

OPTIMIZATION OF PHYTOCHEMICAL EXTRACTION FROM *PANDANUS AMARILLIFOLIUS* ROXB: A COMPARATIVE STUDY OF DRYING TECHNIQUES AND SOLVENT SYSTEMS

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ABSTRACT

Pandanus amaryllifolius Roxb is a tropical plant. Pandan leaves offer potential especially for their function as a source of natural colours, aroma, and antioxidants. 2-Acetyl-1-pyrroline is the primary fragrance compound in Pandan leaves. This scent is essential since it acts as the primary contribution of favourable substances which could be good for physical condition and provide flavour for food products. Its sweetness and flavourful taste are known as a natural origin of flavouring. The extraction and qualitative phytochemical analysis of powdered *Pandanus amaryllifolius* (Pandan) leaves using various drying techniques and solvents are the main goals of the present study. Initially, *Pandanus amaryllifolius* leaves were dried using different drying methods viz., shade dried and Hot Air Oven Dried and the dried samples were powdered and ground. Utilizing four solvents—chloroform, petroleum ether, acetone, and ethanol the powdered plant powder were separated using hot soxhlet extraction methods. Using standard qualitative phytochemical tests, the extracts were examined for the presence of bioactive substances like protein, carbohydrates, steroids, alkaloids, flavonoids, phenols, tannins, saponins, glycosides, and triterpenoids. Results showed that the type of solvent and drying technique had a major influence on the phytochemical content. Despite non-polar solvents like petroleum ether and chloroform were better at extracting terpenoids and steroids, ethanol and acetone extracts displayed an increased number of polar in nature chemical compounds. Again the, the drying methods also plays a crucial role in the extraction of phytonutrients, In the present study, Hot Air Oven dried sample reported higher number of phytochemicals compared to shade dried *P. amaryllifolius* Leaf samples. The research presented here emphasizes how Pandan leaves can be a rich source of natural phytochemicals as well as crucial extraction techniques are to optimizing compound recovery for possible pharmacological and nutraceutical effects.

Keywords: *Pandanus amaryllifolius* Roxb, Drying methods, Shade drying, Hot Air Oven drying, Soxhlet Extraction Method.

INTRODUCTION

Medicinal plants have long been a cornerstone of traditional health systems and continue to contribute significantly to modern pharmaceutical research. One such plant, *Pandanus amaryllifolius* (commonly known as *Pandan*), is well-known in Southeast Asia for its aromatic properties and therapeutic potential. Apart from its culinary uses, Pandan leaves are traditionally used to treat ailments such as headaches, skin problems, and inflammation due to their bioactive compounds. (Harborne, J. B. (1998). Pandan leaves contain a variety of phytol-components including steroids, carbohydrates, polyphenols, alkaloids, tannins, flavonoids and saponins (Aini *et al.*, 2009; Karimi *et al.*, 2011). Among the classes of bioactive compounds, flavonoids and phenolic compounds were intensively studied thanks to their potential benefits for human health. They possess antioxidant properties capable of scavenging free superoxide radicals and reducing the risk of cancer based on its anti-aging properties (Park *et al.*, 2008). Phenolics have the antioxidant activity due to their redox properties, hydrogen donors and singlet oxygen quenchers. Several studies have also found that flavonoids reduce blood lipids and glucose and enhance human immunity (Park *et al.*, 2011). Furthermore, humans are protected by the enzyme system as a result of the effect of flavonoids (Atoui *et al.*, 2005). Determining the use of ethanol, temperature, and length of extraction setup in the extraction process is essential for optimizing desired extract acquisition. Selection of the extraction method is crucial since the extraction results will reflect the method's success rate (Garcia-Salas *et al.*, 2010). Conventional extraction (such as maceration, percolation, reflux, and Soxhlet) generally involves a thermal process and takes a considerable amount of time, which consequently causes damage to the phenolic component. The main problem with Pandan extraction is the longer extraction time and suitability of solvent, which can damage the desired active substance from the extraction process. Extraction is the procedure of selection of the most suitable part one or more compounds from a blend of liquid or solid substances. The present study aims to compare the qualitative phytochemical profiles of *Pandanus amaryllifolius* leaf powder prepared using two drying methods shade drying and hot air oven drying. The powdered samples were extracted using four different solvents—chloroform, petroleum ether, acetone, and ethanol—and subjected to standard qualitative phytochemical screening. The goal is to evaluate the influence of extraction techniques and solvent polarity on the detection of bioactive constituents in *Pandanus amaryllifolius*.

MATERIALS AND METHODS

1. Collection and Preparation of Plant Material

Fresh leaves of *Pandanus amaryllifolius* were collected from the fresh home garden, Aumanai and thoroughly washed with distilled water to remove dust and impurities. The cleaned leaves were divided into two batches for different drying methods: Shade Drying and Hot Air Oven Drying

2. Shade Drying

Leaves were kept in a shade drying, well-ventilated area for 7–10 days until the leaves were crisped and moisture-free.

3. Hot Air Oven Drying

The fresh Leaves were cleaned and dried in a hot air oven at 40–50°C for 24–48 hours.

4. Solvent Extraction

For each dried sample (shade-dried and Hot Air oven-dried), 30 grams of powdered leaves were extracted using four solvents of increasing the orientation viz., Petroleum Ether, Chloroform, Acetone and Ethanol. The extraction were carried out using the Soxhlet extraction method for 6–8 hours for each solvent. The extracts were then filtered and concentrated by evaporation and stored in labeled containers for phytochemical analysis. The given extracted samples were concentrated by evaporating the solvent using Rotary Evaporator at 40 to 60°C for 30 to 60 minutes till all the solvent were evaporated separately, finally, we get the extracts at semisolid state.

The Hot Air Oven Dried Extracts were coded as PHDCH (*P. amaryllifolius* Hot Air Oven Dried Chloroform Extract), PHDPE (*P. amaryllifolius* Hot Air Oven Dried Petroleum ether Extract), PHDA (*P. amaryllifolius* Hot Air Oven Dried Acetone Extract) and PHDE (*P. amaryllifolius* Hot Air Oven Dried Ethanol Extract) and the shade dried *P. amaryllifolius* were coded as PSDCH (*P. amaryllifolius* Shade Dried Chloroform Extract), PSDPE (*P. amaryllifolius* Shade Dried Petroleum ether Extract), PSDA (*P. amaryllifolius* Shade Dried Acetone Extract), PSDE (*P. amaryllifolius* Shade Dried Ethanol Extract). The dried content were weighted and calculated the yield of the dried extract. The extracts were kept and stored in refrigerator at 4 °C for further use. Percentage of yield was calculated by dividing the dry weight of extract (g) by the dry weight of plant biomass and multiplying by 100.

5. Qualitative Phytochemical Analysis:

Test for Carbohydrates (Brain and Turner, 1975)

Benedict's test

To 2 ml of the test samples about 2 ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 minutes. Formation of brick red precipitate indicates the presence of carbohydrate.

Test for Protein (Ansari, 2006)

Biuret Test

To 3 ml of test samples, few drops of 4% NaOH and 1% CuSO₄ solution were added. The sample tubes were observed for violet or pink colour formation.

Test for Glycosides (Ansari, 2006)

Keller-Killiani Test: To 2 ml of test sample, 2 ml glacial acetic acid, one drop 5% FeCl₃ and conc. H₂SO₄ were added. If Reddish brown colour appeared at junction of two liquid layers and upper layer turns bluish green indicates the presence of glycosides.

Test for Steroids (IP, 1996)

Salkowski Test: To 2 ml of extract, 2 ml of chloroform and 2 ml of conc. H₂SO₄ were added. The solutions were shaken well. As a result chloroform layer turned red and acid layer shows greenish yellow fluorescence, indicates the presence of steroids.

Test for Alkaloids (Ansari, 2006)

Mayer's Test: 1ml of extract was mixed with 2ml of 1% HCl and heated gently. To this added 2-3ml of Mayer's reagent. Formation of yellow precipitate indicated the presence of alkaloids.

Test for Flavanoids (Harborne, 1984)

Aluminium Chloride test: To few drops of Conc. HCl, 2ml of Crude extract was mixed, then added a pinch of Magnesium turning and again added few drops of HCl and added few drops of 10% NaOH. Pink color formation which indicated the presence of flavonoids.

Test for Tannins (Mukherjee, 2002)

Lead Acetate Test: On addition of lead acetate solution to the 1ml of extract, if white Precipitate appeared, it indicates the presence of Tannins.

Test for Saponin (Ansari, 2006)

Foam Test: The 1ml of plant extract was shaken vigorously with 1ml of distilled water, persistent foam formation indicates the presence of Saponin.

Test for Phenol (Mukherjee, 2002)

Ferric chloride Test: The 1ml of extract, it was diluted to 5 ml of distilled water. Then few drop of neutral 5% ferric chloride solution was added. A dark green color indicates the presence of phenolic compounds.

Test for Triterpenoids (Harborne, 1984)

To 2 ml of test sample, 2 ml of chloroform was added with few drops of conc. Sulphuric acid at the side of the test tube. An interface with a reddish brown color formation occur, if terpenoids constituent was present.

RESULT AND DISCUSSION

1. Yield of Soxhlet Extracted Shade Dried *P. amaryllifolius* Leaf Extracts

Loui et al. (2004) studied the impact of an integrated liquid and supercritical solvent extraction process to get better antioxidant compounds from winery by-products. They found that the extract's antioxidant activity was influenced by the solvent type, the medium composition. Soxhlet extraction techniques have been widely used to remove some substances from several plant ingredients (Manousi et al., 2019). In the present study, shade dried *P. amaryllifolius* plant powder was allowed for soxhlet method of solvent extraction and the percentage of yield of different extracts varied. Among the different shade dried solvent Extracts, acetone extract of shade dried *P. amaryllifolius* recorded higher Extract yield of 22gm followed by ethanol Extract (19.9gm)>Petroleum Ether Extract (10.3gm)>Chloroform Extract (9.1gm).Table-1

Table -1. Yield of Soxhlet Extracted Shade Dried *P. amaryllifolius* Leaf Extracts

SI NO	Sample code	Dried weight of plant biomass (g)	Dried weight of plant Extract (gm)	% of Yield (g)
1	PSDCH	10	0.91	9.1
2	PSDPE	10	1.03	10.3
3	PSDA	10	2.2	22
4	PSDE	10	1.99	19.9

2. Qualitative Phytochemical Analysis of Different Solvent Extracts of Shade Dried *P. amaryllifolius*

Pandan leaves extract comprises several volatile compounds in the group of alcohols, aromatics, carboxylic acids, ketones, aldehydes, esters, hydrocarbons, furans, furanone, and terpenoids.

This is possibly due to the distinctive aroma of *P. amaryllifolius*, which is a derivative compound of the amino acid phenylalanine, namely 2-acetyl-1-pyrroline (ACPY) (Faras *et al.*, 2014). Varadan *et al.*, (2014), investigated for the nutritional, anti-nutritional and physicochemical characteristics, as well as antioxidant activity and the potent component of *Pandanus amaryllifolius* collected from three different locations in Andaman and Nicobar Islands was identified using Heat Map and PC. In the present study the qualitative phytochemical analysis of shade dried *P. amaryllifolius* leaf extracts was carried out and the result reported that chloroform (PSDCH) and Acetone (PSDA) extract of shade dried *P. amaryllifolius* recorded the presence of Carbohydrates, steroids and Terpenoids and the absence of other seven phytochemicals. Whereas, Petroleum ether extract (PSDPE) of shade dried *P. amaryllifolius* represented the presence of carbohydrate and terpenoids and the least number of phytoconstituents were noticed in the shade dried ethanol extract of *P. amaryllifolius* (PSDE). PSDCH: PSDA>PSDPE>PSDE. The results were displayed in Table: 2 and Figure 1(a-d).

Table.1. Qualitative Phytochemical Analysis of Different Solvent Extracts of Shade Dried *P. amaryllifolius* Sample

SI NO	Phytochemical Test	Sample Code <i>Pandan amaryllifolius</i>			
		PSDCH	PSDPE	PSDA	PSDE
1	Protein	Negative	Negative	Negative	Negative
2	Glycosides	Negative	Negative	Negative	Negative
3	Alkaloids	Negative	Negative	Negative	Negative
4	Flavanoids	Negative	Negative	Negative	Negative
5	Tannins	Negative	Negative	Negative	Negative
6	Phenol	Negative	Negative	Negative	Negative
7	Carbohydrate	Positive	Negative	Positive	Positive
8	Steroids	Positive	Positive	Positive	Negative
9	Saponin	Negative	Negative	Negative	Negative
10	Terpenoids	Positive	Positive	Positive	Negative



Figure-1a



Figure -1b



Figure -1c



Figure-1d

Figure-1-Qualitative Phytochemical Analysis of Different Solvent Extracts of Shade Dried *P. amaryllifolius*

Figure 1. Qualitative Phytochemical analysis of PSDCH

Figure 2. Qualitative Phytochemical analysis PSDPE

Figure 3. Qualitative Phytochemical analysis PSDA

Figure 4. Qualitative Phytochemical analysis PSDE

PSDCH- *P. amaryllifolius* Shade Dried Chloroform Extract

PSDPE- *P. amaryllifolius* Shade Dried Petroleum Ether Extract

PSDA - *P. amaryllifolius* Shade Dried Acetone Extract

PSDE - *P. amaryllifolius* Shade Dried Ethanol Extract

3. Yield of Soxhlet Extracted Hot Air Oven Dried *P. amaryllifolius* Leaf Extracts

The greater the volume of solvent used compared to the amount of material extracted, the greater the yield produced. The solvent's ability to dissolve the material depends on the amount of solvent added to maximally extract the components. The yield of extraction may continue to increase until the solution becomes saturated. After the saturation point of the solution is reached, solvent addition will not increase yield (Amiarsi *et al.*, 2006). In the present study, Hot Air Oven dried *P. amaryllifolius* plant powder was allowed for soxhlet method of solvent extraction and the percentage of yield of different extracts varied. Among the different shade dried solvent Extracts, Petroleum Ether extract of Hot Air oven dried *P. amaryllifolius* recorded higher Extract yield of 4.3 gm, followed by Chloroform Extract (3.7 gm)>Ethanol Extract (3.5 gm)> Acetone Extract (3.4 gm).Table-2

Table -2. Yield of Soxhlet Extracted Hot Air Oven Dried *P. amaryllifolius* Leaf Extracts

SI NO	Sample code	Dried weight of plant biomass (g)	Dried weight of plant Extract (gm)	% of Yield (g)
1.	PHDCH	10	0.37	3.7
2	PHDPE	10	0.43	4.3
3.	PHDA	10	0.34	3.4
4.	PHDE	10	0.35	3.5

4. Qualitative Phytochemical Analysis of Different Solvent Extracts of Hot Air Oven Dried *P. amaryllifolius* Leaf Extracts

According to Aini and Mardiyaningsih (2016), the results of the phytochemical screening indicated that *P. amaryllifolius* have substance tannins, alkaloids, flavonoids, saponins, and polyphenols. In the present study the qualitative phytochemical analysis of Hot air Oven dried *P. amaryllifolius* leaf extracts was carried out and the result reported that Ethanol (PHDE) extract of Hot Air Oven dried *P. amaryllifolius* recorded the presence of maximum of six phytoconstituents viz., glycosides, carbohydrates, steroids, flavonoids, terpenoids and saponins and the absence of other four phytochemicals. Followed by, Chloroform extract (PHDCH) of shade dried *P. amaryllifolius* represented the presence of proteins, glycosides carbohydrates, steroids and terpenoids and the acetone Extract (PHDA) reported the presence of glycosides, carbohydrates, steroids and terpenoids. The least amount of phytoconstituents were noticed in

Petroleum Ether Extract of shade dried *P. amaryllifolius* (PHDPE) revealing the presence of Glycosides, steroids and Terpenoids. Based on the presence of phytochemicals the different extracts were arranged as PHDE>PHDCH>PHDA>PHDPE. The results were displayed in Table: 2 and Figure. 2(a-d).

Table.2. Qualitative Phytochemical Analysis of Different Solvent Extracts of Hot Air Oven Dried *P. amaryllifolius* Sample

SI NO	Phytochemical Test	Sample Code			
		PHDCH	PHDPE	PHDA	PHDE
1	Protein	Positive	Negative	Negative	Negative
2	Glycosides	Positive	Positive	Positive	Positive
3	Alkaloids	Negative	Negative	Negative	Negative
4	Flavanoids	Negative	Negative	Negative	Positive
5	Tannins	Negative	Negative	Negative	Negative
6	Phenol	Negative	Negative	Negative	Negative
7	Carbohydrate	Positive	Negative	Positive	Positive
8	Steroids	Positive	Positive	Positive	Positive
9	Saponin	Negative	Negative	Negative	Positive
10	Terpenoids	Positive	Positive	Positive	Positive



Figure-2a



Figure -2b



Figure -2c



Figure-2d

Figure-2. Qualitative Phytochemical Analysis of Different Solvent Extracts of Hot Air Oven Dried *P. amaryllifolius* Leaf Extracts

Figure 5. Qualitative Phytochemical analysis of PHDCH

Figure 6. Qualitative Phytochemical analysis PHDPE

Figure 7. Qualitative Phytochemical analysis PHDA

Figure 8. Qualitative Phytochemical analysis PHDE

PHDCH- *P. amaryllifolius* Hot Air Oven dried Chloroform Extract

PHDPE- *P. amaryllifolius* Hot Air Oven dried Petroleum Ether Extract

PHDA - *P. amaryllifolius* Hot Air Oven dried Acetone Extract

PHDE - *P. amaryllifolius* Hot Air Oven dried Ethanol Extract

The fragrance of *P. amaryllifolius* leaf also changes along with the processing steps taken. By drying and heating, the fragrance of *P. amaryllifolius* leaves may reduce since it undergoes evaporation and several changes due to the reduction of the ACPY aroma. By drying and heating, the color may also experience a significant change from fresh green to pale green due to the effect of the heat imposed on the *P. amaryllifolius* leaves. Antioxidants from plant sources attract consumers because of their role in maintaining human health (Ghasemzadeh and Jaafar, 2013).

In the extraction process, a solvent is added to form different phases of the material; therefore, the substance that wants to be separated can appear dissolved in the solvent (Toledo, 2007). The type of target compound and its chemical content in the material are determined by the solvent selected. In addition, polarity and conditions of use, such as temperature when using solvents, are the most significant factors affecting extraction efficiency and selectivity (Chemat and Vian, 2014). Comparatively, the Hot Air Oven dried Ethanol Extract of *P. amaryllifolius* recorded the presence of higher number of Phytoconstituents of six followed by the chloroform extract of Hot Air oven dried *P. amaryllifolius*. So the result revealed that the Hot Air Oven dried *P. amaryllifolius* recorded higher amount of Phytoconstituents compared to Shade dried *P. amaryllifolius* Leaf Extracts.

CONCLUSION

Many studies have deliberated the extraction of bioactive compounds from Pandanus plants with more convenient methods. Changing ordinary technology with contemporary one to extract precious compounds represents many benefits, including reduced energy consumed, non-toxic organic solvents, and increased extraction yields. In the present research, we have compared the Hot Air Oven dried *P. amaryllifolius* and shade dried *P. amaryllifolius* leaf Extracts. Extraction of phytoconstituents from varied solvents from different drying methods reported a brief knowledge about representing the best drying method which prevent the loss of phytoconstituents from *P. amaryllifolius* was recognized.

Declarations

Conflict of interests -The authors declare no competing interests.

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