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Research

Pharmacognostical and phenetics assessment of *Pandanus amaryllifolius*

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Abstract

Pandanus amaryllifolius, an aromatic tropical plant belongs to Pandanaceae, Taiwanese used the plant and its preparations to treat fever, severe jaundice, smallpox, headaches, arthritis and dental problem. Essential oil obtained from the leaf is used as a stimulant, antispasmodic is effective against headaches, rheumatism, epilepsy, sore throat, diabetes, diuretics and for skin diseases. It exhibited antiviral, antioxidant, antihyperglycemia, anticancer, antimicrobial activities. The present research papers assigns with macroscopic, microscopic of *Pandanus amaryllifolius* leaf along with phenetics are also studied..

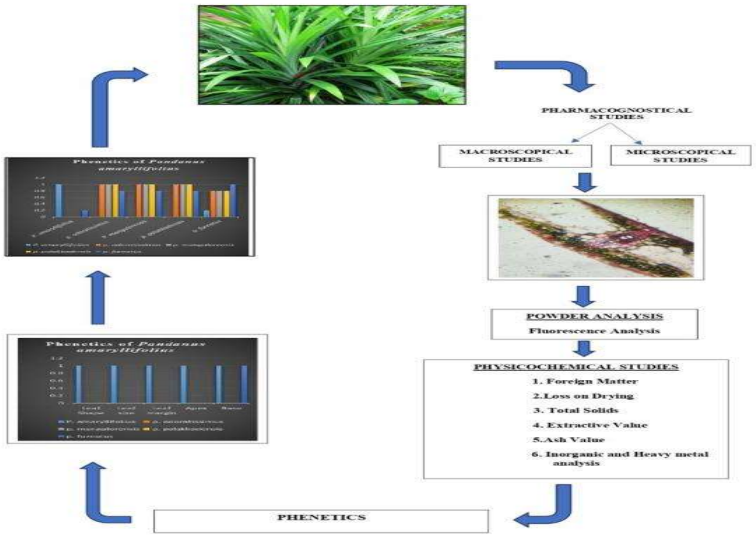
Keywords: *Pandanus amaryllifolius*, Pandanaceae, Pharmacognostical, Phenetics.

INTRODUCTION

Pandanus amaryllifolius Roxb,a plant belong to the Pandanaceae family, mainly native to South-east Asian countries [(1)]. The leaves of *P. amaryllifolius* have mild aroma are widely used in South East Asia for flavouring in culinary purpose [(2)]. The characteristic aroma of pandan is due to the presence of 2-acetyl-1-pyrroline, found in the lower epidermal papillae[(3)]. Taiwanese use this plant and its preparations to treat fever,severe jaundice, smallpox, headaches, arthritis and dental problems [(4)]. The essential oil is used as a stimulant and antispasmodic, effective against headaches, rheumatism, epilepsy and sore throats. The leaves are used to treat diabetes, diuretics and skin diseases[(5)]. In India, leaves preparation are used in rheumatism, its juice is used to treat chest pain,fever, inflammation, stomach cramps and spasms . Infusions of leaves are used internally and externally as sedatives. [(6,7)] Phytochemical studies revealed that *Pandan* has flavonoids, tannin and phenolic compounds [(8)]. It exhibits antiviral [(9)], antioxidant [(10)], antihyperglycemic [(11)], anticancer [(12)], antimicrobial activities [(13)]. The literature survey revealed that the pharmacognostical studies have not been performed so far. It is immediate and essential to explore pharmacognostical parameters of this aromatic plant. Consequently the

present investigation include macroscopical, microscopical evalution, determination of physio-chemical constants including inorganic elements and phenetics are determined.

Graphical abstract



MATERIALS AND METHODS

Authentication and Collection

The leaf was identified and authenticated by a botanist DR.Stephen, Professor, Department of Botany,The American College, Madurai-625002. The herbarium of this specimen was kept in the department for further reference. Leaves were collected from a local garden in Erasanayakkanpatty village, Viralimalai Taluk, Pudukottai Dist, Tamil Nadu in the month of Nov 2023.

Pharmacognostical studies

Fresh leaves were subjected to pharmacognostical studies. Organoleptic, macroscopy and microscopy of the leaves of *Pandanus amaryllifolius* Roxb. were studied.

Organoleptic evaluation

Fresh leaves are collected and checked for their colour, odour and taste by sensory characters.

Macroscopical evaluation

It includes length, width, base, apex, arrangement and venation of the leaf was identified. (figure 1,2 and table 1).

Microscopy evaluation

Free hand made sections were taken,stained with routine staining reagents and were observed under microscope as per Wallis [(14)] presented in (figure 3).Characters were observed using Nikon ECLIPSE E200 trinocular microscope attached to Zeiss ERc5s digital camera under field light.

Determination of leaf constants

Fresh leaves were peeled to observe the stomata, epidermal number, stomatal number and stomatal index were determined as per WHO guidelines [(15)] (table 2 and figure 4).

Preparation of powder

Leaves were collected, washed, shade dried, coarsely powdered and passed through sieve 40.

Powder microscopy

A small amount of the powdered sample was mounted on a microscopic slide with routine reagents as per Khandelwal [(16)]. Photomicrographs of diagnostic characters were detected and documented (figure 5).

Fluorescence analysis

A small quantity of leaf powder was transferred to test tube and 1-2 drops of freshly prepared various solution was added and the colour was observed [17] under visible, UV-254 and 365 nm (table 3).

Determination of Physico-chemical parameters

The leaf powder was analysed by various physio-chemical parameters such as foreign matters, loss on drying, total solids, ash values and extractive values using various solvents were determined by Ayurvedic Pharmacopoeia [18].(table 4).

Determination of inorganic elements and heavy metals analysis

The inorganic elements and heavy metal content was determined as per Atherden [19]; (table 5).

Phenetics

Numerical taxonomy or taxi-metrics, nowadays frequently and perhaps more appropriately referred to as phenetics, refers to the application of various mathematical procedures to numerically encoded character state data for organisms under study. [20]

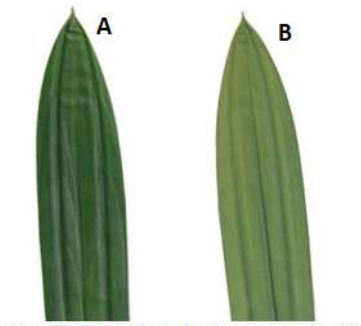
RESULTS AND DISCUSSION

Leaves of *Pandanus amaryllifolius* showed green colour when fresh, slightly aromatic, with sweet taste, linear margin, 25-40 cm in length and 2-5cm width, parallel venation converging with prominent midrib, round leaf base and tip is long no throns, only at the end small throns are present, leaves are single, neatly arranged with smooth and shiny upper surface.

Table 1: Macroscopic studies of *Pandanus amaryllifolius*

S.no	Characters	Observation
1	Color	Green
2	Odour	Slightly aromatic (Basmati)
3	Taste	Sweet
4	Type	Monocotyledon
5	Length	25-40cm
6	Width	2-5 cm
7	Shape	Lanceolate
8	Venation	Parallel venation converging with prominent midrib
9	Base	Round
10	Apex	Only at the end small throns are present
11	Arrangement	Single and neatly arranged
12	Surface	wavy, shiny surface

Fig 1: Habitat of *Pandanus amaryllifolius*



(A-Upper surface B- Lower surface)

Fig 2: Dorsal and ventral view Entire leaves of *Pandanus amaryllifolius* (a portion)

The first leaf on a branch is short, bicarinate prophyll in the adaxial (upper) position. It is successively longer than its predecessor, forming a gradual transition to the normal foilage leaves. The leaf is dorsiventral, hairs are absent, the margin and midrib bear prominent spines.

Microscopy

Transverse section of *Pandanus amaryllifolius* showed following characters

Epidermis

Epidermal cells are rectangular slightly longitudinal extened and arranged in longitudinal files. Leaf is delimited by well developed surface layers each consist of a shallow, strongly cutinized, colourless and often slightly thick walled hypodermal layer three or four cells deep. Adaxial hypodermal layers are thicker than abaxial. In the abaxial epidermis is distinct nonstomatae, costal regions, between the veins, to which stomata are restricted.

Hypodermis

Hypo-epidermal in the outermost two layers consists of flattened cells extending transversely and not longitudinally to the long axis of the leaf. outer hypoepidermal cells immediately surround substomatal chamber, in a uniform shape and arrangement. Anticlinal walls in outer hypodermal layers clearly derived from different initial layers, Khush [21]. The outermost hypodermal layers often sclerotic; inner cells are larger, more cubical in shape and thin-walled.

Mesophyll

Mesophyll runs parallel longitudinal veins, separated by assimilating layers which surround pseudolacunae. Each vein is supported by a well-developed parenchymatous and fibrous buttress, continuous with the hypodermis of each surface. Mesophyll between veins are large, colourless cell tend to arrange into separate transverse plates. These cells collapse in matured leaves so that in thin transverse sections there is an apparent lacuna between each adjacent pair of veins. These pseudolacunae are lined by small chlorenchyma cells, the adaxial layers often conspicuously palisade.

Vascular bundle: Vascular tissues are complex. Bundle sheath cells are present on either sides of the collateral vascular bundle.

Xylem: Protoxylem and metaxylem were found.

Lacunae: Traversed by compact transverse diaphragms, two cells thick, at frequent interval diaphragm include transverse vascular commissures which anastomose with the longitudinal veins. [22]

Stomata: Paracytic stomata confined to the lower surface. [23]

Stoma: Two hypodermal cells below which the chamber originates are longer than adjacent hypodermal cell with large nuclei, include a fine granular deposit.

Guard mother cell: Found immediately above a chamber differs from a neighboring cells of stomata

Neighbouring cell: A cell is surround stomatal apparatus differ from a normal epidermal cell. [24]

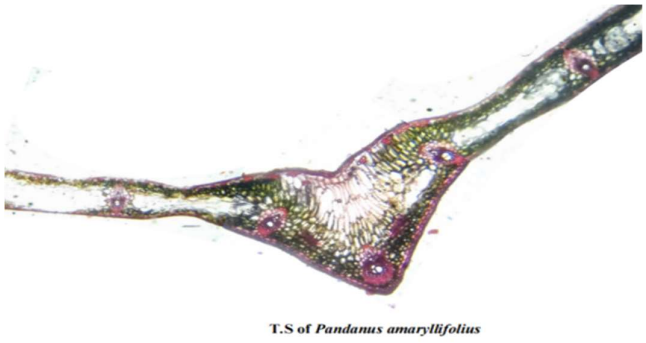


Fig 3: T.S. *Pandanus amaryllifolius* (5x)

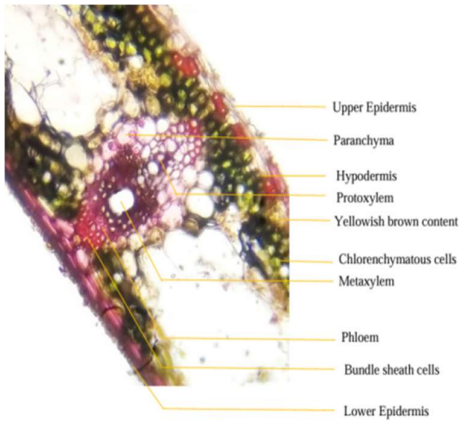


Fig 4: T.S *Pandanus amaryllifolius* (10x enlarged view)

Determination of leaf constant of *Pandanus amaryllifolius*

Quantitative microscopic parameters such as epidermal cells, stomatal number, stomatal index and palisade ratio were analysed.

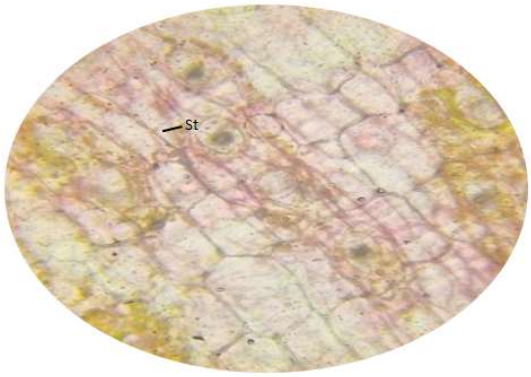


Fig 5: stomata abaxial surface- enlarged view

St-Stomata (Paracytic stomata)

Table: 2 Determination of leaf constants of *Pandanus amaryllifolius*

Parameters	Upper epidermis (/mm2)	Lower epidermis (/mm2)
Epidermal cells	146	161
Stomatal number	12	29
Stomatal index	8	18
Palisade ratio	-	8-12

Powder microscopy of *Pandanus amaryllifolius* leaf

The powder greyish green in colour with a characteristic taste and aromatic pleasant odour, showed epidermis with papillose growth, epidermal fragments of with paracytic stomata, palisade cells in sectional view, mesophyll cells, reticulate and spiral vessels acicular and prismatic calcium oxalate crystals, yellowish content and starch grain (Fig. 5)

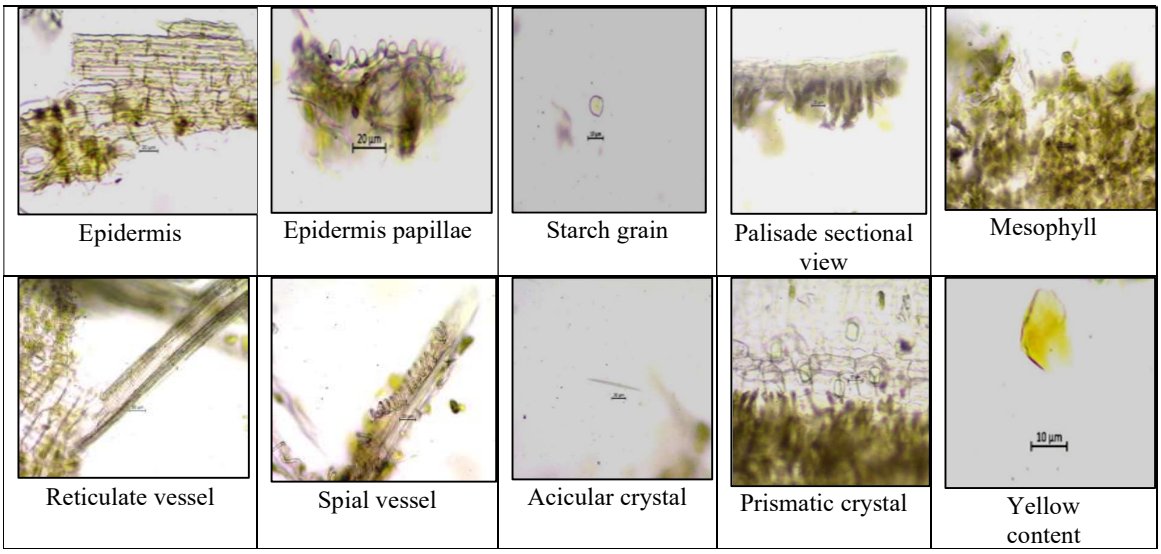


Fig 6: Powder Microscopy of *Pandanus amaryllifolius*

Determiation of flourescence analysis of *Pandanus amaryllifolius* (powder)

Powdered drug of *Pandanus amaryllifolius* plant gave different fluorescence under visible and ultraviolet (UV) radiation.(254nm and 365 nm),when treated with various reagents. Therefore, fluorescence evaluation is used for the identification of powdered drug. Some crude drugs are often assessed qualitatively in this way, and it is an important parameter of pharmacognostic evaluation.

Table 3: Determination of flourescence analysis of *Pandanus amaryllifolius* (powder)

Sample+ Reagent	Visible	UV 254 nm	UV 365 nm
Powder+conHcl	Green	Green	Brown
Powder + Conc. Sulphuric acid	Brown	Black	Black
Powder + Conc. Nitric acid	Yellow	Green	Yellow
Powder + Acetic acid	Brown	Green	Red
Powder + 20% NaOH	Green	Green	Light brown
Powder + Hcl + water	Green	Pale yellow	Green
Powder + H_2SO_4 + water	Yellow	Pale yellow	Green
Powder + HNO_3 + water	Pale yellow	Yellow	Color less
Powder + Alc. NaOH	Green	Red	Yellow
Powder + Water	Green	Green	Light brown

Determination of Physiochemical parameters of the *Pandanus amaryllifolius*

The ash values of the plant were estimated using standard procedures which showed a total ash of 10.22±0.078% , water soluble ash 2.16%w/w and acid insoluble ash of 1.87%w/w. Loss on drying and total solid value of the powder was determined as 5.59±0.733% &94.41±0.233% w/w respectively. Petroleum ether extractive, Ethyl acetate extractive, Chloroform extractive, Ethanol extractive, water extractive the percentage yield of the extractive was found to be 26.141±3.27%, 17.33±1.764%, 22.65±3.236%, 27.72±1.143%, 59.87±2.692% w/w respectively.

Table 4: Determination of Physiochemical parameters of the *Pandanus amaryllifolius*

S.no	Physio-Chemical Parameters	Results (% w/w)
1	Foreign matter	NIL
2	Loss on drying	5.59±0.733
3	Total solid	94.41±0.233
4	Petroleum ether extractive	26.141±3.27
5	Ethyl acetate extractive	22.65±3.236

6	Chloroform extractive	17.33±1.764
7	Ethanol extractive	27.72±1.143
8	Water extractive	59.87±2.692
9	Total ash	10.22±0.078
10	Water soluble ash	2.16
11	Acid insoluble ash	1.87

Determination of inorganic elements and heavy metal analysis

To the ash of *Pandanus amaryllifolius* leaves was treated with 50%v/v hydrochloric acid and kept for 1 hour. It was filtered, filtrate was used for inorganic and heavy metal analysis using various reagents.

Table 5: Determination of inorganic elements and heavy metal analysis

	Presence of elements	Absence of elements
1.	sulphate	aluminium
2.	-	arsenic
3.	-	borate
4.	-	calcium
5.	-	chlorine
6.	-	copper
7.	-	iron
8.	-	phosphate
9.	-	silver

Phenetics

There are around 578 accepted species of *Pandanus* present around the World. Among those species, five species are available in South India.

- *Pandanus amaryllifolius (PA)*
- *Pandanus odoratissimus (PO)*
- *Pandanus mangalorensis (PM)*
- *Pandanus palakkadensis (PP)*
- *Pandanus fureatus (PF)*

The characters such as leaf shape, leaf size, leaf margin, apex and base are considered.
Leaf shape (L. Sh) - Lanceolate; present = 1, absent= 0.
Leaf size (L.size) - 25-40cm and 2-5 cm width; present = 1, absent= 0.
Leaf margin (L.mn) - Entire margin; present = 1, absent= 0.
Leaf apex-small throns at the end ; present = 1, absent= 0.
Leaf base- Rounded; present = 1, absent= 0.

Table 6: Chart of similarity for five species of *Pandanus*

Species	L. shape	L. size	L. margin	Apex	Base
P.amaryllifolius	1	1	1	1	1
P.odoratissimus	0	0	0	0	0
P.mangalorensis	0	0	0	0	0
P. Palakkadensis	0	0	0	0	0
P.fureatus	0	0	0	0	1

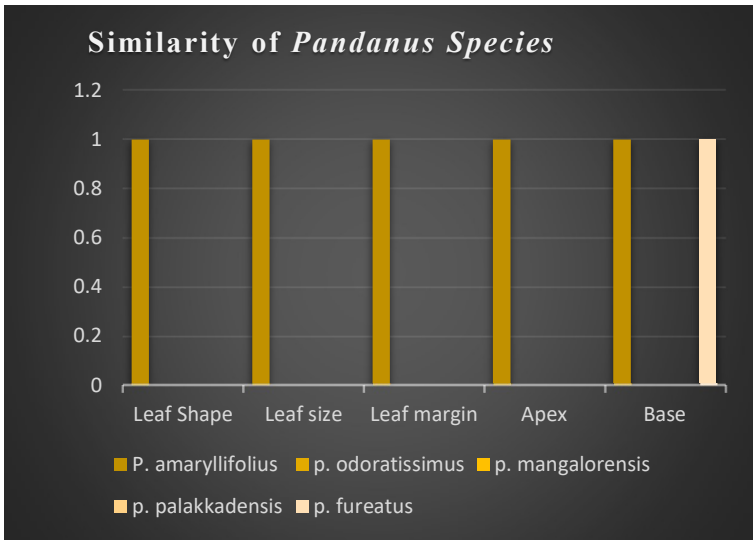


Fig 7: chart of similarity characters of five species of *Pandanus*

Table 7: Chart of dis-similarity for five species of *Pandanus*

Species	P. amaryllifolius	P. odoratissimus	P. mangalorensis	P. palakkadensis	P. fureatus
P.amaryllifolius	1	0	0	0	0
P.odoratissimus	0	0	0	0	0.6
P.mangalorensis	0	0	0.4	0	0.6
P.palakkadensis	0	0	0.4	0	0.6
P. fureatus	0	0	0	0	0.2

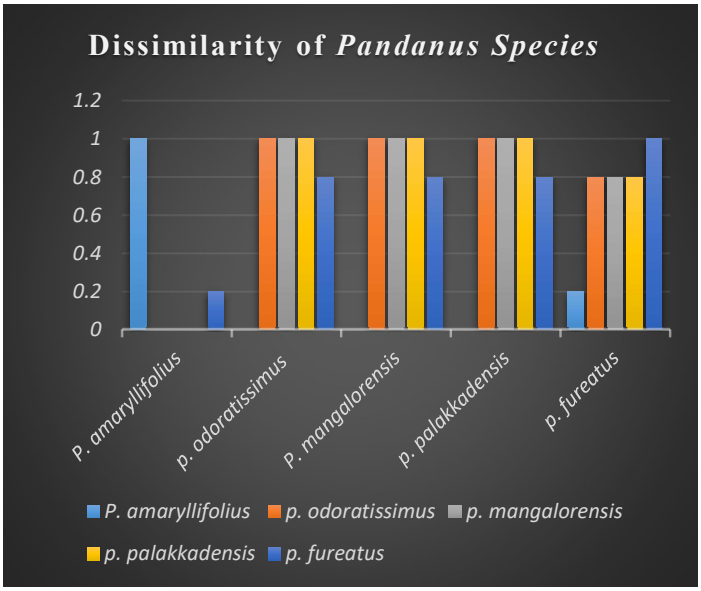


Fig 8: Chart of dissimilarity for five species of *pandanus*

Sample matching coefficient
SSM=NS/NS+ND X 100
Where,
NS - Number of similiarity characters
ND-Number of dissimiliarity characters

N-Number of samples
The matching coefficient of *Pandanus amaryllifolius* with respect to other species was found to be 24%

Key characters of *Pandanus amaryllifolius*
- Aromatic pointed leaves with no toothed edge.
- Plant never flowers and no fruits in *pandanus amaryllifolius.*
- The first leaf on a branch is short, bicarinate prophyll in the adaxial (upper) position.
- It is successively longer than its predecessor, forming a gradual transition to the normal foilage leaves.

Microscopically
- The papillae run parallel along the leaf length and are absent over the veins and midrib.
- The number of papillae varies from one to seven per cell.
- Papillae were also found surrounding the stomata forming a necklace-like structure.
- Paracytic stomata in both leaf surface.
- Hypodermis two layers on both parts of the leaf. 1-2 layers of palisade cells in adaxial and a layer in the abaxial leaf part, sponge exist between both parts.
- The characteristic aroma of pandan is caused by the aroma compound 2-acetyl-1-pyrroline, found in the lower epidermal papillae.

CONCLUSION

Macroscopical and microscopical findings help to identify the plant in whole and powder form. Powder analysis, physicochemical analysis, fluorescence analysis and phenetics helps to prove the authenticity of the plant. Hence the pharmacognostical and phenetics study adds taxonomical information to this plant. Physico- chemical constants, leaf constants add benefits in the identification of this plant. These parameters can serve as standards so as to draw the pharmacopoeial monographs. Phenetical study imparts taxonomical enlightement of this *Pandanus amaryllifolius* can be made to differentiate from other species of *Pandanus.*

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