Original Research

EFFECT OF DRYING METHODS ON THE ANTIOXIDANT PROPERTIES OF *PANDANUS AMARYLLIFOLIUS*

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ABSTRACT:

Objective: Drying is the common way to preserve the quality of aromatic and bioactive compounds in medicinal plants. The goal of this study was to determine the antioxidant activity, phenolic content, chlorophyll content, and phytochemical of different types of drying methods and to propose the best method to produce a good tea.

Method: The leaves were dried by using two types of drying methods which are microwave drying and oven drying at the conditions 35°C for 48 h and 540 W for 4 minutes. Total phenolic content (TPC) and antioxidant activity were determined using Folin Ciacalteu (FC) and 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) methods respectively. Meanwhile, total chlorophyll content (TCC) and Phytochemicals were determined using acetone extraction and phytochemical screening methods respectively.

Results: TPC was higher in microwave drying (0.623 mg gallic acid^{-1} dry weight) and oven drying (0.417 µg gallic acid $^{-1}$ dry weight respectively). In addition, antioxidant activity was higher in IC_{50} values for microwave drying (6.379ug/ml) compared to oven drying (9.339ug/ml) respectively. Furthermore, it was observed that TCC in microwave dried leaves was highest (9.540 mg/g) compared with oven dried leaves (9.204 mg/g).

Conclusion: Among the drying methods, microwave drying produced the dried *P. amaryllifolius* tea of highest quality in terms of phenolic compositions, chlorophyll content, and antioxidant properties. Furthermore, drying methods that use high temperatures to dehydrate *P. amaryllifolius* may cause a dramatic loss of antioxidant activity and phenolic compounds, which reached up to 60%.

KEYWORDS: Medicinal plants: Drying method: Total Phenolic content: Anti-oxidant activity: *Pandanus amaryllifolius*

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INTRODUCTION:

Medicinal plants have been identified as a source of natural antioxidant and antimicrobial agents. The use of medicinal plants in traditional medicines is cheaper than modern medicines. Generally, plants may produce secondary metabolites like flavonoids, flavones, catechins, polyphenols and tannins which constitute an important source of pharmaceutical products in traditional and modern medicine [1]. Furthermore, medicinal plants are a rich source of antioxidants which are used in chronic diseases prevention and treatment like cancer, heart stroke, diabetes, arthritis, cardiovascular diseases, and also inhibit the process of aging. Antioxidants are the chemicals which scavenge the free radicals and help in preventing and treatment of several diseases. Overproduction of free radicals can damage the cells of the human body such as Deoxyribonucleic acid (DNA), white blood cells (WBC) and haemoglobin [2]. Antioxidants are beneficial to protect and prevent a human body against damage by reactive oxygen species. Epidemiologic studies have shown the relationship between the consumption of food rich in antioxidants and the incidence of a variety of disease. Out of various substance known of their antioxidant activity, extraordinary consideration is paid to polyphenols happening in different kinds of tea and herbal infusion [3]. Unlike most other types of tea, herbal teas do not contain any caffeine and its aroma will enhance the taste to drink. Some herbal teas combine with other herbal ingredients to bring about specific purpose such as relaxation, relief fever and for beauty.

P. amaryllifolius is a type of herbal and aroma plant which is used mostly as a flavoring agent for certain rice, herbal tea drinks and bakery products. It is a type of screw pine. The plant does not produce fruits as it undergoes vegetative propagation. The leaves are well known as multipurpose plants as it can be used in dried or fresh leaves form. *P. amaryllifolius* is well known as an aromatic plant which is giving a nutty flavor to the food, drinks and bakery products. The flavor is due to an aromatic compound called as 2-acetyl-1-pyrroline. Besides the aromatic compound, the leaves also have been reported to contain maltodextrin. The fresh leaves of *P. amaryllifolius* are tending to broken easily due to high moisture content. Dehydration is an important treatment of preserving the quality of leaves. Standardization of drying parameter is essential for produce quality leaves. During the hot session, the most common change that occurs is the loss of chlorophyll and aroma. The major challenge in food processing industries is the retention of the naturally occurring aromatic compound in thermally processed food. Therefore, the selection of proper drying treatment is necessary to reduce thermal stress and maintain the bioactive compounds that determine the quality of the product [4].

Therefore, the aim of this research is to study the effect of drying methods on the antioxidant activity and total phenolic compound using DPPH assays and Follin Ciocalteu's reagent.

METHOD AND MATERIAL

Plant material

The fresh leaves of *P. amaryllifolius* were collected from Ampang, Selangor. Only young matured, and good shape leaves were selected for further analysis.

Drying methods

The fresh *P. amaryllifolius* leaves were cleaned with clean water and subjected to two different types of drying methods; microwave and oven drying. The mass of 5 g leaves was weighed using an electronic balance before drying step. In this study, the effect of drying treatments was investigated; Oven drying in a forced convention conventional oven: 35□C for 48 h.Microwave drying using the microwave: 540 W for 4 minutes.

Reagents and materials

Ascorbic acid, Ethanol (HmbG), 1,1-Diphenyl-2-picrylhydrzyl radical (DPPH) (Sigma, USA), and Folin-Ciocalteu reagent

Equipment

Binder FD115 heating oven (Fisher Scientific Sdn Bhd, Selangor, Malaysia), XA 100/2X weighing balance (RADWAG Wagi Elektroniczne, Radom,

Poland) and GENEYS 20 spectrophotometer (Thermo Electron Corporation, Madison, USA).

Preparation of plant extract

The dried leaves for each drying methods were ground to a coarse powder. The powdered material was (5g) was macerated with ethanol for 3 days. The extract was filtered through filter paper and the filtrate was concentrated in a rotary evaporator. The concentrated extract was kept in the refrigerator for further analysis.

Determination of antioxidant activity

The antioxidant activity of *P. amaryllifolius* leaves was determined using the 1,1- diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay. A 0.1 mM DPPH solution was freshly prepared in absolute ethanol. The crude leaves extracts of *P. amaryllifolius* was mixed with absolute ethanol to prepare the stock solution (10mg/100ml). The concentration of extract of leaves extract solution was 100ug/ml. From stock solution, different concentration of extract between a range of 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml, 25µg/ml, 30µg/ml until 100µg/ml was taken in test tubes and undergo serial dilution with absolute ethanol up to 10 ml. 500µl of the freshly prepared DPPH solution was added in each of these test tubes. The samples were kept in the dark for 30 minutes at 37 □C. The absorbance was monitored at 517 nm using UV-Vis spectrophotometer. Ascorbic acid was used as the reference standard and dissolve in ethanol to make a stock solution. A control sample was prepared to contain the volume without any extract and reference ascorbic acid. Absolute ethanol was used as blank. The free radical scavenging activity (%Antiradical activity) was calculated using the formula:

% DPPH radical scavenging = [(Control Absorbance - Sample Absorbance) / (Control Absorbance)] x100

The % inhibitions were plotted against respective concentrations used for the graph, IC_{50} was calculated.

Determination of total phenolic content

Total phenolic content was investigated by using the Follin-Ciacalteu's reagent. The extract was prepared at a concentration of 1mg/ml of stock solution. Different concentration of the stock solution was transferred into the test tubes and 500µl of Follin-Ciacalteu reagent was added and mixed. The mixture was allowed to vortex for 30 s. Then, 1ml of 7.5% (w/v) sodium carbonate was added to the mixture and mixed gently. Subsequently, test tubes were kept for 30 minutes under dark conditions before measure absorbance by using UV-Vis spectrophotometer at 765nm. Gallic acid was used as the reference standard and dissolved with ethanol to make a stock standard solution. The standard calibration curve of Gallic acid was plotted. The results were calculated using the standard calibration curve of Gallic acid and expressed as Gallic acid equivalents (GAE mg/g).

$$\text{TPC} = \frac{(\text{Absorbance of sample} - \text{Intercept}) \times \text{Dilution factor}}{\text{Slope} \times \text{weight of extract}}$$

Determination of total chlorophyll content

1ml of the sample was extracted with 4ml of 80 % (v/v) acetone under the dark condition for 15 minutes. Then, the mixture was centrifuged at 2500 rpm for 10 minutes. The absorbance of the supernatant was read at 655 and 649 nm using a UV-visible spectrophotometer. The total chlorophyll content was calculated by the equation:

Total chlorophyll content (mg/l) = 6.45 (A655) + 17.72 (A649)

Where 6.45 and 17.72 are the factor for chlorophyll a and b respectively.

Qualitative tests: phytochemical screening

Phytochemical screening of different drying methods of *P. amaryllifolius* leaves demonstrated the presence of alkaloids, steroids, flavonoids, tannins, saponins, terpenoids, protein, fatty acids and phenolics by using standard methods with some modifications.

Test for phenolics

1ml of the plant extract, one drop of 5% of ferric chloride ($FeCl_3$) was added. Formation of greenish precipitate indicated the presence of phenolics.

Test for fixed oils and fatty acid

Prepared spot on the filter paper with the test solution and oil staining on the filter paper indicated the presence of fixed oil and fats.

Test for phlobatannins

Plant's leaves extract was filtered into the test tube. 1% of hydrochloric acid was added to the extract and boiled with hot plate stirrer. The formation of red coloured precipitate confirmed a positive result.

Test for proteins

2ml of Biuret reagent was added to the plant leaves extract. Shake well and warm it in a water bath. The appearance of red or violet colour indicates the presence of proteins.

Test for saponins

5ml of distilled water was added to the extract in a test tube. The solution was shaken vigourously and observed for a stable persistent froth. The presence of foaming observed indicated the presence of saponins.

Test for steroids

The crude extract was dissolved in 0.5ml dichloromethane to give a dilute solution and then 0.5ml of acetic anhydride added, followed by three drops of concentrated sulphuric acid. A blue-green coloration indicated the presence of steroids.

Test for tannins

The crude extract was dissolved and added to a test tube containing 20ml of boiling distilled water and then boiled for an hour. A few drops of ferric chloride was added and allowed to stand for proper colour formation. A blue-black colouration indicated the presence of tannins.

Test for terpenoids

The crude extract was added with 2ml of chloroform. 3ml of concentrated sulphuric acid (H_2SO_4) was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

Test for flavonoids

1 ml of sodium hydroxide will be added into the extract. The yellow colour will be turned colourless upon addition of a few drops of dilute hydrochloric acid. This will be indicated the presence of flavonoids.

RESULTS AND DISCUSSION

Total phenolic content of ethanol extract for two types of drying methods which are oven and microwave dried were determined with the Folin-Ciacalteu reagent. The Folin-Ciacalteu (F-C) reagent is sensitive to reducing compounds, polyphenols and thus produces a blue color complex. In alkaline medium, the F-C assays rely on the transfer of reducing equivalents (electrons) from phenolic compound to phosphomolybdic / phosphotungstic acid complexes, presented in the form of blue color complexes that are determined on a UV-visible spectrophotometer by setting the absorbance at 765nm. Gallic acid was used as the reference standard. Based on the measured absorbance, the concentration of phenolics was read (mg/ml) on the calibration line and the phenolics in the extract were expressed in terms of Gallic acid equivalent (mg of GA/g of extract).

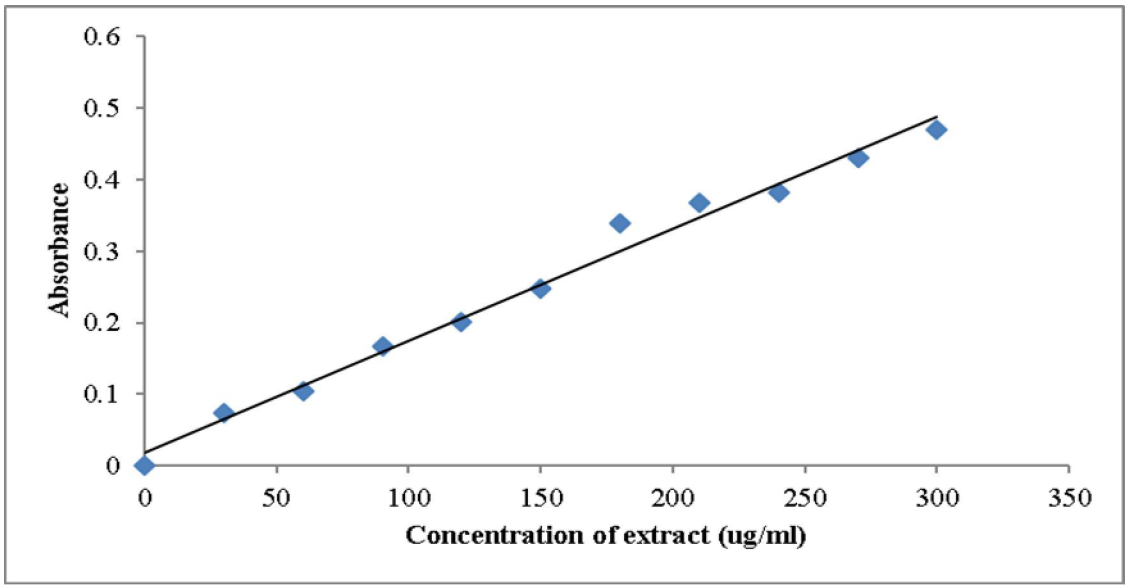

Figure 1: Standard calibration curve of Gallic acid for the determination of total phenolic content

Figure 1 shows mean TPC of plant leaves extracts measured using the GAE equation of y = 0.0016x + 0.0192, whereby x = concentration of plant leaves extracts in mg per ml and y = absorbance at 765nm. A linear calibration curve of Gallic acid with R^2 value of 0.9876 was obtained from the calibration-curve.

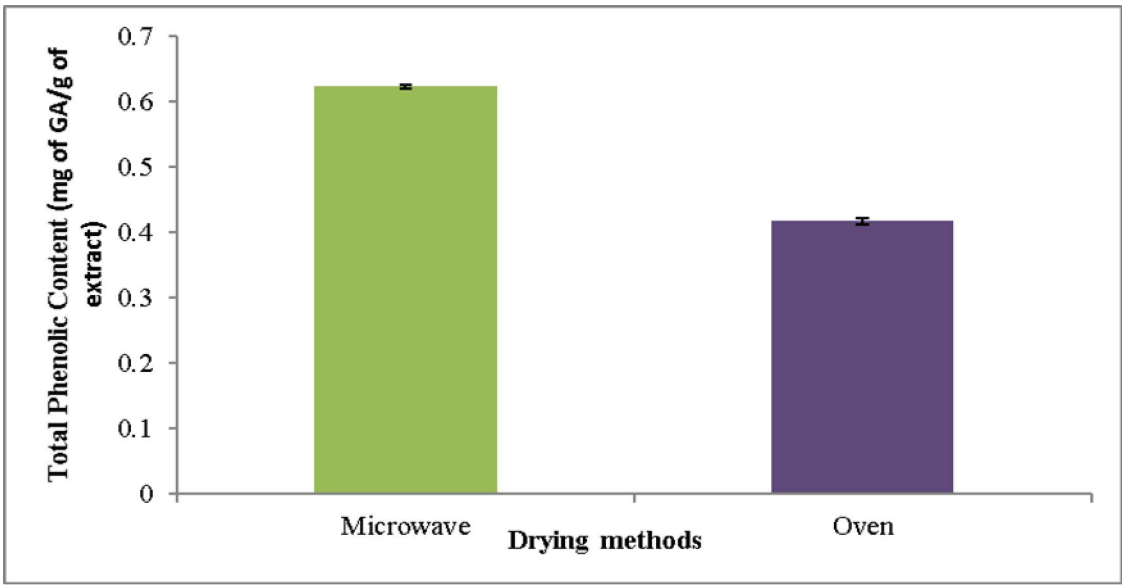

Figure 1: Total phenolic content of *P. amaryllifolius* tea for microwave and oven dried.

Based on figure 2, among the drying methods, a high content was observed in microwave drying in comparison with oven drying. Microwave drying shows the highest total phenolic content which is (0.623 mg/g) and the lowest followed by oven dried (0.417 mg/g).

There is some research work in some project regarding the level of phenolic content in dried *P. amaryllifolius* tea leaves, however, very few studies specify the drying methods. Many studies have reported the losses in phenolic content of dried leaves following thermal treatments. The significant losses mostly in plants and vegetables [5].

1, 1-Diphenyl-2-picryllhydrazyl radical scavenging assays are a widely used method for analyzing the antioxidant activity of plant extracts, since it can accommodate many samples in a short period and can detect the active ingredients at low concentration. The use of DPPH radical provides the fastest method to evaluate the radical scavengers and antioxidants. A freshly prepared DPPH solution exhibit a purple color with an absorption maximum at 517nm. The DPPH radicals were reacted with the reducing agents, the electron becomes paired off and solution loses color depending on the number of electrons taken up[2]. The decrease in absorbance of the DPPH radical caused by antioxidant was due to the scavenging of radical by hydrogen donation. It depends on the intrinsic antioxidant activity of antioxidant as well as on the speed of reaction between antioxidant and DPPH.

DPPH radical is stable free radical and when it reacts with antioxidants compound which can donate hydrogen, it is reduced to diphenylpicrylhydrazine. The changes in colour from deep-violet to light-yellow can be measured spectrophotometrically. Figure 3 shows the comparison of percentage DPPH radical inhibition between microwave and oven drying methods. All the results were reported based on the fresh sample after tea extraction. Based on the results, the test conducted for evaluating antioxidant activity in *P. amaryllifolius* tea leaves showed that the DPPH radical scavenging activity increased with the increase in the concentration of the extract for both drying treatments. The leaves extract exhibited antioxidant activity at all the concentration of test solutions. With the increasing concentration leaves tea extract (5-45 ug/ml), the percentage of antioxidant activity also increased from the ranges (5-59.8%).

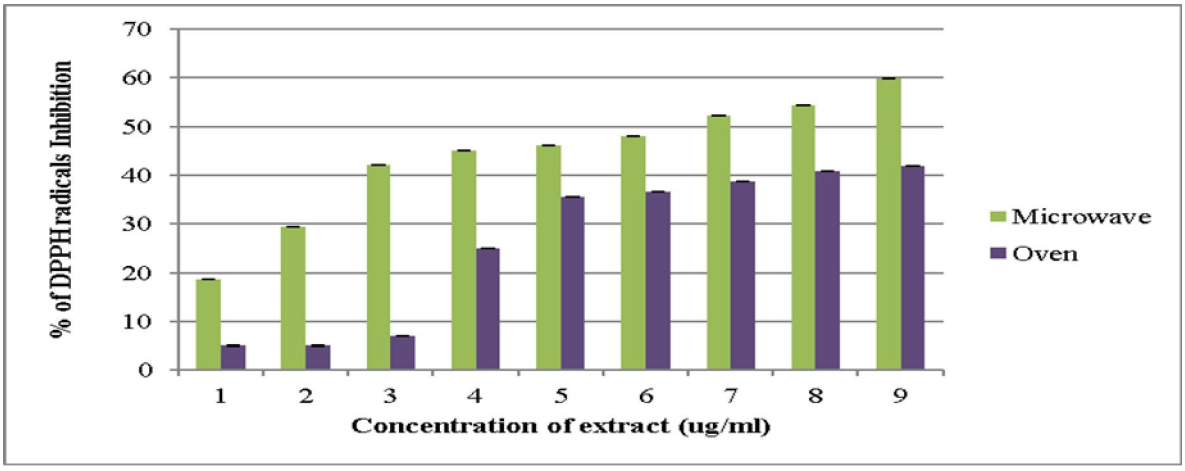

Figure 3: Comparative the antioxidant activity between microwave and oven dried *of P. amaryllifolius.* All the results were expressed in mean ± SD.

From the results above, microwave drying shows the highest percentage antioxidant activity compared to oven drying.

Heat treated leaves of *P. amaryllifolius* tea resulted in losses in the level of antioxidant properties compared to the fresh leaves. Losses in the level of antioxidant were in significant between two drying methods. Antioxidant properties of dried *P. amaryllifolius* leaves were adversely affected by microwave and oven drying. Declines in antioxidant properties have been attributed to the thermal degradation of phytochemicals, degradative enzyme, and loss of antioxidant activity[5].

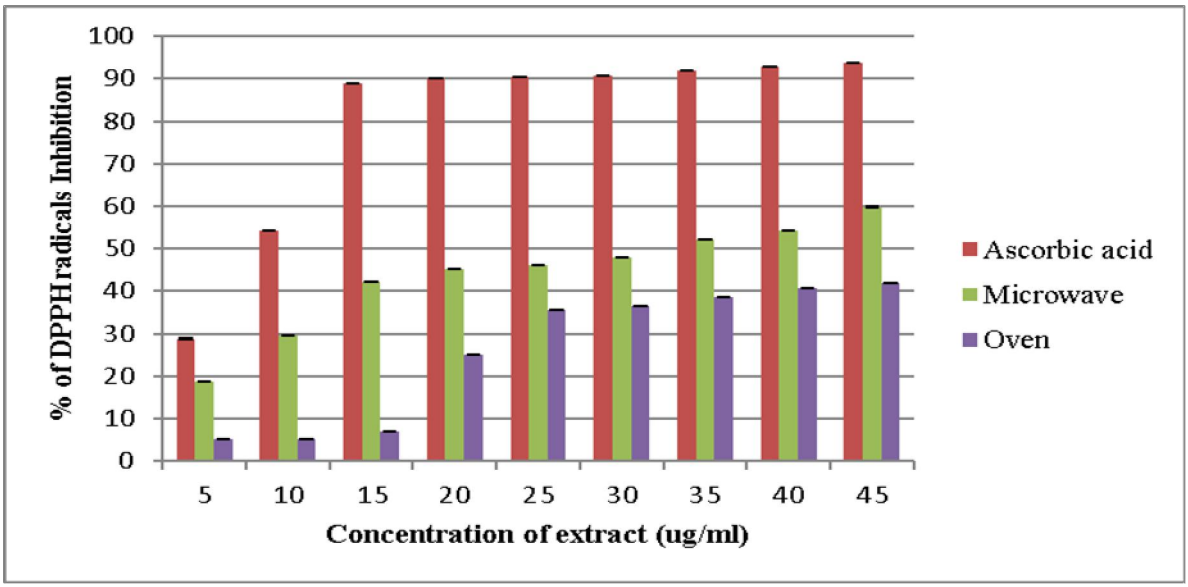

Figure 2: Antioxidant activities of different drying methods of *P. amaryllifolius* tea and standard on DPPH radical.

All the results were expressed in mean ± SD.

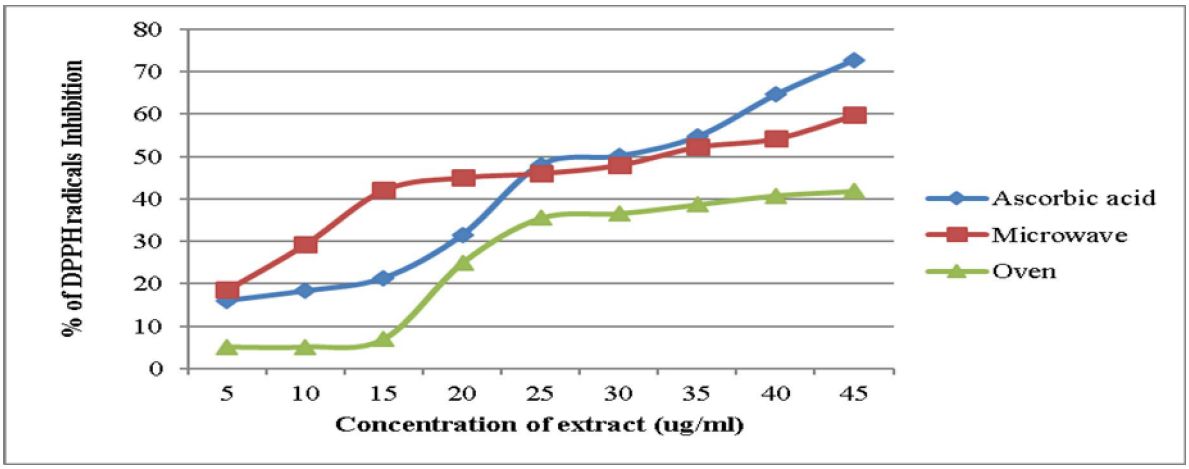

Figure 5: IC_{50} values of different types of drying of leaves of *P. amaryllifolius*

The antioxidant activity of ascorbic acid was shown in the figure above. The ascorbic acid showed the IC_{50} value of 6.0702 µg/ml followed by microwave and oven drying having an IC_{50} value of 6.379 µg/ml and 9.339 µg/ml respectively.

It means drying method extracts of the plant at higher concentration captured more free radicals form by DPPH resulting increasing IC_{50} value and decrease in absorbance. Ascorbic acid acting as a chain breaking antioxidant impairs with the formation of free radicals in the process of formation of intracellular substances throughout the body, including collagen, tooth dentine and bone matrix [6]. Phenolics and flavonoids are a major group of compounds that act as primary antioxidants or free radical scavengers. The quality and quantity of phytochemicals present in the plants may differ between drying methods. However, based on the results, the microwave and oven drying shows the same formation and indications. The presence of phenolics and flavonoid in the plant support the antioxidant, antimicrobial, and anticancer activities of *P. amaryllifolius* tea.

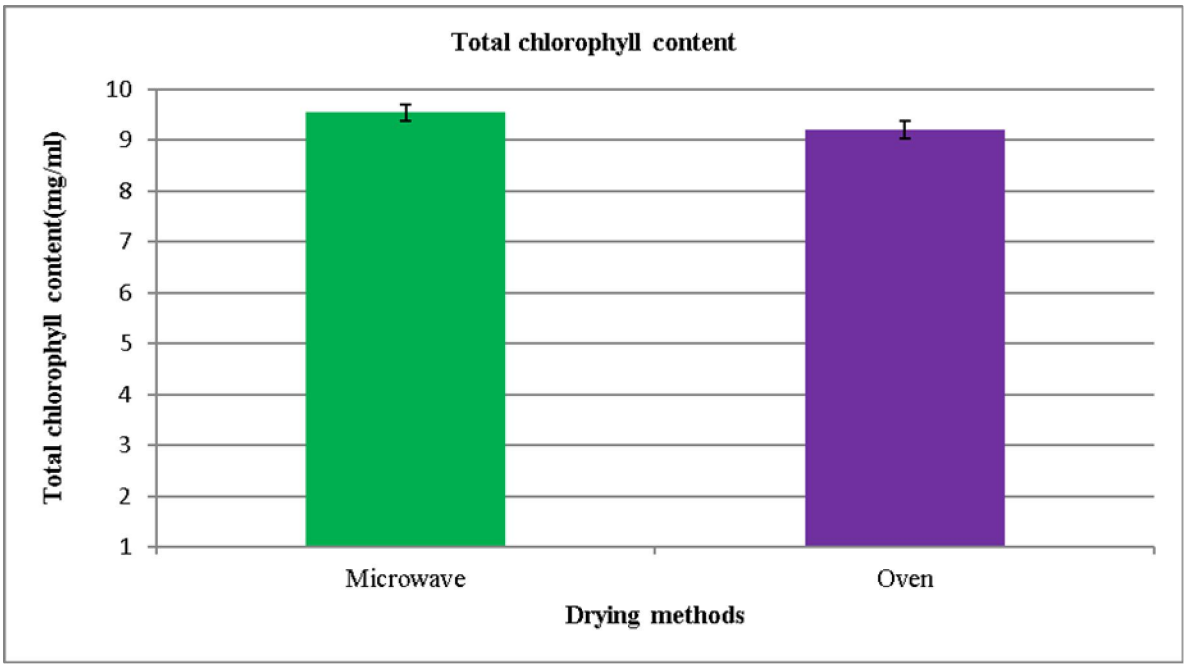

Figure 6: Content of chlorophylls in microwave and oven drying in mean ± SD

Based on figure 6, as can be seen in the figure above, the loss of chlorophyll content was found to be greatest in oven drying followed by microwave drying. The total chlorophyll content was 9.540 mg/g of microwave extract and 9.204 mg/g of oven extract. Therefore, there is only a small difference in chlorophyll content between the drying methods. In most of the cases, any of stress factors like radiation, intensity, light and drying time can lead the decreasing of chlorophyll content. This is reflected in the visual observations of different drying methods. One of the most pleasing attributes of drink and food are colour, the especially appearance of green in dried herb leaves. Thus, the changes in colour may alienate potential customer due to poorly controlled processing and this must be prevented and minimized. The drying time requirement of microwave drying is much lower than in comparison to oven drying and it is because of high rate mass transfer at higher temperature generated due to the electromagnetic field[7] . Studies found that determination of total chlorophyll content is not only important with

respect to the quality of the product but also for better preservation. In the present study, the least preferred sample in terms of colour was sun-dried sample. This is possibly because of its take longer time to dry and exposure to air which cause non-enzymatic browning thereby changing the chlorophyll (green) into a brown olive. In addition, it was observed that as drying temperature increased, chlorophyll degradation also increased. This may be due to chlorophyll is unstable compound difficult to maintain during drying process [8]. In this project, the time drying in the oven is much longer and the colour of green leaves was changed into brown olive but no changes in colour for microwave drying. Therefore, microwave drying methods are more suitable for drying of the leaves.

After performing the analysis of bioactive compound of *P. amaryllifolius* tea leaves extract, the results of the analysis from different drying methods are shown in table 1.

Table 1: Phytochemical analysis of *P. amaryllifolius* tea leaves extract

Phytochemicals	Drying methods		Indications
	Microwave	Oven	
Phenolics	Formation of greenish precipitate	Formation of greenish precipitate	Presence of phenolics
Fixed oils and fatty acid	Did not form of oil staining	Did not form of oil staining	Absence of fixed oils and fatty acid
Phlobatannins	Did not form of red precipitate	Did not form of red precipitate	Absence of phlobatannins
Proteins	Did not form of red and violet colour	Did not form of red and violet colour	Absence of proteins
Saponins	Formation of foaming	Formation of foaming	Presence of saponins
Steroids	Did not form of blue green colour	Did not form of blue green colour	Absence of steroids
Tannins	Did not form blue black colour	Did not form blue black colour	Absence of tannins
Terpenoids	Formation reddish brown colour	Formation reddish brown colour	Presence of terpenoids
Flavonoids	Formation of colorless colour	Formation of colorless colour	Presence of flavonoids

Table 1 presents the phytochemical analysis of pandan. The chemical compounds present in the *P. amaryllifolius* leaves extract are phenolics, saponins, and terpenoids. The presence of phenolics in this plant is likely to be responsible for the free radical scavenging effects observed. Phenolics and flavonoids are a major group of compounds that act as primary antioxidants or free radical scavengers. Furthermore, the presence of these phytochemicals contributes to the free radical scavenging activities of the plants. It had been reported that presence of phenolics, flavonoids, saponins and terpenoids have antimicrobial and anti-diarrheal potency [9]. The presence of these compounds in *P. samaryllifolius* tea indicates that the plants whether microwave or oven dried has medicinal potency and may have the ability as an anti-microbial, anti-diarrheal and anti-cancer.

CONCLUSIONS

Drying is a very useful technique to keep the bioactive compounds in the *P. amaryllifolius* tea extract and to produce an herbal tea with a high phenolic content and a potent antioxidant activity. The effect of drying methods on phenolic compositions, chlorophyll content and antioxidant properties of dried material was found. Therefore, in this project, the microwave drying method was revealed as the best drying to keep the bioactive compound in *P. amaryllifolius* tea

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CONFLICT OF INTEREST REPORTED: NIL; SOURCE OF FUNDING: NONE REPORTED