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Contributed Paper

Effects of Three Drying Treatments on the Polyphenol Content, Antioxidant and Antimicrobial Properties of *Syzygium aromaticum* Extract

Suat-HianTan* [a] and Md. Rezaul Karim [b]

[a] Faculty of Industrial Sciences and Technology, University Malaysia Pahang, Lebuhraya Tun Razak, 26300 Gambang, Kuantan, Pahang Darul Makmur, Malaysia.

[b] Faculty of Science, Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi 6205, Bangladesh.

* Author for correspondence; e-mail: tshian@ump.edu.my; tansuathian@yahoo.com

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ABSTRACT

Clove (*Syzygium aromaticum*) is widely accepted as an ancient and most valuable spice of the Orient. Drying process often affects the antioxidant and antimicrobial properties of the spices and solvent has immense impact on the extractability of phytochemicals. This work explored the water extractability of the phenolics from 3 different tissues (unripe fruit, leaf and stem) of the Malaysian grown clove after 3 drying treatments. The study also evaluated the effects of 3 drying processes (microwave-, oven- and air-drying) on the antioxidant and antibacterial properties of the extracts. The fruit extract exhibited highest total phenolic contents (TPC), caffeoylquinic acid (CQA), total vitamin C contents and antibacterial activity, followed by leaf and stem. On the other hand, total flavonoid content (TFC) was highest in leaf extract, followed by fruit and stem. It was found that the polyphenol contents correlated well with their antioxidant activities as determined by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays. Microwave-drying was found to be the most effective to maintain the antioxidant and antibacterial properties of unripe fruit, leaf and stem of the plant. Methanolic extract of all 3 tissue extracts were found to be active against both Gram-positive and Gram-negative bacteria. The extract was found to be more active against *Pseudomonas aeroginosa*, *Escherichia coli* and *Staphylococcus aureus* compared to *Bacillus subtilis*. It can be concluded from the results that the microwave drying can be the method of choice to maintain the quality of the spice in terms of its antioxidant and antimicrobial properties. The results also demonstrated that water can be used effectively to extract the valuable antioxidants that are beneficial to human health.

Keywords: *Syzygium aromaticum*, antioxidant, antibacterial, microwave-drying

1. INTRODUCTION

The search for healing powers in plants is an idea of choice from the ancient times. Plant extracts have long been used by man to safeguard himself against certain diseases as well as to promote health. Recently, the demand for herbs and medicinal plants are increasing with the increasing number of people that are using herbal remedies in their daily life. It was reported that approximately 80% of the world's population, especially those in third world countries largely rely on herbs for maintaining their health [1]. Many aromatic, medicinal and spicy plants contain confirmed strong antioxidant possessing components [2]. Most of the medicinal plants may possess high antoxidant power due to the redox properties of phenolic compounds which act as reducing agents, hydrogen donors and singlet oxygen quenchers [3].

Syzygium aromaticum has been accepted as one of the most ancient and valuable spices of the Orient [4] and looked upon as a source of natural antioxidants. *S. aromaticum* (tropical cloves) is the champion of all the antioxidants known till date. Dorman et al. [5] reported that *S. aromaticum* exhibits powerful antioxidant activity that can be compared to the activities of butylated hydroxyanisole (BHA) and pyrogallol, the synthetic antioxidants.

Although there is a debate on the efficacy of different methods followed in different studies in assessing these antioxidants [6], it is beyond any doubt that different food materials possessing antioxidant compounds are contributing greatly to our health. In addition, lack of correlation between activities determined on the same material by different assays and between activities determined by the same assay in different laboratories might be the root of debate [7] but the necessity of the antioxidants in human health is undebatable. However, the required daily amount (RDA) of the antioxidants has not been confirmed yet [8]. It is worth mentionable that many available results are inconsistent, and their antioxidant capacity is improperly specified [6]. Inspite of these debates and questions, it is necessary to determine antioxidants or other medicinally potential constituents in relation to the processing or preservation of those plant materials which are being consumed regularly.

In addition, *S. aromaticum* plays an important role in medicine for its antibacterial, antiseptic and antibiotic properties. According to a study conducted by Shafi et al. [9], *S. aromaticum* possesses the antibacterial activity. Moreover, Muruganadan et al. [10] reported the anti-inflammatory activity of *S. aromaticum*.

Drying is one of the conventional preservation methods for extending the shelf life of different foods and spices retaining their nutrional quality. The study on the effects of various drying methods on the antioxidant and antibacterial properties of different tissues of *S. aromaticum* has not been reported previously. The aim of this study was to determine the effects of conventional and cheaper drying methods (microwave-, oven- and air-drying) on the antioxidant compounds of unripe fruits, leaves and stems of *S. aromaticum* and their *in-vitro* antioxidant capacity by using DPPH and FRAP assay methods. In addition, the correlation between antioxidant compounds and their antioxidant activity and the antibacterial activity of soxhlet extract of the tissues against Gram-positive and Gram-negative bacteria were also aimed at. Thus, the study represents the first systematic analysis of the effects of drying treatments on the contents of total phenolics, total flavonoid, caffeoylquinic acid, total vitamin C,

and on the antioxidant and antibacterial activities of different tissues of *S. aromaticum*. Besides, it has to prove that water extraction has high efficacy to extract the active compounds which the community can safely consume to fight against the reactive oxygen species.

2. MATERIALS AND METHODS

2.1 Sample Collection

Fresh leaves, stems and unripe fruit samples of *S. aromaticum* were collected from the orchard at Universiti Putra Malaysia (UPM) campus, Serdang, Malaysia. The handpicked samples were subjected to various drying treatments on the same day.

2.2 Drying Treatments

The samples were thoroughly washed 2-3 times with running tap water in order to clean debris and contaminants and the extra water was removed from the sample by using Moslin cloth before drying. Three different drying methods namely, microwave-, oven- and air-drying were employed. In microwave-drying, the samples were placed in a microwave oven (Triple distribution system, Samsung) and subjected to 800 W power input for 2 min . In oven-drying, the samples were placed in an oven (Memmert Model UN 55) at 40 °C for 24 h. In air-drying, the samples were air-dried for 72 h in the laboratory at ambient temperature of 25-30 °C. The drying process continued until a constant weight of the samples was obtained. The dried samples were powdered separately using blender (Pensonic PB-323GL) and were stored at room temperature for further analysis.

2.3 Sample Extraction

A 100 mg portion of each of the samples in triplicate was weighed out using an electronic balance (Mettler Toledo). Then, 25 ml of distilled water were added to each sample in a 50 ml conical flask and heated at 50-60 °C for 30 min. The extracts were filtered through Whatman No.1 filter paper. The filtrates were stored at 4 °C for further analysis of polyphenols and antioxidant activity. The soxhlet extraction method was also used to extract the samples for the determination of antibacterial activity. A 30 g portion of the powdered samples was extracted with 175 ml of methanol in a soxhlet extractor for 4 h and the extracts were stored at 4 °C for further use.

2.4 Extraction Efficiency

In order to determine the extraction efficiencies of boiling water the residues obtained after first extraction was extracted again for the second and third time. The extraction efficiency (in percentage) was calculated based on the total phenolic content of the first, second and third extractions.

2.5 Determination of Phenolic Compounds

Total phenolic content of the sample extracts was determined by the Folin-Ciocalteu assay [11] and the value was expressed as the amount in mg of gallic acid equivalent per 100 g dry weight. Briefly, 0.3 ml of the sample extract was added to 1.5 ml of 10 times diluted Folin Ciocalteu's reagent and 1.2 ml of 7.5% sodium carbonate, mixed well by vortex mixer and incubated for 30 min at room temperature. Absorbance was recorded at 765 nm. The assay was done in triplicate.

The total flavonoid content was determined in triplicate by a modified method reported by Tan et al, [12] and the value was expressed as mg of quercetin equivalent per 100 g sample dry weight. Briefly, 0.5 ml of the diluted sample was

added to 4.5 ml of distilled water and 0.3 ml of 5% sodium nitrite. After 5 min, 0.3 ml of 10% aluminium chloride was added. At 6 min, 2 ml of 1.0 M sodium hydroxide and 2.4 ml of distilled water were added and vortexed. The mixture was left at room temperature for 30 min. Absorbance was recorded at 510 nm.

Caffeoylquinic acid content of different sample extracts was assayed using the molybdate assay method [13]. The assay was done in triplicate and the value of caffeoylquinic acid was expressed as mg of the chlorogenic acid equivalent (CGAE) per 100 g of sample dry weight. A 0.3 ml portion of the extract was added to 2.7 ml of molybdate reagent and incubated for 10 min at room temperature and absorbance was measured at 370 nm.

The total vitamin C content in different tissue extracts of *S. aromaticum* was determined using a modified method of Davis and Masten [14] where 1 g of the dried sample was extracted with 5 ml of 1.0% phosphate citrate buffer (pH 3.5) in a mortar and pestle. The extract was centrifuged at 15,000 × g for 10 min at 4 °C. Then 1.0 ml of the supernatant was added to 0.2 ml of 1.72 mM 2,6-dichloroindophenol and the absorbance was read at 518 nm. The assay was performed in triplicate and the value expressed as mg of ascorbic acid equivalent per 100 g of sample dry weight.

2.6 Determination of Antioxidant Activity

The DPPH free radical-scavenging (FRS) assay was done following the method of Miliauskas et al. [15] with some modifications. An aliquot of 2.0 ml of 0.0059% DPPH was added to 1.0 ml of the diluted extract and incubated for 30 min in dark. Absorbance was recorded at 517 nm. The DPPH assay was conducted in triplicate. FRS ability was expressed as IC_{50} and the value was calculated as ascorbic acid equivalent antioxidant capacity (AEAC) in mg of ascorbic acid/ 100 g of sample using the formula given below:

$$\text{AEAC (mg AA/100g)} = IC_{50(\text{ ascorbate})} / IC_{50(\text{ extract})} \times 100{,}000$$

The FRAP assay was done by following the method as described by Chan et al. [11]. Briefly, 0.2 ml of the extract was added to 3.0 ml of FRAP reagent and incubated at 37 °C for 30 min. Absorbance was recorded at 593 nm. The assay was conducted in triplicate and the antioxidant capacity was expressed as percent and was calculated by using the equation below.

$$\text{Percent antioxidant (\%)} = [(A_{593} \text{ of sample } A_{593} \text{ of control}) / \text{A593 of sample}] \times 100$$

2.7 Determination of Antibacterial Activity

The Kirby-Bauer disc-diffusion method [16] was used to determine the antimicrobial activity of the soxhlet extracted sample. The paper disc (6 mm diameter) was dipped into a concentrated sample (0.5 g/ml in methanol). The disc was then transferred onto the inoculated agar. The disc impregnated with 1 mg/ml Streptomycin was used as a positive control and the disc immersed in methanol was used as a negative control. Then, the plate was inverted and incubated for 24 hours at 37 °C and the zone of inhibition was recorded and expressed as mm. Each test was carried out in triplicate and the mean value was calculated.

2.8 Statistical Analysis

All the experimental results were expressed as means ± standard deviation (SD).

The data were analyzed using one way ANOVA. The mean values were compared using Duncan's multiple range test at 5% ($p = 0.05$) significance level with the aid of Statistical Package for the Social Sciences (SPSS) software version 16.0 for windows.

3. RESULTS AND DISCUSSION

3.1 Effect of Drying on Polyphenol Extractability

Polyphenol content of various tissues of *S. aromaticum* has been reported previously [17], but there is no report on the effect of drying treatments on polyphenol content of various tissues, especially the unripe fruit. The data in Table 1 demonstrate the effect of drying treatments on the polyphenol content and the efficiency of warm water (50-60 °C) as a solvent for the extraction of polyphenolics from different tissues. It was evident from the data that the applied drying processes did not affect the polyphenol content and the extractability of polyphenols by water. However, microwave dried sample gave slightly higher yield of polyphenol compared to oven- and air-dried samples. Similar results were reported by Chan et al. [11], for *Etlingera elatior* leaves (ginger species) extract. Lin et al. [18] reported similar results for green tea.

Table 1. Effect of drying on polyphenol content and water extraction efficiency of different tissue of *S. aromaticum*.

Drying Treatment	Tissue	Extraction	TPC (mg GAE/100g)	Yield (100%)
Microwave	Leaves	1st	264.93 ± 15.46	86.74 ± 4.43
		2nd	36.43 ± 0.18	11.72 ± 0.06
		3rd	9.63 ± 0.21	3.10 ± 0.07
	Stems	1st	252.19 ± 2.17	89.14 ± 0.77
		2nd	22.36 ± 0.20	7.90 ± 0.07
		3rd	8.37 ± 0.38	2.96 ± 0.14
	Fruits	1st	325.19 ± 1.54	86.80 ± 0.41
		2nd	38.71 ± 1.12	10.33 ± 0.30
		3rd	10.74 ± 0.59	2.87 ± 0.16
Air	Leaves	1st	228.00 ± 4.12	87.44 ± 1.58
		2nd	24.80 ± 0.18	9.51 ± 0.07
		3rd	7.95 ± 0.15	3.05 ± 0.06
	Stems	1st	213.05 ± 2.07	88.35 ± 0.86
		2nd	20.52 ± 0.28	8.51 ± 0.12
		3rd	7.56 ± 0.09	3.31 ± 0.04
	Fruits	1st	319.57 ± 0.87	90.26 ± 0.25
		2nd	25.88 ± 0.21	7.31 ± 0.06
		3rd	8.59 ± 0.04	2.43 ± 0.01
Oven	Leaves	1st	252.52 ± 2.21	86.86 ± 0.76
		2nd	29.76 ± 0.06	10.23 ± 0.02
		3rd	8.46 ± 0.19	2.91 ± 0.06
	Stems	1st	216.24 ± 6.38	88.06 ± 2.60
		2nd	21.49 ± 0.43	8.75 ± 0.17
		3rd	7.84 ± 0.08	3.19 ± 0.03
	Fruits	1st	323.76 ± 3.75	88.63 ± 1.03
		2nd	32.21 ± 0.36	8.82 ± 0.10
		3rd	9.32 ± 0.15	2.55 ± 0.04

Values of total phenolic content (TPC) are means ± SD (n = 3).

Water is a solvent with the highest degree of polarity and the polyphenols possessing phenolic group contribute to the polarity of the compound. There are so many polar compounds other than polyphenols in a plant sample. Water, being the cheapest and nontoxic solvent, gets the preference over other solvents in extracting phytochemicals of biological importance. This article reports the extraction efficiency of water on the phenolic compounds of 3 dried samples. Furthermore, the study of efficiency of water to extract *S. aromaticum* is useful to justify its action on the human body as it contains 70% water of its total body weight. More than 87% of the total phenol was extracted in the first step. The first extraction gave higher yield compared to second and third steps. It was evident from the data that 3 times extraction ensured almost a complete extraction of the total phenolics of the tissues. In addition, the unripe fruits retained the highest phenolic content in all the extractions regardless of their drying process. Thus, hot water extraction of the clove was found effective enough to extract the secondary metabolites as it could yield more than 87% of the total phenolics in the first step. Of course aqueous alcoholic extraction can show better performance. Because, the polarity of aqueous -alcohol is modified through mixing of two solvents of different degrees of polarity. Our target was to assess the effect of conventional drying process of the spices on their bioactive compounds such as polyphenols and their antioxidant and antimicrobial properties. Therefore, we ignored this aqua-methanol solvent.

3.2 Antioxidant Species

This is, for the first time, a systematic analysis of different antioxidant species present in different tissues of *S. aromaticum* after 3 different drying treatments. It is evident from the data in Table 2 that the total phenolic content was the highest in microwave-dried fruit followed by oven-dried leaf. Stem demonstrated the lowest phenolic content irrespective of drying treatment. It is also evident from the data that the microwave dried leaf, stem and unripe fruit exhibited higher flavonoid content compared to that of oven- and air-dried tissues of *S. aromaticum*. Banji and Adebayo reported that air-dried Clove bud extracted with methanol or with acetone demonstrated higher flavonoid content than its aqueous extract [25]. They also demonstrated that the flavonoid content of the bud tissue is higher than its phenolic content, which is contrary to our findings. Flavonoid is less polar than phenolics and methanol is also less polar solvent than water. Therefore, it is logical that flavonoid be extracted better in methanol than the phenolics in water. According to Samanta et al. [19], flavonoids play an important role in the formation of remarkable colors in flowers and fruits. The pigments responsible for the green color of leaves and unripe fruits are chlorophyll. Since chlorophyll pigment also contribute to color reaction for flavonoid, leaves gave higher values of total flavanoid content. The *S. aromaticum* grown in Malaysia contained lesser amount of total flavanoid compared to its total phenolics which is in agrement with the results reported for the same specie grown in Nigeria [20].

The highest caffeoylquinic acid content was found in fruit followed by leaf and stem regardless of the drying treatments (Table 2). However, microwave-dried samples retained higher amount of caffeoylquinic acid compared to that of oven- and air-dried samples. Interestingly, caffeoylquinic acid content was found to increase or decrease proportionately with the total phenolic

content of all extracts. In general, caffeoylquinic acid can be categorized under the phenolic acids. This is because, one of the phenolic acid categories is the hydroxycinnamic acid which is made up of caffeic and quinic acids [21]. Later, these caffeic and quinic acids react to form caffeoylquinic acid, an ester [13]. Thus, most of the fruits and vegetables that contain higher phenolic content also exhibit a significantly high amount of caffeoylquinic acid naturally. Chan et al. [13] reported that the caffeoylquinic acid content for all the five leaf extracts of *Etlingera* species were proportional to their total phenolic content.

Table 2. Antioxidant compounds of unripe fruits, leaves and stems of *S. aromaticum*.

Drying Treatment	Tissue	TPC (mg GAE/100g)	TFC (mg QE/100g)	CQAC (mg CGAE/100g)	Total Vitamin C Content (mg AA/100g)
Microwave	Leaves	264.93 ± 15.46^{b}	2.35 ± 0.08^{a}	74.79 ± 3.45^{bc}	8.58 ± 0.06^{e}
	Stems	252.19 ± 2.17^{c}	1.84 ± 0.03^{d}	60.13 ± 2.05^{d}	6.98 ± 0.05^{g}
	Fruits	325.19 ± 1.54^{a}	2.23 ± 0.05^{ab}	86.71 ± 3.09^{a}	9.47 ± 0.02^{a}
Air	Leaves	228.00 ± 4.12^{d}	1.89 ± 0.11^{cd}	56.71 ± 1.73^{d}	8.14 ± 0.05^{f}
	Stems	213.05 ± 2.07^{e}	1.05 ± 0.27^{f}	55.46 ± 1.71^{d}	6.59 ± 0.04^{i}
	Fruits	319.57 ± 0.87^{a}	1.50 ± 0.17^{e}	72.63 ± 1.44^{c}	8.88 ± 0.04^{c}
Oven	Leaves	252.52 ± 2.21^{c}	2.09 ± 0.05^{bc}	73.04 ± 2.02^{c}	8.72 ± 0.05^{d}
	Stems	216.24 ± 6.38^{e}	1.70 ± 0.06^{de}	59.25 ± 2.88^{d}	6.78 ± 0.04^{h}
	Fruits	323.76 ± 3.75^{a}	1.74 ± 0.10^{d}	80.00 ± 7.73^{b}	9.05 ± 0.04^{b}

Note: Values of total phenolic content (TPC), total flavonoid content (TFC), caffeoylquinic acid content (CQAC) and total vitamin C are means ± SD (n = 3). The different letters within the column indicate that the values are significantly different ($p \leq 0.05$).

The fruit also exhibited the highest vitamin C followed by leaves regardless of the drying treatments (Table 2). However, the oven-dried leaves contained highest vitamin C followed by microwave- and air-dried fruits. The stems contained the lowest vitamin C where the amount is highest for microwave-drying followed by oven- and air-drying treatments. Vitamin C is a heat labile compound and microwave drying generates higher heat than oven (set at 40 °C). That may be the reason for lower vitamin C content in microwave-drying than that in oven-drying. However, despite the lowest temperature, air-drying takes longer time to dry the samples that might cause a loss of the vitamin C during air-drying. Leena and Uptala [4] reported that the amount of vitamin C in *S. aromaticum* is 80.81mg/100 g while, Bhowmik et al. [22] reported the amount to be 11.7 mg/100 g. Vitamin C is very sensitive to heat and light that may affect its content during extraction and assay. Therefore, reports on the vitamin C content of the same plant material may vary depending on the experimental procedure and handling process.

3.3 Antioxidant Activity

The antioxidant activity of the tissue extracts was determined using two *in vitro* antioxidant activity assays (Table 3). In FRAP assay, antioxidant activity was expressed as percent. It was found that the unripe fruit

extract exhibited the highest antioxidant capacity regardless of the drying treatments, followed by the leaf and stem. On the other hand, microwave-drying retained higher percentage of antioxidant capacity compared to that of oven- and air-drying treatments. A good antioxidant capacity of *S. aromaticum* extracts was demonstrated in many other reports by using FRAP assay method [23, 24], but the effect of drying on the FRAP activity of different tissues is reported for the first time in this study. Contrary to our findings, Banji and Adebayo reported that air-dried Clove bud extracted with methanol or with acetone demonstrated higher antioxidant capacity than that of its aqueous extract [25]. This variation may be due to the difference in the antioxidant species extracted in different solvents. According to Hossain et al. [23], the efficient hydrogen-donating ability and metal-chelating property resulted in a good antioxidant activity of *S. aromaticum* extracts.

Table 3. Antioxidant activities of unripe fruits, leaves and stems of *S. aromaticum*.

Drying Treatment	Tissue	FRAP (% of antioxidant)	DPPH IC_{50} (mg/mL)	DPPH AEAC (g AA/100g)
Microwave	Leaves	81.89 ± 0.07^{b}	0.002557	105.24 ± 4.16^{ab}
	Stems	80.19 ± 0.06^{e}	0.002626	102.38 ± 1.03^{abc}
	Fruits	82.19 ± 0.07^{a}	0.002456	109.58 ± 4.67^{a}
Air	Leaves	79.56 ± 0.18^{f}	0.003293	82.30 ± 9.19^{d}
	Stems	78.46 ± 0.03^{g}	0.004455	63.91 ± 19.96^{e}
	Fruits	80.37 ± 0.09^{d}	0.003151	85.91 ± 9.00^{cd}
Oven	Leaves	81.38 ± 0.09^{c}	0.002968	90.91 ± 6.99^{bcd}
	Stems	80.08 ± 0.16^{e}	0.003027	89.20 ± 7.50^{bcd}
	Fruits	81.91 ± 0.11^{b}	0.002759	97.56 ± 4.30^{bcd}

Note: Values of ferric reducing antioxidant power (FRAP) and ascorbic acid equivalent antioxidant capacity (AEAC) are means ± SD (n = 3). The different letters within thecolumn indicate that the values are significantly different ($p \leq 0.05$).

The antioxidant activity of the tissue extracts determined by DPPH assay reveals that the fruits possessed the highest ascorbic acid equivalent antioxidant capacity (AEAC), followed by leaf and stem regardless of the drying treatments (Table 3). The results also demonstrated that microwave-dried samples exhibited higher free radical scavenging activity compared to that of oven- and air-dried samples. The IC_{50} values of the extracts were compared with that of ascorbic acid. Based on the results, microwave-dried fruit, leaf and stem exhibited lower IC_{50} value than that of ascorbic acid. Lower IC_{50} value means higher AEAC [24], indicating that microwave-dried sample extracts were stronger antioxidants than that of ascorbic acid. Kim et al. [26] reported that the hot water (80-100 °C) extracted *S. aromaticum* exhibited the highest DPPH free radical scavenging activity (84.22%) among all 13 plant spices analyzed. A correlation study is also done on the content of the antioxidant species and their antioxidative capacity and the data are presented in Table 4. This study clearly showed that the antioxidant compounds correlated well with the antioxidant activities (Table 4).

The results obtained were comparable with the ginger species, where the total phenolics content was directly proportional to the AEAC; the higher the total phenolics content, the higher the AEAC of the extracts [11].

Table 4. Correlation between antioxidant compounds and antioxidant activities of unripe fruits, leaves and stems of *S. aromaticum*.

Correlation	TPC (mg GAE/100g)	TFC (mgQE/100g)	CQAC (mg CGAE/100g)	Total Vitamin C Content (mg AA /100g)
FRAP (% of antioxidant)	0.721	0.768	0.888	0.796
AEAC (mg AA/100g)	0.506	0.716	0.513	0.475

Note: TPC (total phenolic content), TFC (total flavonoid content), CQAC (caffeoylquinic acid content), GAE (galic acid equivalent), CGAE (chlorogenic acid equivalent), AA (ascorbic acid), FRAP (ferric reduction antioxidant power), AEAC (ascorbic acid equivalent antioxidant capacity).

3.4 Antimicrobial Activity

Effect of drying on the antimicrobial properties of the methanolic extracts has also been analysed. For the assay of antimicrobial activity against Gram-positive and Gram-negative bacteria the tissues were extracted in methanol by soxhlet and the results are presented in Table 5. The extracts showed a significant inhibition against both types of bacteria. However, the inhibition was found to be more effective against *S. aureus*, *E.coli* and *P. aeroginosa* compared to *B. subtilis* (Table 5). Pandey and Singh [27] also reported that the *S. aromaticum* extracted with methanol was able to inhibit *S. aureus*, *P. aeroginosa* and *E. coli*. Similar results were obtained by El-Chaghaby et al. [28] for the antibacterial activities of *Annona squamosa* L. leaves. Methanol, being a moderately polar solvent, can extract some less polar components capable of demonstrating antimicrobial properties. However, the water extracted samples in this study showed no inhibition against any type of bacteria (data not shown). The reason for the lack of inhibition is not clear. However, we assume that the antimicrobial agent present in methanol extract might be less polar or nonpolar and are absent in the water extract. Similar results were reported by El-Chaghaby et al. [27] for water extract of *Annona squamosa* L. leaves against the organisms tested except *Streptococcus faecalis* and *E. coli*. Another report by Shaikh et al. [29] revealed that water extract obtained from dried *Cichorium intybus* seeds was active against *S. aureus* among four tested microorganisms. Khalid and Kiong [30] also reported that *S. aromaticum* oil exhibited appreciable antibacterial activity against *E. coli, Salmonella spp*, *Klebsiella pneumoniae*, *S. aureus*, *Streptococcus* and *B. subtilis*.

Table 5. Antibacterial activity of soxhlet-extracted unripe fruits, leaves and stems of *S. aromaticum* against Gram-positive and Gram-negative bacteria.

Drying Treatment	Tissue	Zone of inhibition (mm)			
		Gram-positive bacteria		Gram-negative bacteria	
		B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*
Microwave	Leaves	4.63 (++)	10.47 (+++)	5.50 (++)	9.1 (+++)
	Stems	3.50 (++)	9.67 (+++)	5.37 (++)	8.4 (+++)
	Fruits	5.30 (+++)	12.33 (+++)	6.43 (++)	10.0 (+++)
Air	Leaves	3.30 (+)	7.50 (+++)	5.10 (++)	8.2 (+++)
	Stems	2.30 (+)	7.43 (+++)	4.37 (++)	8.0 (+++)
	Fruits	3.90 (++)	10.00 (+++)	5.50 (++)	9.0 (+++)
Oven	Leaves	3.80 (++)	9.33 (+++)	5.17 (++)	8.7 (+++)
	Stems	3.43 (+)	10.00 (+++)	5.07 (++)	8.0 (+++)
	Fruits	4.63 (++)	11.63 (+++)	5.77 (+++)	10.0 (+++)
Streptomycin		7.06	8.80	8.15	9.62
Methanol		-	-	-	-

Note: The antibacterial activity of the extracts was classified as strong (+++), moderate (++) and weak (+).

Based on the results, it can be concluded that the microwave- and oven-dried tissues retained most potential antimicrobial compounds compared to that of air-dried ones. Again, fruit extract was found to be the most potent compared to those of leaf and stem irrespective of drying treatments. According to Kamatao et al., [31] eugenol is the main consistituent of *S. aromaticum* that demonstrates antioxidant and antibacterial activities.

Overall, microwave- and oven-drying were found to retain higher antioxidant compounds and antimicrobial activity as well. It can be assumed that shorter drying treatments give better quality through quick drying. Air drying takes longer time that causes deterioration of the bioactive components. This is supported by the premilinary results on the stability of the phenolics during storage (data not shown). Phenolic content started to decline after 3 days of storage at 4 °C and room temperature. However, when they were kept at -20 and -80 °C, the phenolic content was stable up to 12 days.

4. CONCLUSIONS

In order to increase the shelf life of agricultural commodities various preservation techniques are applied. Drying is one of the techniques that extends the shelf life by reducing active water in the commodities. However, drying may cause loss of flavor, taste and above all, its nutritional values. Keeping in mind these points one should select a drying process for extending shelf life. Clove being an important spice its bioactive constituent must be analyzed upon different drying treatments. The drying processes applied in this study are very conventional. Polyphenols contribute a lot in health and disease inspite of recent debates on the mode of its action and required daily allowance. Therefore, TPC and other antioxidant species were evaluated after drying. The most effective drying treatment was found to be microwave-drying as it was able to retain higher amount of antioxidant compounds and antimicrobial activity than oven- and air-drying. This is possibly because, microwave-drying requires lesser time and the heat is

uniformly distributed throughout the drying materials. A correlation between the conent of antioxidant compounds and their antioxidant activities was also established. The dried tissues also demonstrated antimicrobial activity.

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