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STUDY OF PHYTOCHEMICAL CONTENT AND EVALUATION OF ANTIOXIDANT ACTIVITY OF SOME AROMATIC PLANT EXTRACTS

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ABSTRACT

Phytochemicals and antioxidants from plant sources are of increasing interest to consumers because of their roles in the maintenance of human health. Most of the secondary metabolites of herbs are used in a number of pharmaceutical products. Secondary metabolites composition and content of flavonoids and phenolic acids were evaluated and determined in Pandanus amaryllifolius and rosemary extracts by simple colorimetric methods. Total phenolic and total flavonoid content were determined using Folin-Ciocalteau and aluminum chloride colorimetric assay; The antioxidant activity of the extracts was determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assays.

KEYWORDS: Pandanus amaryllifolius, rosemary, flavonoids, Antioxidant activity, polyphenols.

1. INTRODCTION

Most plants are major sources of natural products used in pharmaceuticals, agrochemicals, flavour and fragrance ingredients, food additives and pesticides. Secondary metabolites are unique to a species or group, and they are important for defence, protection and competition.[1] Most of these compounds are commonly used as flavourings, medicines, or recreational drugs. Secondary metabolites are important in plant use by humans. Most pharmaceuticals are based on plant component structures, and secondary metabolites are widely used especially in Asia.[2] Phenolic compounds are famous group of secondary metabolites with wide pharmacological activities. Flavonoids are an important group of

secondary metabolites and are a source of bioactive compounds in plants.[3] They are also a kind of natural product with antioxidant properties capable of scavenging free superoxide radicals, having anti-aging properties as well as reducing the risk of cancer. Park et al.[4] showed that some flavonoid components in green tea are effective in inhibiting cancer or induce mechanisms that may kill cancer cells and inhibit tumor invasion. It was found that flavonoids reduced blood-lipids and glucose, and enhanced human immunity.[5,6] The effect of flavonoids on human health is the result of their ability to induce human protective enzyme systems.[7] Several studies have suggested that flavonoids such as catechin and rutin are able to control cancer cell growth in the human body.[8-10]

Among the inventory of medicinal plants, rosemary (*Rosmarinus officinalis L.*) is a shrub of the Lamiaceae family, which is widely used in traditional medicine for its biological properties.[5] Pandan (Pandanus amaryllifolius Roxb.) is a tropical plant of the family Pandanaceae in the screw pine genus. Pandan leaf, often known as screw pine, because they resemble the pineapple with the spiral arrangement of long, narrow and strap-shaped green leaves.[11] The sweet and delightful flavour of pandan leaves, which is well-known as a source of natural flavouring, is widely used in various parts of South-East Asian countries including India. The aim of our work is to study the chemical composition of the essential oil of rosemary and Pandan leaves to demonstrate the wealth of these plants into polyphenols and evaluate the antioxidant of their extracts.

2.MATERIALS AND METHODS

2.1. Plant material: The rosemary and pandan leaves available in local markets were collected and shade dried and was cut into small pieces. These were used for further analysis.

2.2. Extraction of essential oil: Essential oil of *Rosmarinus officinalis L.* or Pandanus amaryllifolius Roxb. were extracted by hydrodistillation using a Clevenger apparatus type. A flask of 2 L is charged with 250 g of freshly harvested plant material and distilled water (about 2/3 of the flask), the whole is boiled for 2 hours. After extraction, the obtained condensate is constituted by the essential oil, immiscible with water of yellow color, and the hydrosol of white color loaded by soluble compounds (or partially soluble) in water which can be concentrated by performing a salting out.

2.3. Phytochemical tests: To realize the phytochemical tests, extracts of rosmary or pandanus are diluted in suitable solvents.

2.4. Flavonoids: The presence or absence of the flavonoids in extract can be detected by a simple and rapid test with magnesium. This test involves putting 5 cm^3 of each extract in a test tube, 1 cm^3 of concentrated HCl and 0.5 g of magnesium turnings are added. The color changes to red or pink indicates the presence of flavonoids.[8]

2.5. Saponins: The saponins are characterized by a foam index. In a test tube, a little water is added to 2 cm^3 of extract, stirred well and the mixture is allowed to stand for 20 mins. The content of saponin is measured by measuring the height of the foam formed.[8]

- No foam: negative test (-)
- Foam height lower than 1 cm: weak positive test (+)
- Foam height between 1 and 2 cm: positive (++)
- Foam height over than 2 cm: very positive test (+++)

2.6. Quantitative estimation of total polyphenols: The quantitative determination of polyphenols was done with Singleton and Rossi methods.[10] To 0.5 ml of each sample dilution or gallic acid (used as a standard), 1 cm^3 of Folin-Ciocalteu reagent is added and diluted 10 times. After 2 min, 0.8 cm^3 of sodium carbonate solution (7.5%, w/v) was added and the whole was incubated for 30 min in darkness at room temperature. A reagent blank is prepared in parallel under the same conditions with distilled water instead of the extract solution, and the absorbance of each solution is determined at 765 nm. The same procedure is repeated to all standard Gallic acid solutions (0 – 0.25 mg/ cm^3). The reading of the absorption allows to determining the concentration of polyphenols in each solution with reference to a calibration graph plotted from gallic acid solutions previously prepared. The total phenolic content is expressed as milligrams of gallic acid equivalents per gram of dry matter (mg GAE/g DM).

2.7. Quantitative estimation of flavonoids content: The flavonoid content was determined by colorimetry according to the method of Zhishen et al.[12] 1 cm^3 of each sample dilution or of the quercetin is mixed with 3 cm^3 of distilled water. After stirring, a volume of 0.3 cm^3 of sodium nitrite solution (5%, w/v) is added and the mixture was stirred well for 5 min, 0.2 cm^3 of aluminium chloride solution (10% w/v) is added, the whole is incubated in darkness at room temperature for 30 min, and then 0.5 cm^3 of sodium hydroxide is added to the mixture. The absorbance of the solution is measured at 510 nm. In this case the calibration straight is plotted from the quercetin solutions (0 - 0.5 mg/ cm^3) previously prepared. The total

flavonoids content is expressed as milligrams of quercetin equivalents per gram of dry matter (mg QE/g DM).

2.8. Evaluation of antioxidant activity: The antioxidant activity of different extracts was studied by the reduction method of DPPH• (1,1-diphenyl-2-picrylhydrazyl), described by Sanchez-Moreno.[13] This, solutions with increasing concentrations are prepared by dilution for various extracts and ascorbic acid (0 - 0.15 mg/ cm^3) used as standard. The solution of DPPH• is prepared by solubilization of 6 mg of DPPH• in 200 cm^3 of ethanol.

To the small quantity of the extract in a test tube, 2 cm^3 of freshly prepared solution of DPPH is added. A negative control (or blank) is prepared in parallel with ethanol. Both solutions are then incubated in the dark for 30 min and the absorbance is measured at 517 nm. Three optical density measurements were determined for each solution.

The evaluation of the antioxidant activity is expressed as percentage inhibition of the DPPH radical according to the following relationship.[14]

Percent (%) inhibition of DPPH activity = $[(A_0 - A_1)/A_0] \times 100$ %

Where A_0 is the absorbance value of the blank sample or control reaction, and A_1 is the absorbance value of the test sample.

This formula allows to draw the straight line which represents the variation of inhibition percentage as a function of the different concentrations of each sample (y = ax + b). From the straight line, it is possible to deduce the concentration which can reduces 50% of DPPH• for each sample studied and for ascorbic acid. This concentration called ***IC50*** is generally calculated according to the following equation

IC50 = (50 – b)/a

Where ***IC50*** is concentration required to reduces 50% of DPPH•, ***a*** is the slope and ***b*** is the intercept.

3. RESULTS AND DISCUSSION

3.1. Quantitative results for extractions: The effects of operating parameters such as drying conditions and extraction time on the quantity and quality of essential oils extracted by means of steam extraction were studied using rosemary and pandan leaves as the plant material. The aspects of the results studied here include both quantitative and qualitative analyses obtained by laboratory analysis and colorimetric methods. The variations in quantity

of volatile compounds which may be driven by steam are influenced by various factors such as the flowering period, the soil, the climate[15] and the extraction method.[16] The yield of hydrosol (0.30%) is less than that of essential oil. It is constituted of soluble compounds or partially soluble in water isolated by salting out. There is thus obtained 0.85% of volatile products.

Phytochemical tests: The phytochemical tests, carried out on polar extracts of rosemary and pandan leaf extracts, gave the results presented in Table 1.

Table. 1: Results of phytochemical tests (+): presence (-): absence.

	Rosemary leaf extracts			Pandan leaf extracts		
	Flavonoids	**Tannins**	**Saponosids**	**Flavonoids**	**Tannins**	**Saponosids**
Aqueous extract	+ (Red)	+ (Blue)	+++	+ (Red)	+ (Blue)	+++
Essential oil	-	+ (Green)	+++	-	+ (Green)	+++

3.2. Phenolic compounds content: From the regression equation (y = 6.812x + 0.038, R2 = 0.989) of the calibration graph drawn from a series of increasing concentration solutions of gallic acid, the content of total polyphenols in each extract have been calculated. This value is expressed in milligrams of gallic acid equivalents per gram of dry matter (mg GAE/g DM).

The quantification of flavonoids is obtained through the calibration graph, which is the regression equation (y = 1.575x-0.008, R2 = 0.989) formed by a primary standard "quercetin" at different concentrations. Only the methanol extracts responded to phytochemical test of flavonoids in this assay. The value of the flavonoids content is expressed in milligrams of quercetin equivalents per gram of dry matter (mg QE/g DM). The total polyphenols and the flavonoids content of each extract are presented in Table 2. These results show that methanol extracts provide the most important polyphenol levels in both extractions (over 70% against less than 30% for aqueous extracts). In total, more polyphenols obtained by steam distillation against that obtained by maceration. Which indicates, the higher yield obtained by this extraction method. The results of the quantitative determination of flavonoids extract show that methanol extract is richer in flavonoids compared to the aqueous extract. This shows that the extraction by steam distillation can extract higher amounts of flavonoids. Therefore, the steam distillation seems to be the best method of extracting polyphenols.

Table. 2: The total polyphenols and the flavonoids content in the extracts of rosemary and pandan leaves.

	Rosemary leaf extracts		Pandan leaf extracts	
	Polyphenols content (mg GAE/g DM)	Flavonoids content (mg QE/g DM)	Polyphenols content (mg GAE/g DM)	Flavonoids content (mg QE/g DM)
Aqueous extract	2.645 ± 0.088	2.209 ± 0.167	3.107 ± 0.057	2.576 ± 0.201
Essential oil	0.871 ± 0.096	-	1.415 ± 0.078	-

3.3. The antioxidant capacity of the extracts of rosemary and pandan leaves

Evaluation of the antioxidant activity of rosemary and pandan leaf extracts was performed by the DPPH• radical reduction test using the ascorbic acid as standard. The measurements of the absorbance of the various solutions prepared, are obtained using a colorimeter by following the reduction of this radical which is accompanied by the change of the purple color (DPPH•) to a yellow color (DPPH-H) measured at 517 nm.

The values obtained allowed us to plot the curves of the assay (absorbance versus concentrations) for ascorbic acid, rosemary and pandan extracts. These curves, having an exponential pace, are represented by two linear parts: a descending line representing the reduction of DPPH• radical and the horizontal one that indicates that reducing the DPPH• is total. These results show that ascorbic acid has a very powerful anti-radical activity with IC50 equal to 0.098 mg/cm^3 which is close to the reported value[17] which is of the order of 0.08 mg/cm^3. Among the five extracts, essential oil has the lowest antioxidant power.

Table. 3: IC50 values of rosemary and pandan.

	Rosemary leaf extracts			Pandan leaf extracts		
	Equation	R2 values	IC50 (mg/mL)	Equation	R2 values	IC50 (mg/mL)
Ascorbic acid	497.3x + 1.542	0.987	0.098			
Aqueous extract	357.7x + 1.271	0.986	0.247	347.7x + 1.256	0.978	0.267
Essential oil	0.475x + 0.861	0.896	110	0.502x + 0.869	0.897	114

It has been shown that antioxidant molecules such as ascorbic acid, flavonoids and tannins reduce and discolored DPPH• due to their ability to give hydrogen.[18] Therefore, the polyphenols in the extracts of rosemary and pandan are probably responsible for the antioxidant activity of these extracts. However, it seems that the high antioxidant activity of the aqueous extract is probably due to the significant presence of saponins in this extract, which have an antioxidant effect.[19]

4. CONCLUSION

In current study pandan and rosemary leaves extracts showed good potential of bioactive compounds such as flavonoids, tannins and polyphenols. The wide ranges of the secondary metabolites content and antioxidant activities of rosemary and pandan extracts could be due to many factors including locations, altitude, temperature, age of plant, climate and variation of plant variety. The ranges of phenolic acids and flavonoid content and antioxidant activity will be useful for standardization of rosemary and pandan extracts for further pharmaceutical productions. These results also show the possibility of increasing the content of natural antioxidants by optimizing the growing conditions of these plants. More information on other bioactive component of these plants would help us to establish a greater degree of accuracy on this matter.

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