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Effect of Different Drying Methods on Chemical Composition and Antimicrobial Activity of Bush Tea (*Athrixia phylicoides*)

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Abstract

The effect of different drying methods; sun, freeze, shade and oven drying on total polyphenol content, tannins, total antioxidants and phytochemicals content of bush tea (*Athrixia phylicoides* DC.) were evaluated. Assessment of minimum inhibitory concentration (MIC) assay was also done on bush tea samples. Results showed that different drying processes significantly affected phytochemical compositions of bush tea. Highest total phenolic content (8.34 mg/100 g) sample was found on freeze and shade dried bush tea compared to less than (6.50 mg/100 g) in sun or oven dried samples. Similar response was shown in total antioxidants with shade (0.628 µmol/g) and freeze (0.626 µmol/g) drying having significantly highest content of total antioxidants as compared to sun (0.440 µmol/g) and oven (0.444 µmol/g) dried samples. MIC values falls within 3.1 to 6.3 mg/mL with gram-positive bacteria being more vulnerable to the inhibitory effect of extract regardless of bush tea drying method than gram-negative. Anti-microbial activities of bush tea observed in this study will be useful to develop tea extracts for deterrence of diseases causing microorganisms hence shade drying methods will provide easy drying bush tea samples which can be adopted by farmers for commercial purposes.

Keywords: Antioxidants; Bush tea; Drying methods; Minimum inhibitory concentration; Phytochemicals

Introduction

Bush tea (*Athrixia phylicoides* DC.) leaves contains 5-hydroxy-6,7,8,3',4',5'-hexamethoxyflavon-3-ol (Mashimbye *et al.*, 2006), 3-0-demethyldigicitrin, 5,6,7,8,3',4'-hexamethoxyflavone and quecertin (Mavundza *et al.*, 2010), tannins (Mudau *et al.*, 2007; Chabeli *et al.*, 2008), total polyphenols (Mudau *et al.*, 2006; Maudu *et al*., 2012) and total antioxidants (Maudu *et al.*, 2010; Mogotlane *et al.*, 2007) compounds with antimicrobial activity (Mavundza *et al*., 2010; Nchabeleng *et al*., 2013). Indigenous South Africans have significant use of this plant as a traditional medicine (Roberts, 1990; Van Wyk and Gericke, 2000; Mbambezeli, 2005).

McGaw *et al.* (2007) reported that bush tea contains no caffeine as well as pyridoxine making it more appropriate as a healthy beverage. More so, occurrence of antioxidants in bush tea has positive favourable health benefits (Mudau and Mariga, 2012). According to Mudau *et al.* (2006), active chemical compounds present in herbal tea influence the quality of herbal tea. They also serve as the potential indicators of medicinal prospective due to their antioxidant activities (Mudau *et al.*, 2006; MaKay and Blumberg, 2007; Kokotkiewicz and Luczkiewicz, 2009). However, yield and chemical composition from herbal plants are related to a variety of factors which include the drying process (Rocha *et al*., 2011).

Drying is an important part of tea post-harvest handling, with the processing having impact on antioxidant content and appearance which all have effect on viability of the industry (Chong and Lim, 2012). It is the most common and fundamental way to preserve quality of aromatic and medicinal plants (Müller and Heindl, 2006; Rocha *et al.*, 2011). In other common herbal tea in South Africa, Joubert and de Villiers (1997) reported that drying affects quality attributes on rooibos tea whilst in honeybush tea, drying methods did not significantly influence the quality of tea (du Toit and Joubert, 1998). In other herbal teas, sun-drying resulted in deterioration of antioxidant properties (Chong and Lim, 2012) and various drying methods have different effects on aromatic and medicinal plants (Stafford *et al.*, 2005; Rocha *et al.*, 2011). Hence for viability of the bush tea production, there is need to establish drying techniques for recommendations to producers.

Data that describe the standard production protocol for drying bush tea have not been established. Therefore, the objective of this study was to compare effect of different bush tea drying methods on phytochemical composition and anti-microbial activities of bush tea extract.

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Materials and Methods

Fresh bush tea samples were collected from the wild at Muhuyu village (22°53'60S and 30°25'0E, 724 m.a.s.l.) in Limpopo province, South Africa. The fresh leaves of uniform shape, color and size were selected and subjected to four different drying methods viz., sun, freeze, shade and oven drying. In sun drying, bush tea leaves were placed in the sun/daylight. Midday temperature reached 35°C for 5 days during the sun drying period. For, freeze drying, leaves were placed in freezer dryer. In shade drying, leaves were placed in wooden trays and protected from direct sunlight. For oven drying, leaves were placed in an oven tray and heated at a temperature between 45 to 65°C overnight. All treatment were laid out in a completely randomized design (CRD) and replicated five times. Dry samples were thereafter analysed for chemical compositions. Assessment of minimum inhibitory concentration (MIC) assay was also done (Eloff, 1988). Comparisons of total phenolic contents and antioxidant activities were made to the fresh bush tea extracts reported by Mavundza (2010).

Chemical Compositions Assays

Determinations of total polyphenol content were done using methods reported by Singleton and Rossi (1965) and modified by Waterman and Mole (1994). Tannins were determined using Vanillin HCL method described by Prince *et al.* (1978) and total antioxidants were determined using the method described by Awika *et al.* (2004).

Phytochemicals Assays

The chlorophyll, riboflavin, niacin, ascorbic acid, carotenoid contents were determined using the standard AOAC methods (1984).

Minimum Inhibitory Concentration Assay

Gram-positive; *Staphylococcus aureus* (ATCC 12600), *Bacillus cereus* (ATCC 11778), *B. subtilis*, *B. pumilis* (ATCC 21356), *Enterococcus faecalis* (ATCC 29212) and Gram-negative; *Pseudomonas aeruginosa* (ATCC 25922), *Escherichia coli* (ATCC 11775), *Klebsiella pneumonia* (ATCC 27736) microorganisms were used. The assays were carried out using micro-dilution methods on 96 well microplates (Eloff, 1988).

Data Analysis

Data were subjected to analysis of variance (ANOVA) using PROC GLM (General linear model) procedure of SAS version 9.2 (SAS Int, 2011).

Results

Chemical Compositions

Drying of bush tea after harvesting had significant effect on the chemical composition of the extract, with generally a higher composition than in fresh samples which had 2.30 mg/100 g of total phenolic content. Total phenolics were higher in shade (8.34 mg/100 g) and freeze (8.34 mg/100 g) dried bush tea as compared to sun (6.42 mg/100 g) and oven (5.62 mg/100 g) dried samples (Table 1). The same response was shown in total antioxidants with shade (0.63 µmol/g) and freeze (0.63 µmol/g) drying having significantly highest content of total antioxidants as compared to sun (0.44 µmol/g) and oven (0.44 µmol/g) dried samples. However, even though there was slightly more tannin content (0.4 mg/100 g) in freeze dried bush tea samples than in other drying techniques; the different drying approaches did not exhibit significant differences on tannin contents of bush tea leaves.

Shade (83.4 mg/100 g) and freeze (83.4 mg/100 g) dried bush tea samples had significantly highest chlorophyll contents compared to sun (69.9 mg/100 g) and oven (68.3 mg/100 g) dried samples (Table 2). The difference between the highest and lowest contents was 14.1 mg/100 g of dried sample. The ascorbic acid, niacin, carotenoids and riboflavin contents of the sampled bush tea did not vary regardless of the drying method used. Average phytochemical compositions within the plant samples were 76.3 mg/100 g, 1.7 µmol /g, 1.5 mg/100 g, 1.8 mg/100 g and 2.4 mg/100 g for chlorophyll, ascorbic acid, niacin, carotenoids and riboflavin contents respectively.

Minimum Inhibitory Concentration

MIC values of extract on different microorganisms are shown in Table 3. The crude extract showed positive inhibitory activity against the entire gram-positive and gram-negative tested microorganism regardless of drying method subjected to the bush tea sample with MIC values ranging from 3.1 to 6.3 mg/mL. All the gram-negative microorganisms gave a MIC value of 6.3 mg/mL whilst the lowest MIC values of 3.1 mg/mL were obtained in gram-positive microorganisms except for *B. cereus* with MIC value of 6.3 mg/mL.

Discussion

Drying of bush tea resulted in a higher composition of chemical constituents. During drying, cell membranes rapture and degrade thereby releasing compounds during extraction (Stafford *et al.*, 2005). However, freeze and shade drying proved to be more efficient in retaining the total polyphenols and antioxidants within the bush tea samples than oven and sun drying procedures. In most studies carried out on various aromatic and medicinal plant types (Yousif *et al.*, 2000; Asami *et al.*, 2003; Abascal *et al.*, 2005), freeze drying has been shown as mostly recommended method in retention of plant compounds. Similarly, van Golde *et al.* (2004) reported that approximately 70% of polyphenols in red wine can be preserved through freeze drying. In grape peel, losses of

Table1: Response of chemical composition to selected dying methods of bush tea

Drying methods	Total polyphenols content (mg/100 g)	Total antioxidants (µmol/g)	Tannin contents (mg/100 g)
Sun drying	6.42b	0.44b	0.34a
Shade drying	8.34a	0.63a	0.34a
Oven drying	5.62b	0.44b	0.34a
Freeze drying	8.34a	0.63a	0.38a
Significant level	0.0001	0.0001	0.5038
LSD 5%	1.01	0.08	0.07

Means in a column followed by the same letter are not significantly different (P>0.05).

Table 2: Effect of drying on phytochemical compositions of bush tea

Drying methods	Chlorophyll (mg/100 g)	Ascorbic acid (µmol/g)	Niacin (mg/100 g)	Carotenoids (mg/100 g)	Riboflavin (mg/100 g)
Sun drying	69.9b	1.8a	1.6a	1.8a	1.9a
Shade drying	83.4a	1.8a	1.7a	1.9a	2.6a
Oven drying	68.3b	1.6a	1.4a	1.8a	2.6a
Freeze drying	83.4a	1.6a	1.4a	1.8a	2.4a
Significant level	0.0001	0.9794	0.8969	0.9981	0.3195
LSD 5%	4.2	1.6	1.0	1.1	0.9

Means in a column followed by the same letter are not significantly different (P>0.05).

Table 3: MIC values of the crude extract from bush tea dried irrespective of drying methods

Bacterial species	Gram +/-	Minimum inhibitory concentration (mg/mL)
Staphylococcus aureus	+	3.1
Bacillus cereus	+	6.3
Bacillus subtilis	+	3.1
Bacillus pumilis	+	3.1
Enterococcus faecalis	+	3.1
Pseudomonas aeruginosa	-	6.3
Escherichia coli	-	6.3
Klebsiella pneumonia	-	6.3

polyphenols, tannins and antioxidants have been reported in oven dried samples at higher temperature (Laurrauri *et al.*, 1997).

Post-harvest processes like drying take authoritative impact on the quality of the product influencing its value (Müller and Heindl, 2006) which is mostly shown on its appearance. The present study showed that sun and oven drying can reduce the chlorophyll content of bush tea, adversely affecting the color. Mahanom *et al.* (1999) observed loss of chlorophyll in all oven dried medicinal plants leaves. Similar results were reported by Joubert and de Villiers (1997) in rooibos tea, where less chlorophyll was present in sun-dried than in tea dried under controlled conditions. Ramana *et al.* (1988) suggested that such loss of colour must be minimized as it may adversely distance prospective customers due to possibility of below par processing. In addition to yielding extracts high in phenols and active ingredients; meadowsweet (*Filipendula ulmaria*) and willow (*Salix alba*) herbs developed an appropriate colour for fusion into a beverage when dried under temperatures of 30°C compared to the 70°C (Harbourne *et al.*, 2009).

Besides the benefits of antioxidants, bush tea samples were also beneficial as antibacterial regardless of drying method used in this trial. According to Fabry *et al.* (1998), all plant extracts with MIC values below 8 mg/mL are considered to possess some antimicrobial activity. MIC values have demonstrated that the gram-positive bacteria appeared to be more susceptible than the gram-negative ones to the inhibitory effect of the extract. Similar results were reported by Tshikalange *et al.* (2005) who also noted on different susceptibility of these bacteria while studying the antibacterial activities of other herbal plants. These difference in susceptibility can be ascribed to the morphological differences between the gram-positive and gram-negative bacteria (Palombo and Semple, 2001; Tadeg *et al.*, 2005), with the later having an outer phospholipidic membrane that carries structural lipopolysaccharide components making the cell walls impermeable to lipophilic solutes (Nostro *et al.*, 2000).

Benefits of tea extracts have been noted previously by Toda *et al.* (1989), with the extract inhibiting enteric pathogens such as *Staphylococcus aureus, S. epidermis and Plassiomonas shigelloides.* Amongst other things, tea extracts have been found effective against *Helicobacter pylori* which are linked to gastric, peptic and duodenal ulcer diseases (Diker and Hascelik, 1994). Biological activities of the cariogenic streptococci can also be inhibited by polyphenols in green tea thereby preventing teeth from decaying (Sakanaka *et al.*, 1996; Mitscher *et al.*, 1997). According to Baydar *et al.* (2004), the extent of this inhibition can be attributed to phenolic composition within the plant with phenols as the predominant active compounds in medicinal plants (Rios and Recio, 2004). This makes bush tea a very promising herbal tea as it is a rich source of phenols.

In conclusion, shade and freeze drying methods have shown to be more useful for retention of phytochemicals within bush tea samples. Crude extracts of bush tea had a broad spectrum in their actions on antibacterial activities of either gram positive bacteria or gram negative bacteria regardless of drying method subjected to the bush tea. Therefore, sound processed bush tea could be used as accessible sources of natural antimicrobial and antioxidants.

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