 8.6% in rhizome. Total ash, acid insoluble ash and water soluble ash content were also determined and recorded. Total ash was approximately ten and two times more than water soluble ash. All these values were useful for the quality control system reading the physiological ash and impurities whenever it was used for medicinal purpose. The solubility in different solvents of Peda-gaw-gale plants was found to be significantly soluble in ethyl acetate and pet-ether.

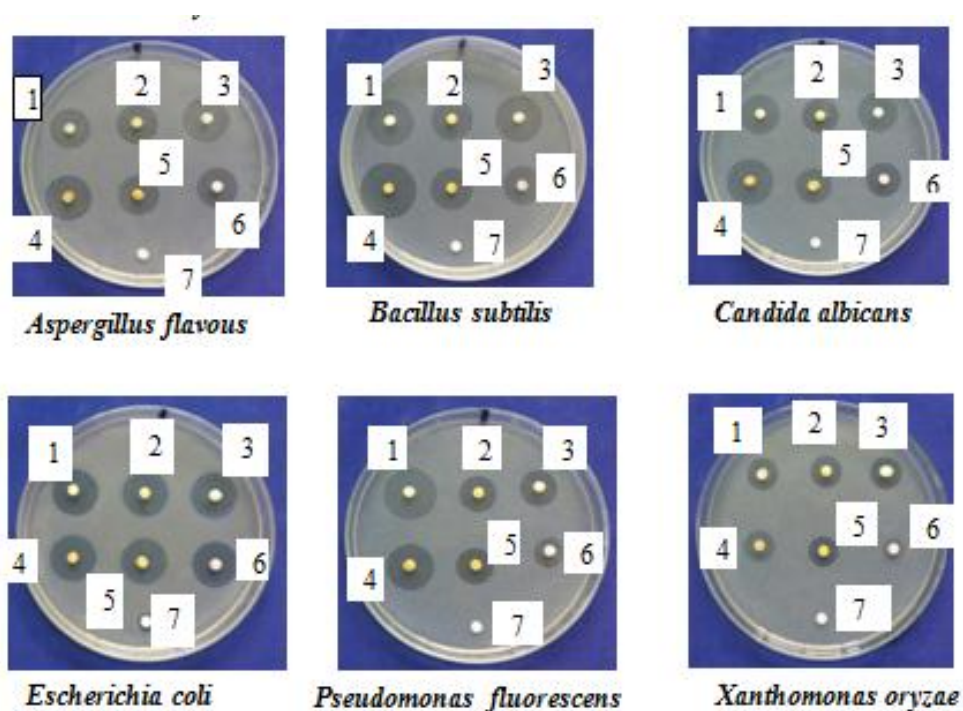
Table. (2) Physicochemical characters the *Alpinia officinarum* Hance.

No.	Physicochemical characters	Rhizome%
1.	Moisture content	8.6
2.	Total ash	10.3
3.	Acid insoluble ash	6.85
4.	Water-soluble ash	4.5
5.	Water soluble matter	3.3
6.	Ethanol soluble matter (95%)	6.7
7.	Methanol soluble matter (95%)	6.7
8.	Acetone soluble matter	5.0
9.	Ethyl acetate soluble matter	16.7
10.	Chloroform soluble matter	5.0
11.	Petroleum ether (60-80°C) soluble matter	13.3

Microbiological studies

Antimicrobial activities of leaves extracts of *Alpinia officinarum* Hance.

In this experiment, six different types of test microorganisms, *Aspergillus flavous*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Pseudomonas fluorescens* and *Xanthomonas oryzae*. Watery extracts of rhizome show antibacterial activity in *Escherichia coli* and other microorganisms did not show activity in watery extracts. Ethanol extracts were more effective antimicrobial activity than other solvents extracts. Ethanol extracts showed the highest inhibitory zone of 28mm on *Bacillus subtilis* and ethanol extracts showed the lowest inhibitory zone of 16mm on *Xanthomonas oryzae*.



Paper disc-6mm

Fig.2 Antimicrobial activity of Rhizome extracts of *Alpinia officinarum* Hance.

Table.3 Antimicrobial activity of Rhizome extracts of *Alpinia officinarum* Hance.

Solvents	Microorganisms					
	<i>Aspergillus flavous</i>	<i>Bacillus subtilis</i>	<i>Candida albicans</i>	<i>Escherichia coli</i>	<i>Pseudomonas fluorescens</i>	<i>Xanthomonas oryzae</i>
1. Acetone	20	24	22	26	20	16
2. Chloroform	20	22	20	26	16	16
3. Ethyl acetate	22	24	22	26	16	16
4. Ethanol	22	28	24	26	18	16
5. Methanol	20	22	20	26	16	14
6. Pet-ether	20	20	18	24	12	10
7. Watery	-	-	-	8	-	-

Discussion and Conclusion

The plant *Alpinia officinarum* Hance. involved in this research were collected from Hlegu Township in Yangon Region during the flowering and fruiting period from July to December. In this investigation, the morphological, phytochemical and antimicrobial activities of rhizomes have been undertaken. This plant belongs to the family Zingiberaceae. It is perennial herbs usually aromatic. Leaves are simple, parallel venation. Inflorescences are terminal panicle. Flowers are bisexual and zygomorphic. Calyx 3-lobed, bell shaped and corolla 3-lobed, the tube cylindrical. Androecium is staminode fused to form labellum and one fertile stamen. Gynoecium is tricarpeal, syncarpous, axile placentation, style filiform and lying in the groove of the fertile stamen these characters were in agreement with those describe by Hooker, (1885), Kirtikar and Basu, (1975); www.ibiblio/herbmed/electic/kings/alpinia-offi.html.

In this research, the preliminary phytochemical examination was carried out on the powder of rhizome. The phytochemical tests indicate the presence of saponins, tannins, glycosides, phenolic compounds and alkaloids, α -amino acid, reducing sugars, terpenoids and steroids, starch, flavonoids and carbohydrates. Trease & Evans, (2002); Harbone, (1973). The physicochemical character's of powdered samples has solubility properties. Powdered sample of rhizome were more soluble in ethyl acetate and pet-ether.

In microbiological study, the ethanol extracts of *Alpinia officinarum* Hence. rhizome showed more effective antimicrobial activity than other extracts. Seven different solvents extracts of rhizome showed significant activities in

Escherichia coli and *Bacillus subtilis*. From this investigation it may be inferred of *Alpinia officinarum* Hance. rhizome can be effective in the formulation of medicine for the treatment of disease, namely, bronchitis, urinary tract infection, diarrhea, food poisoning, infections of bone and joints, abscesses, inflammation, tuberculosis, leprosy, eye infection and non pathogenic. These findings are in agreement with those described by Cruickshank (1975); HANOI (1999). The above data would be helpful in further study of the research and development in the field of medicine.

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References

- British Pharmacopoeia** 1968. General Medicinal Council, London.
- Cruickshank, R., J.P. Duguid. B.P. Marmion. And R. H.A. Swain (1975) **Medicinal Microbiology**. Churchill Livingstone Ltd.
- Dutta, A.C. 1979, **Botany for degree students. (5thed)**. New Delhi: Oxford University Press.
- Harbone, J.B., (1973) *Phytochemical Methods (A Guide to Modern Techniques of Plant Analysis)*. Chapman and Hall (London)
- Hooker. J.D. 1885, **Flora of British India. Vol. IV**, L. REEVE & Co. Ltd London.
- Hundley and Chit KoKo. 1978. **List of Trees, Shrubs, Herbs and Principle Climbers, etc.** Government Printing Press, Yangon.
- Kirtikar, K.B and Basu, B.D. 1935. **Indian Medicinal Plants. Vol. 3**. Lalit Mohan Basu, M.B.49, leader Road, Allahabad, India.
- Kress and Yin Yin Kyi, Daw. 2003. **A Checklist of the Trees, Shrubs, Herbs and Climbers of Myanmar**, Department of Systematic Biologh-Botany, National Museum of Natural History Washington, DC.
- Lawrence, G.H.M (1964) *Taxonomy of vascular plants*. The Macmilan Company, New York.
- Trease and Evans, (2002) **A Text Book of Pharmacognosy**. 15th Edition, Edinbrugh London New York.
- www.ibiblio/herbmed/electic/kings/alpinia-offi.html.
- Wallis, T.E. (1967). *Textbook of Pharmacognosy*. 5th Edition. J.& A. Churchill Limited London.