Original Research Article

Effect of spice form and extraction period on Total Phenolic Content of Selected Ugandan Spices

ABSTRACT

Introduction: Spice consumption is one of the globally recognized healthy nutritional practices. Most spices contain phenolic compounds that may prevent or prolong the onset of the non-communicable diseases. The harvesting, processing and preparation procedures of such spices may influence the phenolic amount extracted and eventual biological availability. Literature about how extraction period and spice form affects total phenolic content yield in water infusions is scanty.

Aim: The current study determined the effect of spice form and length of extraction time on the total phenolic content (TPC) yield of the selected Ugandan spices infused in water.

Methods: Samples of *Ocimum sanctum*, *Allium sativum*, *Cymbopogon citratus* and *Zingiber officinale*, were collected in triplets from Kanungu, Bushenyi and Lugazi Districts, in Uganda. Fresh and dry samples of these spices were infused in hot water for four minutes and 40 minutes and sieved with Whatman paper, No. 1. Phenolic content was measured with a spectrophotometer in Makerere University, Biochemistry Department, following Folin-ciocalteu method, using garlic acid as the reference standard. Results were analyzed using GraphPad Prism 8.0.1 software.

Results: Higher TPC yield was generally observed in dry samples compared to the flesh ones and 40 minute extracts of both fresh and dry samples also had higher TPC content compared to the four minutes' ones. The highest TPC yield was observed in *Cymbopogon citratus* (12.21±0.754 mg GAE/g) among the dry samples and *Ocimum sanctum*(10.02±2.452 mg GAE/g) among the fresh samples, extracted for 40 minutes.

Conclusion: Longer extraction time and sample dryness maximize TPC yield. *Ocimum sanctum* and *Cymbopogon citratus* may benefit consumers by improving their antioxidant status.

***Keywords**: Phenolic content, extraction period, spice form, Ocimum sanctum, Allium sativum, Zingiber officinale, Cymbopogon citratus*

1. INTRODUCTION

Currently, consumption of herbs and spices is considered one of the globally cherished healthy nutritional practices. Most of these herbs and spices contain phytochemicals which offer the health benefits including but not limited to prevention or prolonged onset of many chronic diseases such as cardiovascular diseases, diabetes, cancers, neurodegenerative diseases, aging, etc. (1). Among the phytochemicals polyphenols constitute the largest group and exert their action through a wide spectrum of mechanisms (2). However, how these spices are harvested, processed and/or prepared matters. Some of the procedures may extract more or less, or degrade some active ingredients hence the determination of the effect of spice form and length of extraction time on the total phenolic content (TPC) yield is essential (3).

Phenolics are secondary plant metabolites, essentially for protection against fungi and bacteria, growth, and development. They exhibit high antioxidant activity needed by human beings for good health (4). For example, caffeic acid, synthesized in most plant species especially coffee and tea, selectively inhibits leukotrienes biosynthesis, thereby regulating asthma and other allergic reactions. In addition, it has anti-carcinogenic properties, preventing hepatocellular carcinoma and colon cancer (5). Catechins reverse peripheral endothelial dysfunction especially in smokers, improve blood pressure and suppress myocardial inflammation and fibrosis (6).

Garlic phenolics scavenge reactive oxygen species (ROS), boost immunity, reduce cancer risks, heart diseases, aging and cholesterolemia. In addition, they are helpful in coughs and colds, and cleanse blood vessels, have antibacterial, antiviral, and anti-mutagenic properties. Garlic antioxidant support the body´s defense mechanism against oxidative damage. The most biologically active molecule in garlic is alliin, which is usually transformed during the cutting process, by alliinase enzyme to allicin which is responsible for the characteristic garlic aroma and taste (7).

Ocimum sanctum (Tulsi or 'Holy Basil) is another plant with interesting medicinal values. It is known as the "Queen of plants" or "mother medicine of nature" because of its vast medicinal values (8). According to Bhattacharyya, et al, (2010), *O. sanctum* is very helpful in prevention or therapy of oncologic diseases

(9). In many traditions, its aqueous extracts have been useful in treatment of poisoning, stomachaches, colds, headaches, malaria, inflammation, and heart disease (10). Its oils can be used as expectorants, analgesics, anti-emetics, and antipyretics; stress reducers and inflammation relievers. They are anti-asthmatic, anti-hyperglycemic, anti-hypertensive, hepatoprotective, anti-hyperlipidemic, and act as immune boosters (8). It is useful in treatment of bronchitis, malaria, diarrhea, dysentery, skin disease, arthritis, eye diseases, insect bites, etc. It has anti-fertility, anticancer, antidiabetic, antifungal, antimicrobial, cardioprotective, analgesic, antispasmodic and adaptogenic properties (10).

Zingiber officinale (Ginger) a rhizome plant that belongs to the family Zingiberaceae is another globally utilized spice, with anti-inflammatory, antioxidant, anti-carcinogenic, anti-diabetic and hypotensive properties. The main constituent responsible for these therapeutic potentials in *Z. officinale* is Eugenol (1-hydroxy-2-methoxy-4-allylbenzene). *Z. officinale* activates apoptosis and enhances the ability of p53 to prevent cancer. It is hepatoprotective, antibacterial and enhances gastric emptying (11).

Cymbopogon citratus (Lemongrass) is one of the most effective globally used antifungal antidotes. The major biologically active essential oils in this spice are E-Citral (52.9%) and Z-Citral (39.38%). These oils are anticlastogenic (preventing chromosomal damage), antidepressant, mood-boosting and can completely inhibit the thriving of aflatoxin producing fungi and their production of aflatoxin B_1 (12). In addition, it contains other active compounds e.g. terpenes, alcohols, carbonyl compounds, esters, flavonoids like quercetin, and phenolics. It has hypoglycemic, hypolipidemic, anxiolytic, sedative and antioxidant activities (13).

Humans consume approximately 25 mg–1 g on a daily basis depending on the composition of their diet for the day (fruit and fruit juices, tea, coffee, vegetables, spices, grains, red wine, legumes, cereals, etc.).The way they are processed and prepared for consumption determines their phenolic bioavailability (14). Most procedures used in evaluating total phenolic content of plant materials has been done on organic extracts, yet for consumption, they are usually prepared using water. Therefore, this study aimed at determining the effect of spice form (fresh or dry) and extraction period (four and forty minutes) on the total phenolic content of the selected Ugandan spices, infused in water.

2.0 Materials and Methods

2.1. Materials

Garlic acid, anhydrous sodium carbonate, and Folin Ciocalteu reagent, were purchased from Sigma Aldrich, (Steinheim, Germany). All these chemicals were of analytical grade.

2.2 Collection of plant materials

Samples of *Ocimum sanctum*, *Allium sativum*, *Cymbopogon citratus* and *Zingiber officinale*, were collected in triplicates from Kanungu, Bushenyi and Lugazi Districts, in Uganda. They were identified by a botanist in the department of Botany, Makerere University and copies kept in the departmental repository. Samples from each of the materials were kept in a refrigerator at 8ºC till analysis while others were dried indoors.

2.3 Sample preparation

One gram of each fresh or indoor dried (crushed) sample was measured into a beaker, followed by 100mls of hot distilled water. The paste was shaken occasionally for four minutes or kept at boiling temperature in a water bath for 40 minutes, and then sieved using Whatman paper, No. 1.

2.4 Determination of total phenolic content

Total phenolic content (TPC) was determined using Folin Ciocalteu method (15). Sample extract (0.5 mL) was separately pipetted into three different test tubes followed by the addition of distilled water (3.5 mL), Folin Ciocalteu reagent (0.5mL) and 7% Na_2CO_3 (0.5mL), consecutively. The resultant solution was incubated in the dark for colour development under room temperature (25 ºC) for 90 minutes. Absorbance of the solution was read at 730 nm using a Hitache, U2001 spec 1212510-02 spectrophotometer. Garlic acid (cat. no:6546548769138) was

used as the reference standard and TPC in one gram of fresh or processed plant material was calculated and expressed as mg garlic acid equivalent (GAE) per gram, dry sample. Total phenolic content was determined from the garlic acid calibration curve, of 2, 4, 6, 8 and 10 µg/mL concentration with linear equation of $y = 33.596x - 0.0099$, R^2=0.9862.

2.5 Data management and analysis

All quantitative data were analyzed using GraphPad Prism 8.0.1 software. Data was expressed as mean ± standard error of mean (SEM) while variation in a set of data was analysed through the one-way Analysis of Variance. The difference among the means was considered at 95% confidence level using the post-hoc methods of Tukey's Multiple Comparison.

2.6 Ethical approval

Approval to conduct the study was obtained from Gulu University Research and Ethics Committee (GUREC) with number: GUREC-110-18.

3.0 Results and discussion

Spices, especially those with high phenolic contents, have recently received a lot of attention because of their nutritional and medicinal values. Their anti-inflammatory, anticancer, anti-hypertensive, antibacterial and antioxidant properties make their study a worthwhile venture. In the current study, the total phenolic content yield of the selected local spice form (fresh or dry) and length of extraction time (four and 40 minutes) were determined.

As presented in table 1, all the 40 minutes' extracts of fresh samples yielded about threefold TPC more than the amount yielded by four minutes' extracts. Likewise, the 40 minutes dry sample extracts yielded higher TPC than the four minutes extracts. This could be because the

longer the spice particles stay in hot water, the greater the likelihood of solvent water molecules interacting with and dissolve out the phenolic molecules out.

In addition, the dry/processed samples yielded better TPC amounts than fresh ones whether extracted for four or 40 minutes with the exception of *Allium sativum*. This indicated that the drier the sample, the better the hot water molecules will dissolve the phenolics from its tissues. Fresh materials contain a lot of water (water content), which may neither be replaceable nor removable. In addition, the percentage of phenolic molecules per unit weight of the fresh samples is low. Differences in TPC yields among species are probably due to variations in maturity and genetics.

C. citratus (12.21±0.754 mg GAE/g) yielded the highest TPC among the processed. The 40-minute TPC yield for dry/processed *C. citratus* was much higher than the four minute one, ($P < .001$). The 40-minute extract of the fresh form was much lower in TPC yield than the 40 minutes' dry extract ($P < .001$) but tenfold the four minute's extract of the fresh one. This indicate that 40 minutes extraction of both fresh and processed *C. citratus* is more beneficial than its four minutes extraction. The longer the time of extraction and the drier the sample, the higher the TPC yield. This is probably because the solute phenolic molecules get more time to interact with the solvent and dissolve out of the plant tissues. In addition, heat increases kinetic energy and entropy of both the solvent water and solute phenolic molecules, enhancing their removal from the otherwise tough tissues of *C. citratus.* The finding of the current study about the TPC yield of processed *C. citratus* is higher than that of Godwin, et al, 2014, who reported that the TPC of *C. citratus* in hot water preparations ranges from 2.6 to 7.3 mg GAE/g (16). The difference could be due to the variation in extraction time period, growth location and genetics.

Fresh *O. sanctum* yielded over 38-fold TPC when extracted for 40 minutes (10.02 ± 2.452 mg GAE/g), as compared to when extracted for four minutes (0.2575 ± 0.021 mg GAE/g) ($P < .001$). The TPC yield in fresh and processed *O. sanctum* extracted for 40 minutes was quite close (10.02±2.452 and 11.93±0.754, mg GAE/g, respectively), indicating that it may not matter whether fresh or processed form is available for consumption. This could be because, when a longer period in the solvent is provided, the water molecules can easily penetrate the soft tissues of fresh or dry *O. sanctum* almost equally, dissolving out the phenolic molecules. The TPC reported by Abeywardhana, et al, (2014) is 8.34 ± 0.14 mg GAE/g DW (17) which was quite close to the findings for this study. The variation could be because of the differences in the plant age, genetics, location, soil nature, harvesting, drying and storage conditions, or the differences in experimental procedure.

Forty minutes extraction yield of dry *Z. officinale* was two-fold the four minutes' one. It therefore meant that it is more beneficial to use processed *Z. officinale* than the fresh one. Qadir, et al, (2017) reported TPC for dried/processed *Z. officinale* as 2.81 ± 0.07 mg GAE/g (18), which is in close agreement with the 40-minutes' finding of the current study. Maizura, et al, (2011) reported (101.6 ± 0.6 mg GAE/100 g), in the aqueous extract (19), which is quite close to the four minute extract of dry finding of the current study.

When fresh *A. sativum* was extracted with hot water for 40 minutes, it yielded 1.208±0.102mg GAE/g, while the dry/powdered samples yielded 1.139±0.040mg GAE/g, which was quite close but lower, and an exception to the rest. This suggests that longer heating of the processed form may to some extent destroy the phenolic content. The finding is slightly lower than that of Mishra et al, (2017) who reported that the TPC of fresh *A. sativum* was 78.45 mg GAE/100 g

(20) which is close to the finding of the current study. The variations in the findings may be due to the differences in cultivars, soil, location, genetics or the procedures.

3.1 Table 1. Effect of spice form and extraction time on total phenolic content

Table 1 presents effect of spice form and extraction time (minutes) on total phenolic content (TPC) in mg GAE/g.

Spice type	**Fresh**		**Processed**	
	4-minutes	40-minutes	4-minutes	40-minutes
Zingiber officinale	0.5204±0.064	2.221±0.057	1.044±0.013	2.647±0.099
Ocimum sanctum	0.2575**±0.021	10.02**±2.452	2.2*±0.075	11.93*±0.754
Allium sativum	0.2798±0.009	1.208±0.102	0.7709±0.030	1.139±0.040
Cymbopogon citratus	0.3476±0.012	3.56^{a}±0.383	2.894*±0.026	12.21^{*a} ±0.754

Data are expressed as mean ± SEM, n=9, ^{a,} **and *P<0.05 is considered significant

4.0 Conclusion

This study shows that for maximum benefit, it is better to use indoor dried samples than fresh ones. It also shows that both fresh and dry samples give more total phenolic content (TPC) when they stay longer in boiling water. For longer periods of heating/extraction (40 minutes), it does not matter whether one has fresh or dry *O. sanctum*. Processed *O. sanctum* and *C. citratus* are almost equally good when extracted for 40 minutes and yield much higher TPC than *A. sativum* and *Z. officinale*, whether in the fresh or dry form. It therefore suggests that consumption of *O. sanctum* and *C. citratus* may be the most important among the selected spices, in protecting the body from oxidative stress.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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