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## Analyzing of the Antioxidant Activity, Flavonoid Content and Caffeine Content of Guava Leaves for Four Different Drying Methods

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## ABSTRACT

Effects of four different drying methods which are usually employed for the drying of tea leaves for getting different grades of tea leaves were studied in case of *Psidium gujava*, Natural drying method, Steam processing method, Temperature variation method, High temperature method. The first process was the conventional shade drying method, in all the process vacuum drying was involved in the final stages. Steam processing method was found to be more superior as compared to other methods.

Keywords: Guava leaves, Drying methods, Antioxidant, Caffeine content.



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#### INTRODUCTION

Degradation of active constituents in the presence of moisture content is seen in case of medicinal plants. Drying processes have been used since ancient times to preserve the chemical bioactive molecules produced in the plants the moisture content and the heat treatment seems to effect the active constituents composition in different ways thereby the need for the most cost effective and best drying method which can be used for the maximum yield of active constituents is necessary to be evaluated. These processes have been employed in case of tea leaves but this study was conducted on Guava leaves. The variation in the antioxidant activity Flavonoid and Caffeine content have been studied. The caffeine content can be used for establishing the anti-diabetic activity in further studies. Effect of different drying methods have been conducted on *Mentha piperita*, *Mentha spicata* and *Thymus vulgaris but the processes are different. This study involves unique drying method not studied earlier*.

**Aim**: To evaluate and establish the variations in the Antioxidant activity, Flavonoid and Caffeine content of *Psidium gujava* leaves by various drying methods.

#### MATERIALS AND METHOD

Guava leaves, Ammonium Molybdate, Sodium Hydrogen Orthophosphate, Sulphuric acid, L-Ascorbic acid, Quercetin, Sodium Nitrite, Aluminium Chloride, Caffeine, Sodium Carbonate, Distillation apparatus, Heating Mantle, Water bath, Magnetic Stirrer, UV-visible Spectrophotometer.

#### Methods:

#### Natural shade drying:

The leaves were shade dried for 6 days and then using a vacuum dryer further drying achieved for one hour at 60°C.

#### **Steam drying method:**

Steam was applied to the leaves using water bath and after one minute the leaves were then cooled and treated at 40°C for two hours followed by rolling for fifteen minutes. Using vacuum dryer further drying achieved for one hour at 60°C.

#### **Temperature variation Method**:

Successive treatment of the leaves with water at the temperature of 90°C for 15 second (20 ml water for every 1 g of the drug), and cold water at 18°C for 5 seconds. The drained leaves were treated at 40°C for two hours followed by rolling for fifteen minutes. Using vacuum dryer further drying achieved for one hour at 60°C

#### **High temperature drying**

The leaves were treated at 40°C for 4 hours coarsely grinded using a blender and fermented at 29°C-31°C, for 100 minutes and then using a vacuum dryer further drying achieved for one hour at 60°C.

#### Preparation of crude plant extract:

The dried plant materials were powdered using a grinder. About 10 g of the dried ground plant materials from each drying process were soaked in Methanol (100 ml) for 5 days at room temperature. The soaked material was stirred every 18 hours using a sterilized glass rod. The final extracts were passed through Whatman filter paper No.1. The filtrates obtained were concentrated using rotary vacuum evaporator at 40°C and stored at 4°C.

## Determination of the Antioxidant activity of the crude extract <sup>3-4</sup>

Increasing concentration of Ascorbic acid in the order 1, 8, 16, 24  $\mu$ g/ml were prepared and a standard calibration curve drawn using excel 2010. Ascorbic acid standard solution and reagent solution (198.7 mg Sodium hydrogen phosphate, 247.17 mg Ammonium molybdate and 0.16 ml of concentrated sulphuric acid made up to 5 ml with distilled water) 0.3 ml and 3.0 ml respectively we mixed. Similarly, 0.3 ml of the extract solution (1mg/ml) was taken and treated with 3.0 ml of the reagent solution and the mixture was incubated at 95 C for 1hour. Absorbance was measured at 695 nm and the antioxidant capacity expressed as ascorbic acid equivalent.

#### **Determination of Total Flavonoid content** <sup>5-6</sup>

Different concentrations of standard Quercetin (100, 150, 200, 250, 300)  $\mu$ g/ml was prepared in Methanol and 1mg/ml was prepared in distilled water. Standard/Extract (0.05 ml) was mixed with distilled water (1.35 ml), 5% w/v Sodium Nitrite (0.05 ml) and 10% w/v Aluminium Chloride (0.05 ml) and allowed to stand in dark for ten minutes. The absorbance was read thereafter at 510 nm using water 0.05 ml as blank.

## Analysis of Caffeine 7-9

10 g of the dried grinded guava leaves were taken and 0.3 g Sodium Carbonate (Na<sub>2</sub>CO<sub>3</sub>) and 25 ml water were mixed and allowed to stand in dark for fifteen minutes. The mixture was distilled at 30 °C after Chloroform (3.0 ml) was added. The distillate (0.08 ml), Hydrochloric acid (80  $\mu$ l/0.5 N) and distilled water (1.84 ml) were mixed and the absorbance was read at 274 nm.

#### **RESULTS AND DISCUSSION**

Ascorbic Acid(standard/Extract	Absorbance at 695 nm	Concentration in µg/ml	Report
0 μg/ml	0	NA	NA
1 μg/ml	0.0132	NA	NA

#### **Table 1: Determination of Antioxidant Activity**

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8 μg/ml	0.0249	NA	NA
16 µg/ml	0.0434	NA	NA
24 µg/ml	0.0702	NA	NA
Natural shade drying	0.0116	2.67 µg/ml	2.67mgAscorbic acid equivalent/100mg of extract
Steam drying method	0.0346	11.19 µg/ml	11.19mgAscorbic acid equivalent/100mg of extract
Temperature variation Method	0.008	1.33 µg/ml	1.33 mg Ascorbic acid equivalent/100mg of extract
High temperature drying	0.0186	5.26 µg/ml	5.26 mg Ascorbic acid equivalent/100mg of extract





The Natural shade drying gave the maximum Antioxidant activity in Psidium Guajava leaves.

Quercetin(302.236	Absorbance	Concentration	Report
g/mol) standard/Extract	at 510 nm	in µg/ml	
0 μg/ml	0	NA	NA
10 µg/ml	0.0056	NA	NA
20 µg/ml	0.0114	NA	NA
30 µg/ml	0.0238	NA	NA
40 µg/ml	0.0304	NA	NA
50 μg/ml	0.0422	NA	NA
Natural shade drying	0.0157	6.41 µg/ml	641.38µg(2.12mM) Quercetin equivalent/100mg of extract
Steam drying method	0.0175	7.03 µg/ml	703.44 μg (2.32mM)Quercetin equivalent/100mg of extract
Temperature variation Method	0.0143	5.93 µg/ml	593.10 μg (6.12mM) Quercetin equivalent/100mg of extract
High temperature drying	0.0129	5.44 µg/ml	544.83 μg (1.80mM) Quercetin equivalent/100mg of extract

Table 2:	Determination	of Flavonoid	content

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The Natural shade drying method gave the maximum Flavonoid content in Psidium Guajava leaves.



## Figure 2: The standard curve of Quercetin

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Caffeine standard/Extract	Absorbance 274 nm	Concentration in µg/ml	Report
0 μg/ml	0	NA	NA
0.8 µg/ml	0.1093	NA	NA
1.6 µg/ml	0.1643	NA	NA
$2.4 \mu g/ml$	0.2472	NA	NA
3.2µg/ml	0.2935		
Natural shade drying	0.2925	3.03 µg/ml	Approx. 75.86 µg /10 g of extract
Steam drying method	0.3363	3.51 µg/ml	Approx. 87.95 $\mu$ g /10 g of extract
Temperature variation Method	0.2854	2.95 µg/ml	Approx. 73.81 $\mu$ g /10 g of extract
High temperature drying	0.2394	2.44 µg/ml	Approx. $61.12 \ \mu g / 10 \ g \ of$ extract



## Figure 3: The standard curve of Caffeine

The Natural shade drying gave the maximum Caffeine content in Psidium Guajava leaves.

## CONCLUSION

The Natural shade drying showed maximum Flavonoid, Caffeine content and also highest antioxidant activity is seen.

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## REFERENCE

- H. Hajimehdipoor et.al. Comparative study on the effect of different methods of drying on phenolic content and antioxidant activity of some edible plants. IJSPR. Oct. 2012; 23(3): 3712-3716.
- Darfour Bernard et.al. The Effect of Different Drying Methods on the Phytochemicals and Radical Scavenging Activity of Ceylon Cinnamon (*Cinnamomum zeylanicum*) Plant Parts. European Journal of Medicinal Plants. 2014; 4(11): 1324-1335.
- Eliza J., Daisy P., Ignacimuthu S. Antioxidant activity of costunolide and eremanthin isolated from Costus speciosus (Koen ex. Retz) Sm. Chemico-biological interactions. 2010; 188(3):467-472.
- Rishi Pal, Mangal Sain Hooda, Anil Bhandari et al. Antioxidant potential and free radicals scavenging activity by pod extracts of Acacia Senegal Willd. International journal of pharmaceutical, chemical and biological sciences, 2012; 2(4): 500-506.

- Shoib A. Baba, Shahid A. Malik. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of Arisaema jacquemontii Blume. Journal of Taibah University for Science. 2015;9:449–454.
- Samidha Kamtekar, Vrushali Keer, Vijaya Patil. Estimation of Phenolic content, Flavonoid content, Antioxidant and Alpha amylase Inhibitory Activity of Marketed Polyherbal Formulation. Journal of Applied Pharmaceutical Science.2014; 4(9): 061-065.
- https://books.google.co.in/books?id=Rgs\_rVOceZwC&lpg=PA20&ots=Exayjki1\_L& dq=determination%20of%20caffeine%20at%20274%20nm&pg=PA20#v=onepage& q=determination%20of%20caffeine%20at%20274%20nm&f=false. Accessed on 22-09-2016.
- Igelige Gerald, David Ebuka Arthur, Adebiyi Adedayo. Determination of Caffeine In Beverages: A Review.AJER.2014; 3(8): 124-137.
- H. N. Wanyika et.al. Determination of caffeine content of tea and instant coffee brands found in the Kenyan market. African Journal of Food Science.2010; 4(6): 353-358.

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