

Morphological and Phytochemical Study of *Pongamiapinnata* Pierre. and Its Antimicrobial Activity

Ohn Mar Than¹, Aye Myat San²

Abstract

This is a study on medicinal plant *Pongamiapinnata* Pierre. It belongs to the family Fabaceae. Vernacular name of this plant is Thin-win in Myanmar and Tha-win in Rakhine. *Pongamiapinnata* Pierre. was collected from Sittway Township, Rakhine State. The plant parts of *Pongamia* is very common in Ayurvedha and Siddha Indian Medicine practices. In this paper, morphology characters of leaves, stems, roots, fruits and seeds of fresh specimen have been described. In addition, all the plant parts of *Pongamiapinnata* Pierre. were screened for their phytochemical composition and were evaluated for the physicochemical properties. Antimicrobial activity of *Pongamiapinnata* Pierre. was also investigated. In morphological study, *Pongamiapinnata* Pierre. is tree, stem woody; leaves alternate, imparipinnate compound, both surfaces glabrous; inflorescences axillary; flowers are purplish white, fragrant; stamens monadelphous; ovary superior, 1 to 2 ovules; fruit flattened, indehiscent, and 1 to 2 seeded. In phytochemical investigation, alkaloids, amino acids, carbohydrate, flavonoids, glycosides, phenolic compounds, saponins, steroid, starch and terpenoids were found in the parts of leaves, stem and fruit but these constituents were found in root except phenolic compounds. Physicochemical properties of leaves, stems, roots and fruits such as moisture content, total ash, acid insoluble ash, water soluble ash and different solubility of extractive value in various organic solvents were determined. The results indicate that the highest moisture contents were found in stems. The powder of all parts of plant was soluble in all solvents test. Antimicrobial activities *Pongamiapinnata* Pierre. are also investigated by using agar-disc diffusion method with six types of test organisms. The different solvent extracts of leaves show antimicrobial activities on all test microorganisms. Therefore, the medicinal plant, *Pongamiapinnata* Pierre. possesses many medicinal values.

Key words: *Pongamiapinnata* Pierre., morphological and phytochemical study, antimicrobial activity

Introduction

Plants have a great potential for producing new drugs for human benefit. The use of medicinal plants as a source of drugs is as old as mankind. The demand for more and more drugs from plant sources is continuously increasing. More than 50% of drugs used in western pharmacopeia are isolated from plants (Mitter, 2012). Many thousands of medicinal plants are widely distributed in the world. There are 12,000 different plants growing in different climate zone in Myanmar and most of them have been regarded as medicinal plants (Kress, *et al.* 2003).

Pongamiapinnata Pierre. belongs to family fabaceae about 550 genera and 13000 species (Lawrence, 1969). *Pongamiapinnata* Pierre. is well-adapted to arid zones and has many traditional uses. The plant parts of *Pongamia* is very common in Ayurvedha and Siddha Indian Medicine practices. *Pongamiapinnata* Pierre. exhibits many therapeutic activities especially for the treatment of malaria caused by *Plasmodium falciparum*. It is often used for landscaping purposes as a windbreak or for shade due to the large canopy and showy fragrant flowers. The bark can be used to make twine or rope and it also yields a black gum that has historically been used to treat wounds caused by poisonous fish. The wood is said to be beautifully grained but splits easily when sawn thus relegating it to firewood, posts, and tool handles. (www.ijpsr.com)

While the oil and residue of the plant are toxic and will induce nausea and vomiting if ingested, the fruits and sprouts, along with the seeds, are used in many traditional remedies. Juices from the plant, as well as the oil, are antiseptic and resistant to pests.

Seed extract of this plant has hypotensive effects and produce uterine contractions. Powdered seed is used in bronchitis, chronic fever, whooping cough and chronic skin diseases

¹ Associate Professor, Department of Botany, Sittway University

² Demonstrator, Department of Botany, Sittway University

and painful rheumatic joints. Seed oil is used in scabies, leprosy, piles, ulcers, chronic fever, liver pain and lumbago. (<https://bioinfopublication.org/>)

Many herbal remedies individually or in combination have been recommended in various medical treatises for the cure of different diseases. The *Pongamiapinnata* Pierre., commonly known as 'Karanj', has been recognized in different system of traditional medicines for the treatment of different diseases and ailments of human beings. It contains several phytoconstituents belong to category flavonoids and fixed oils. The fruits and sprouts of *Pongamiapinnata* Pierre. were used in folk remedies for abdominal tumours in India, the seeds were for keloid tumours in Sri Lanka and a powder derived from the plant was for tumours in Vietnam. In Sanskrit India, seeds were used for skin ailments (<http://www.greenpharmacy.info.>).

The aim of this research is to find out the effective medicinal properties and utilization of *Pongamiapinnata* Pierre. as folk medicine. Therefore, to fulfill this aim, the research was carried out according to such objectives: to verify the specific morphological characters and to examine the histological characters, to reveal the phytochemical constituents, to determine the physicochemical properties and to evaluate the antimicrobial activity of *Pongamiapinnata* Pierre.

Materials and Methods

Morphological Study

The specimens of *Pongamiapinnata* Pierre. were collected during the flowering and fruiting period from April 2017 to January 2018. The specimens were identified by using the available literature such as Backer, 1965; Hooker 1875; Hundley and Chit Ko Ko, 1987; John Kress *et al.*, 2003 at the Department of Botany, Sittway University.

Phytochemical investigation of leaves, stems, roots, fruits and seeds of *Pongamiapinnata* Pierre.

The preliminary phytochemical investigation of *Pongamiapinnata* Pierre. were carried out to determine the presence or absence of phytochemical constituents such as alkaloids, flavonoids, glycosides, cyanogenic glycoside, saponins, reducing sugars, carbohydrates, α -amino acid, phenolic compounds, tannins and terpenoids and steroids. The preliminary phytochemical examination was carried out at the Department of Chemistry, Sittway University, according to the methods of Central Council for Research in Marimi Bettola *et al.*, 1981, Unani Medicine, 1987, and Trease and Evans, 2002.

Physicochemical properties of leaves, stems, roots, fruits and seeds of *Pongamiapinnata* Pierre.

Physicochemical properties such as determination of moisture content, total ash, water soluble ash content, acid insoluble ash content and solubility of nonpolar and polar solvents such as ethanol, methanol, pet-ether, ethyl-acetate, chloroform, acetone and water soluble matter content of powder samples were carried out by the method of British Pharmacopeia (1980).

Antimicrobial activity of leaves of *Pongamiapinnata* Pierre.

Antimicrobial activities of leaves of *Pongamiapinnata* Pierre. were investigated with six different solvents extracts on six pathogenic microorganisms by using paper-disc diffusion method (Curickshank *et al.*, 1975) at the Department of Botany, University of Yangon.

Table. Types of microorganisms, diseases and their respective code numbers

No.	Test Organisms	Diseases	Source
1.	<i>Agrobacterium tumefaciens</i>	Plant tumor cell (Crown gall disease)	-
2.	<i>Bacillus subtilis</i>	Pathogenic group, anthrax in animals and fever	JAP-0225215
3.	<i>Escherichia coli</i>	Cholera, diarrhea and vomiting, urinary tract infections, septic wounds and bed-sores.	ATCC-25922

No.	Test Organisms	Diseases	Source
4.	<i>Pseudomonas fluorescens</i>	Bacteria for leaf blight	-
5.	<i>Samonellatyphi</i>	Typhoid	ST.3/ Sep.69
6.	<i>Candida albicans</i>	Skin infection, vaginal candidiasis, alimentary tract infections	IFO- 1060

Results

Morphological characters of *Pongamiapinnata* Pierre.

Scientific Name	-	<i>Pongamiapinnata</i> Pierre.
Common Name	-	Indian beech tree, Pongam tree
Family	-	Fabaceae
Myanmar Name	-	Thin-win
Rakhine Name	-	Tha-win

Trees, perennials, latex absent, 2 m to 5 m height. Leaves alternate, imparipinnate compound, leaflets opposite, 5 to 7 terminal leaflet larger than the other, ovate to elliptic oblong, lamina 10.5 cm to 16.5 cm in length and 5 cm to 10 cm in wide, acuminate, slightly broader at the base than at the apex, petiolate, petiole 0.8 cm to 1 cm in length and 0.1 cm to 0.3 cm in wide, stipulate, pulvinate, caducous. Inflorescences axillary, raceme 10 cm to 15 cm, axis pubescent. Flowers are purplish white, bracts small, ebracteolate, pedicellate, complete, bisexual, irregular, zygomorphic, caducous, hypogynous, pedicellate 0.8 cm to 0.9 cm long. Sepals (5), synsepalous, sepaloid (brown), valvate, calyx tube campanulate, minutely 5 tooth, inferior, calyx tube 0.3 cm to 0.5 cm long, calyx lobe 0.3 cm to 0.5 cm in wide. Petals (5), purplish white, apopetalous, imbricate, standard suborbicular, wing obliquely oblong, keel obtuse, inferior. Stamens (10), monadelphous, vexillary stamens free at the base but jointed with other to a close tube, anther ditheous, yellow, versatile, longitudinal dehiscence, inferior. Carpel 1, style incurved, stigma capitate, small, pubescent, , basal placentation, 1 ovule in a locule, superior. Fruits pod, obliquely oblong, slightly curve, flattened, thick, pointed at both ends, green, brown when mature, sub-fleshy or almost woody, indehiscent. Seed one or two reniform, thick flattened. (Figures 1-10)

Flowering periods	-	From March to May
Fruiting periods	-	Throughout the year
Collection site	-	Sittway University Campus N 20° , 08', 49.6" and E 92° , 51', 41.8"

Morphological characters of *Pongamiapinnata* Pierre.



Fig. 1 Habit



Fig. 2 leaves



Fig. 3 Inflorescences



Fig. 4 flowers



Fig. 5 Calyx



Fig. 6 Corolla



Fig. 7 Androecium



Fig. 8 Carpel



Fig. 9 Fruits



Fig. 9 Seeds

Phytochemical investigation of *Pongamiapinnata* Pierre.

In phytochemical test, the presence of alkaloids, flavonoids, steroids, terpenoids, glycosides, carbohydrates, saponins, tannins and phenolic compounds were found in all parts of the plant. The results were shown in table 1.

Table 1 Phytochemical Tests of the leaves, stems, roots, fruits and seeds of *Pongamiapinnata* Pierre.

No	Test	Extracts	Test reagent	Observation	Remark				
					L	S	R	F	S
1.	Alkaloids	1% HCl	Mayer's reagent Wanger's reagent Dragendraff reagent	White ppt, deep blue ppt, deep brown ppt	+	+	+	+	+
2.	Flavonoids	EtOH	Mg ribbon and conc: HCL	Pink colour	+	+	+	+	+
3.	Glycosides	EtOH	10 % lead acetate	White ppt	+	+	+	+	+
4.	Phenolic compound	H ₂ O	5% FeCl ₃ solution	Deep blue	+	+	-	+	+
5.	Saponins	H ₂ O	Distilled water	Frothing	+	+	+	+	+
6.	Carbohydrates	H ₂ O	10 % a-naphthol and conc: H ₂ SO ₄	red ring	+	+	+	+	+
7.	Starch	H ₂ O	Iodine solution	Deep blue	+	+	+	+	+
8.	α-amino acids	H ₂ O	Ninhydrin reagent	Pink spot	+	+	+	+	+

No	Test	Extracts	Test reagent	Observation	Remark				
					L	S	R	F	S
9.	Terpenoid	CH ₂ Cl ₂	Benedict's solution	Pink colour	+	+	+	+	+
10.	Steroid	Pet-ether	Acetic anhydride + Conc H ₂ SO ₄	Greenish blue	+	+	+	+	+

+ = present, - = absent, ppts = precipitates, L= Leaves, S = Stems, R = Roots, F = Fruits, S = seeds

Physicochemical investigation of *Pongamiapinnata* Pierre.

In the determination of some physicochemical properties, moisture content, total ash, acid insoluble ash, water soluble ash and solubility in different solvents of leaves, stems, roots and fruits of *Pongamiapinnata* Pierre. were studied. The results were shown in table 2.

Table 2 Physicochemical properties of leaves, stems, roots, fruits and seeds of *Pongamiapinnata* Pierre.

No.	Physicochemical properties	Content %			
		Leaves	Stems	Roots	Fruits and Seeds
1	Moisture content	15.83	18.12	16.05	16.77
2	Total Ash	6.14	15.15	17.26	7.49
3	Acid-insoluble ash	2.7	3.72	7.78	0.7
4	Water-soluble ash	39.3	42.1	34.1	31.4
5	Ethanol soluble content	9.43	2.31	3.08	8.05
6	Chloroform soluble content	1.73	0.48	0.46	1.31
7	Petroleum ether soluble content	0.92	2.12	1.87	2.73
8	Acetone soluble content	1.38	2.12	0.83	4.10
9	Distilled water soluble content	13.17	4.80	6.51	6.89

Antimicrobial activities of various solvent extracts from leaves of *Pongamiapinnata* Pierre.

The results of the screening of the antimicrobial activities of six different solvent extracts from leaves of *Pongamiapinnata* Pierre. were shown in the following table.

Table 3 Antimicrobial activities of various solvent extracts from leaves of *Pongamiapinnata* Pierre.

No.	Microorganism	Inhibition Zone (mm)					
		Acetone	Chloroform	Ethanol	Ethyl acetate	Methanol	Water
1.	<i>Agrobacterium tumefaciens</i>	16	12	8	8	16	8
2.	<i>Bacillus subtilis</i>	14	14	10	8	18	10
3.	<i>Escherichia coli</i>	8	12	-	-	12	-
4.	<i>Pseudomonas fluorescens</i>	14	12	-	-	13	-
5.	<i>Salmonella typhi</i>	14	10	8	-	10	-
6.	<i>Candida albicans</i>	16	14	12	8	16	10

Paper disc size = 6 mm

Discussion and Conclusion

In morphological study, *Pongamiapinnata* Pierre. is tree, stem woody; leaves alternate, imparipinnate compound, both surfaces glabrous; inflorescences axillary, flowers are purplish white, fragrant; stamens monadelphous; ovary superior, 1 to 2 ovules; fruit flattened, indehiscent, and 1 to 2 seeded. These characters were in agreement with those mentioned by Wealth of India 1950, Hooker 1875, Heywood 1978, Dassanayake 1987, and, Flora of Hong Kong, 2008.

According to phytochemical test of *Pongamiapinnata* Pierre., the presence of alkaloids, flavonoids, steroids, terpenoids, glycosides, carbohydrates, saponins, tannins and phenolic compounds were found in the leaves, stems, and fruits and seeds. All these phytoconstituents were found except phenolic compound in the roots of *Pongamiapinnata* Pierre. These metabolites have been shown to be responsible for the therapeutic activity of plants (Trease and Evans, 2002)

In quantitative analysis, moisture content, total ash, acid insoluble ash, water soluble ash and solubility matter in different solvents were investigated. The results showed that the highest moisture contents were found in stems. The powders of all parts of plant were soluble in all solvent tests. The leaves powders showed highest solubility in water. The fruits were more soluble in pet-ether and acetone than other parts of the plants. The solubility in polar solvent, ethanol, and aqueous of extractive values were more soluble than non-polar solvents, pet-ether, chloroform and acetone. These physicochemical properties were examined by using the method of British Pharmacopoeias 1980 and WHO, 2007.

In the screening of antimicrobial activity, acetone, chloroform and methanolic extract exhibited antimicrobial activity on all test organisms. Among them, methanolic extract significantly showed antimicrobial property against all test organisms with the inhibition zone diameters ranged between 10-18mm. Methanolic extracts indicated antimicrobial activity on four test organisms: *Agrobacterium tumefaciens*, *Bacillus subtilis*, *Samonellatyphi* and *Candida albicans*. Ethyl acetate and aqueous extract exhibited antimicrobial activity on *Agrobacterium tumefaciens*, *Bacillus subtilis* and *Candida albicans*. Therefore, *Pongamiapinnata* Pierre. can be used for the preparation of effective antimicrobial sources.

In conclusion, *Pongamiapinnata* Pierre. possesses secondary metabolites for the therapeutic activity and leaves extracts have beneficial effect in antimicrobial activity such as antibacterial and antifungal activity. Therefore, the medicinal plant, *Pongamiapinnata* Pierre. possesses many medicinal values and it can be used as a folk medicine not only for local people but also for animals and plants. So, other bioactive compounds should be isolated from the various plant parts of *Pongamiapinnata* Pierre. that they may serve as lead for the development of new that highly address to unmet therapeutic needs.

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References

- Backer, C.A. (1965). "Flora of Java", Vol.II, Neordhoff N V. Groningen Company, Netherland.
 Cruickshank, R. J. P. (1975). "Medicinal Microbiology". 11th ed. Churchill Livingstone Ltd., London.
 Dassanayake, M.D. and F.R. Fosberg, (1987). "A Revised Handbook to the Flora of Ceylon", Vol.V, Amerind Publishing Co. Pvt. Ltd, New Delhi.
 Hooker, J.D., (1875). "Flora of British India", Vol.I.L, Reeve and Company, England.

- Hundley, H.G and Chit KoKo. (1987). "List of Trees, Shrubs, Herbs and Principal Climbers, etc", Government Printing Press. Yangon.
- Kress, W., R. John, A.D. Ellen Farr and Yin Yin Kyi. (2003) "A Check List of Trees, shrubs, Herbs and Climbers of Myanmar", National Museum of Natural History Smithsonian Institution. Washinton D.C., U.S.A.
- Marini-Bettolo, G.B *et al.*(1981). "Plant screening by chemical and chromatographic procedure under field condition" *J. Chromato* 213. 113. 127.
- Trease and Evans. (2002). "Pharmacognosy", SpottiswoodeBallantyne Ltd, Colchester and London.
- British Pharmacopoeia. (1980). The Pharmaceutical Press. London and Bradford.17, Bloomsbury Square.London.W.C.I.
- Central Council for Research in Unani Medicine.(1987). "Phytochemical Standards of Unani Formulations". New Delhi, Ministry of Health and Family welfare.
- Hong Kong Herbarium. (2008). "Flora of Hong Kong" Vol.2 Edited by Hong Kong Herbarium Agriculture, Fisheries and Cinservation Department and South China Botanical Garden Chinese Academy of Sciences.
- Wealth of India. (1950). "A Dictionary of Indian Raw Materials and Industrial Product", Vol.II, Delhi.
- World Health Organization. (2007). WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues.

Websites

1. <https://britannica.com>
2. [http://www. Ijpsr.com](http://www.Ijpsr.com)
3. <http://bioinfopublication.org/files/articles/>
4. [http://www. Greenpgarmacy.info](http://www.Greenpgarmacy.info)
5. <http://www.globinmed.com>
6. <https://www.ncbi.nlm.nih.gov/pubmed/16414771>