Effect of Spice form and Extraction Period on Total Phenolic Content of Selected Ugandan Spices

Article *in* European Journal of Medicinal Plants · April 2022

DOI: 10.9734/ejmp/2022/v33i330456

7 authors, including:

Justus Murokore
Mbarara University of Science & Technology (MUST)
2 PUBLICATIONS **2** CITATIONS
SEE PROFILE

Alex Paul Wacoo
Makerere University
31 PUBLICATIONS **347** CITATIONS
SEE PROFILE

Clement Olusoji Ajayi
Mbarara University of Science & Technology (MUST)
34 PUBLICATIONS **64** CITATIONS
SEE PROFILE

European Journal of Medicinal Plants

33(3): 25-32, 2022; Article no.EJMP.84466
ISSN: 2231-0894, NLM ID: 101583475

Effect of Spice form and Extraction Period on Total Phenolic Content of Selected Ugandan Spices

Biryomumaisho Justus Murokore [a,b], Peter Vuzi California [a], Alex Paul Wacoo [c], Raphael Wangalwa [d], Clement Olusoji Ajayi [e], Hannington Gumisiriza [f] and Agnes Nandutu Masawi [a*]

[a] Department of Biochemistry and Sports Science, School of Biological Sciences, College of Natural Sciences, Makerere University, P.O. Box-7062, Kampala, Uganda.
[b] Department of Biochemistry, Faculty of Medicine, Mbarara University of Science and Technology, P. O. Box-1410, Mbarara, Uganda.
[c] Department of Medical Biochemistry, School of Biomedical Sciences, College of Health Sciences, Makerere University, P.O Box-7062, Kampala, Uganda.
[d] Department of Biology, Faculty of Science, Mbarara University of Science and Technology, P. O. Box-1410, Mbarara, Uganda.
[e] Department of Pharmacy, Faculty of Medicine, Mbarara University of Science and Technology, P. O. Box-1410, Mbarara, Uganda.
[f] Department of Chemistry, Faculty of Science, Mbarara University of Science and Technology, P. O. Box-1410, Mbarara, Uganda.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2022/v33i330456

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/84466

Original Research Article

Received 20 January 2022
Accepted 29 March 2022
Published 08 April 2022

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 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: This 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.

**Corresponding author: E-mail: agnes.nandutu@mak.ac.ug, masawi12agnes@gmail.com;*

Methods: Samples of *Ocimum gratissimum*, *Allium sativum*, *Cymbopogon citratus* and *Zingiber officinale*, were collected in triplicates 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 at Makerere University, Biochemistry Department, following Folin-ciocalteu method, using gallic 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±0.75 mg GAE/g) among the dry samples and *Ocimum gratissimum* (10.02±2.45 mg GAE/g) among the fresh samples, extracted for 40 minutes.

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

Keywords: Phenolic content; extraction period; spice form; Ocimum gratissimum; Allium sativum; Zingiber officinale; Cymbopogon citratus.

1. INTRODUCTION

Currently, the consumption of herbs and spices is considered one of the globally cherished healthy nutritional practices. Most of these herbs and spices contain phytochemicals that offer 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, catechins reverse peripheral endothelial dysfunction, especially in smokers, improve blood pressure and suppress myocardial inflammation and fibrosis [5].

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 [6].

Ocimum gratissimum L. (African Tulsi or 'Holy Basil) is another plant with interesting medicinal values. *O. gratissimum* is very helpful in the prevention or therapy of cancers like prostate, cervical, lung, breast, and colorectal cancers. Its leaves have mosquito repellant, antibacterial, antifungal, antifertility, antiviral, immune-modulatory, anti-diabetic, anti-allergic, renoprotective, neuro-protective and cardio-protective and insecticidal activities. Leaves of *Ocimum* species are useful in the treatment of colds, coughs, bronchitis, asthma, influenza, eye diseases, dysentery and diarrhea, [7]. The main active ingredient in *O. gratissimum* is Eugenol (65.135%), though it also contains Ocimene (7.20%), Caryophyllene (6.64%), and Germacrene D (12.03%) [8].

Zingiber officinale Roscoe (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. Its effectiveness is due to medicinal phytochemicals especially, 6-gingerol, 6-shogaol. *Z. officinale* activates apoptosis and enhances the ability of p53 and p23 to prevent cancer. It is hepatoprotective, antibacterial and enhances gastric emptying [9].

Cymbopogon citratus (DC.) Stapf (Lemongrass) is one of the most effective globally used antifungal antidotes. The major biologically active essential oils in this spice are E-Citral (52.90%) 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 [10]. 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 [11].

Humans consume approximately 25 mgg^{-1} daily, 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 [12]. Most procedures used in evaluating the total phenolic content of plant materials have 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. MATERIALS AND METHODS

2.1 Materials

Gallic 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 gratissimum*, *Allium sativum*, *Cymbopogon citratus* and *Zingiber officinale,* were collected in triplicates from Kanungu, Bushenyi and Lugazi Districts, in Uganda. They were identified and authenticated by a botanist in the Department of Plant Sciences, Microbiology and Biotechnology, Makerere University and specimens were deposited at the Departmental Herbarium of Makerere University, Kampala, Uganda where they were assigned the following voucher numbers; *C. citratus* 001, *O. gratissimum* 002*, Z. officinale* 003 *and A. sativum* 004*.* Samples from each of the materials to be analyzed were kept in a refrigerator at 8ºC while others were dried, under a shade indoors at room temperature.

2.3 Sample Preparation

One gram of each fresh or indoor dried (crushed) sample was measured into a beaker, followed by 100 mL 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 [13]. 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 at room temperature (25 ºC) for 90 minutes. The absorbance of the solution was read at 730 nm using a Hitache, U2001 spec 1212510-02 spectrophotometer. Gallic 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 gallic acid calibration curve, of 2, 4, 6, 8 and 10 µg/mL concentration with linear equation of $y = 33.596x - 0.0099, R2 = 0.9862$.

2.5 Data Management and Analysis

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

3. 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

[7]. 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.

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

Spice type	Fresh		Processed	
	4-minutes	40-minutes	4-minutes	40-minutes
Zingiber officinale	0.52±0.06	2.22±0.06	1.04±0.01	2.65±0.10
Ocimum gratissimum	0.26±0.02**	10.02±2.45**	2.20±0.08*	11.93±0.75*
Allium sativum	0.28±0.01	1.21±0.10	0.78±0.03	1.14±0.04
Cymbopogon citratus	0.35±0.01	3.56±0.38[a]	2.89±0.03*	12.21±0.75*[a]

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

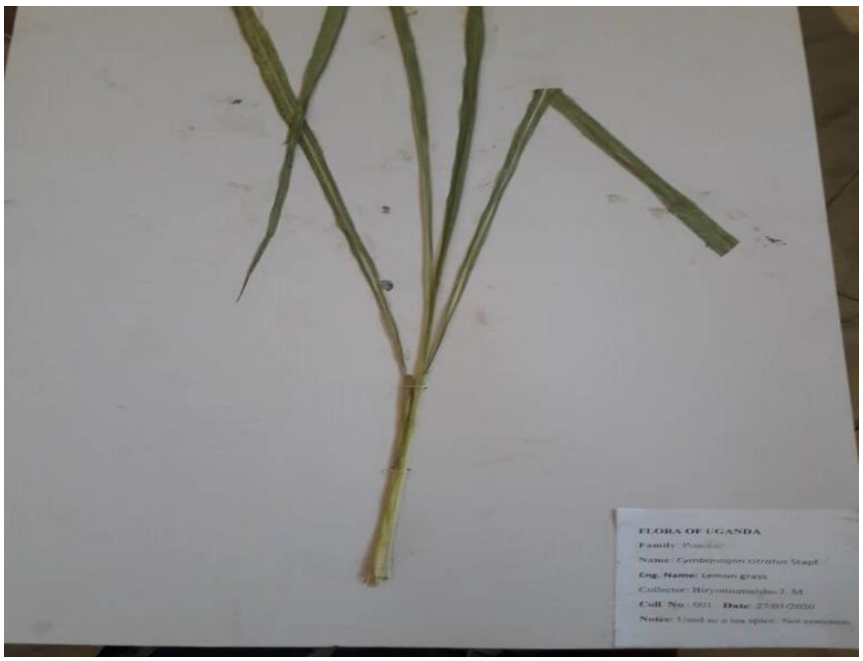

Fig. 1. *Cymbopogon citratus* (DC.) Stapf herbarium specimen

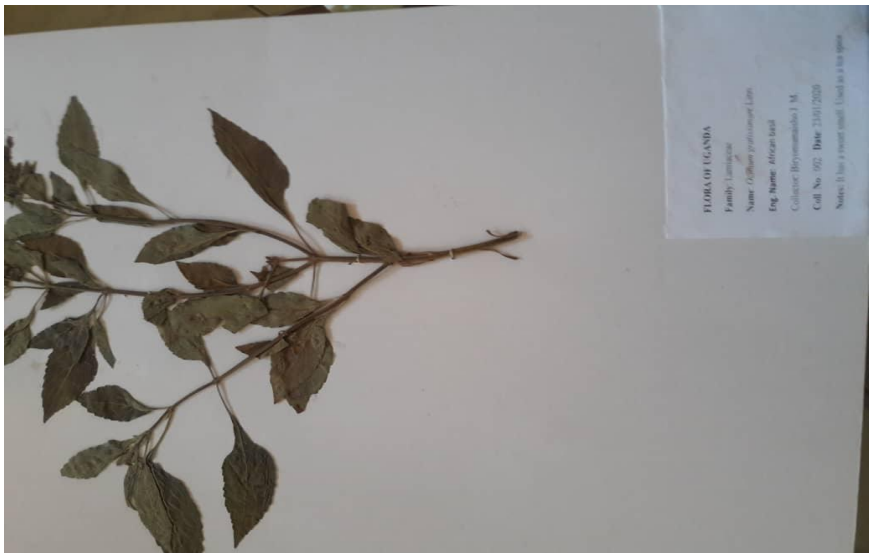

Fig. 2. *Ocimum gratissimum* L. herbarium specimen

Fig. 3. *Zingiber officinale* Roscoe herbarium specimen

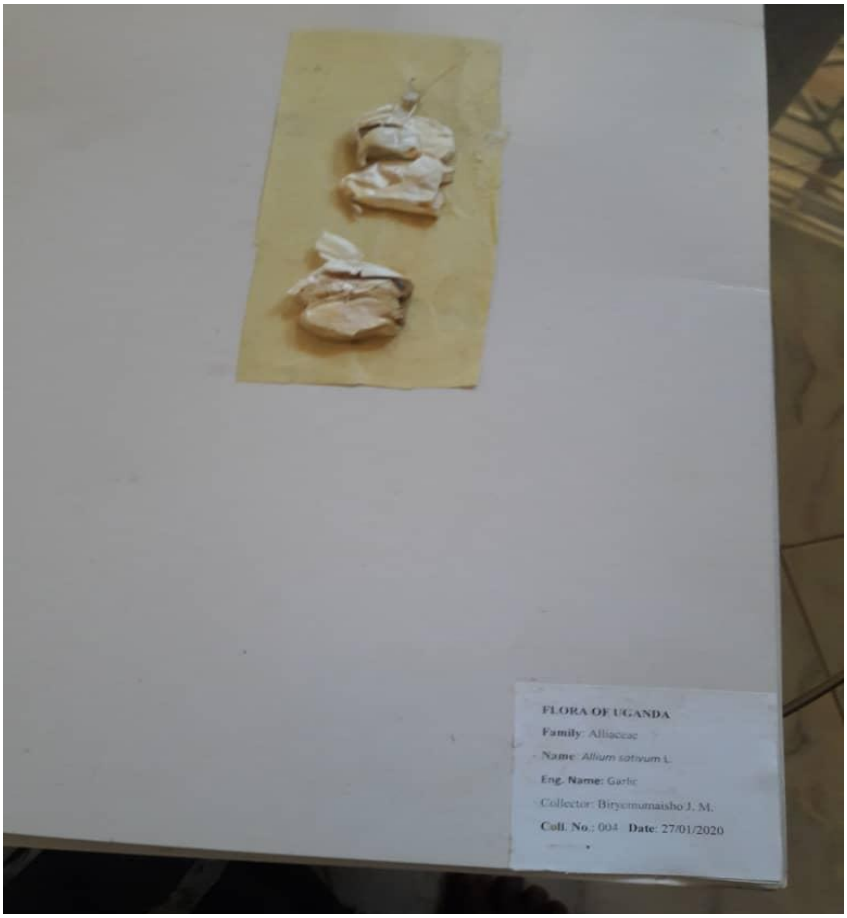

Fig. 4. *Allium sativum* L. herbarium specimen

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 interact and dissolve the phenolic molecules [14]. In addition, the dry/processed samples yielded better TPC amounts than fresh ones whether extracted for four or 40 minutes except *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 the environment, leaf maturity and genetics [15].

C. citratus (12.21±0.75 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 ten-fold the four-minute extract of the fresh one. This indicated that 40 minutes of extraction of both fresh and processed *C. citratus* is more economical than four minutes of 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 [16], who reported that the TPC of *C. citratus* in hot water preparations ranges from 2.6 to 7.3 mg GAE/g. The difference could be due to the variation in extraction time, growth location and genetics.

Fresh *O. gratissimum* yielded over 38-fold TPC when extracted for 40 minutes (10.02 ± 2.45 mg GAE/g), as compared to when extracted for four minutes (0.26 ± 0.02 mg GAE/g) (P < .001). The TPC yield in fresh and processed *O. gratissimum* extracted for 40 minutes was quite close (10.02±2.45 and 11.93±0.75 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. gratissimum* almost equally, dissolving out the phenolic molecules. The TPC reported by Uyoh *et al*. [17] is 9.09-27.41 µg GAE/mg a range within which two of the findings (40 minute dry and fresh extracts) of this study belong. 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 the experimental procedure.

Forty minutes extraction yield of dry *Z. officinale* was two-fold the four minutes one. Therefore, it is more economical 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 (101.60 ± 0.6 mg GAE/100 g), in the aqueous extract [19], which is quite close to the four-minute extract of the dry finding of the current study.

When fresh *A. sativum* was extracted with hot water for 40 minutes, it yielded 1.21±0.10 mg GAE/g, while the dry/powdered samples yielded 1.14±0.04 mg 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 procedures.

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

4. 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),

irrespective of fresh or dry *O. gratissimum,* the TPC is essentially the same. Processed *O. gratissimum* 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 dried form. Therefore, the use of *O. gratissimum* and *C. citratus* could be the most essential among the selected spices, in protecting the body from oxidative stress.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is 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 the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by Sweden International Development Agency, with no strings attached.

CONSENT

It is not applicable.

ETHICAL APPROVAL

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Costa C, Tsatsakis A, Mamoulakis C, Teodoro M, Briguglio G, Caruso E, et al. Current evidence on the effect of dietary polyphenols intake on chronic diseases. Food and Chemical Toxicology. 2017; 110:286-99.
2. Yashin A, Yashin Y, Xia X, Nemzer B. Antioxidant activity of spices and their impact on human health: A review. Antioxidants. 2017;6(3):70.
3. Rajbhar K, Dawda H, Mukundan U. Polyphenols: Methods of extraction. Sci Revs Chem Commun. 2015;5(1):1-6.
4. Leri M, Scuto M, Ontario ML, Calabrese V, Calabrese EJ, Bucciantini M, et al. Healthy effects of plant polyphenols: molecular mechanisms. International journal of molecular sciences. 2020;21(4):1250.
5. Suzuki Ji, Ogawa M, Futamatsu H, Kosuge H, Sagesaka YM, Isobe M. Tea catechins improve left ventricular dysfunction, suppress myocardial inflammation and fibrosis, and alter cytokine expression in rat autoimmune myocarditis. European Journal of Heart Failure. 2007;9(2): 152-9.
6. Kovarovič J, Bystricka J, Vollmannova A, Toth T, Brindza J. Biologically valuable substances in garlic (*Allium sativum* L.)–A review. Journal of Central European Agriculture. 2019;20(1):292-304.
7. Patel F, Modi NR. Estimation of total phenolic content in selected varieties of Ocimum species grown in different environmental condition. Journal of Pharmacognosy and Phytochemistry. 2018;7(5):144-8.
8. Dung P, Dao T, Le T, Tran H, Dinh T, Pham Q, et al., editors. Extraction and analysis of chemical composition of Ocimum gratissimum L essential oil in the North of Vietnam. IOP Conference Series: Materials Science and Engineering; 2021: IOP Publishing.
9. Prasad S, Tyagi AK. Ginger and its constituents: Role in prevention and treatment of gastrointestinal cancer. Gastroenterology Research and Practice. 2015;2015.
10. Sonker N, Pandey AK, Singh P, Tripathi N. Assessment of Cymbopogon citratus (DC.) stapf essential oil as herbal preservatives based on antifungal, antiaflatoxin, and antiochratoxin activities and in vivo efficacy during storage. Journal of Food Science. 2014;79(4):M628-M34.
11. Trang DT, Hoang TKV, Nguyen TTM, Van Cuong P, Dang NH, Dang HD, et al. Essential oils of lemongrass (Cymbopogon citratus Stapf) induces apoptosis and cell cycle arrest in a549 lung cancer cells. BioMed Research International. 2020; 2020.
12. Shahidi F, Ambigaipalan P. Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects–A review. Journal of Functional Foods. 2015;18:820-97.
13. Way ML, Jones JE, Nichols DS, Dambergs RG, Swarts ND. A comparison of

laboratory analysis methods for total phenolic content of cider. Beverages. 2020;6(3):55.
14. Borrás-Enríquez AJ, Reyes-Ventura E, Villanueva-Rodríguez SJ, Moreno-Vilet L. Effect of ultrasound-assisted extraction parameters on total polyphenols and its antioxidant activity from mango residues (*Mangifera indica L. var. Manililla*). Separations. 2021;8(7):94.
15. Irakli M, Kargiotidou A, Tigka E, Beslemes D, Fournomiti M, Pankou C, et al. Genotypic and environmental effect on the concentration of phytochemical contents of lentil (*Lens culinaris* L.). Agronomy. 2021;11(6):1154.
16. Godwin A, Daniel G, Shadrack D, Elom S, Afua N, AB K, et al. Determination of elemental, phenolic, antioxidant and flavonoid properties of Lemon grass (*Cymbopogon citratus Stapf*). International Food Research Journal. 2014;21(5).
17. Uyoh EA, Chukwurah PN, David IA, Bassey AC. Evaluation of antioxidant capacity of two Ocimum species consumed locally as spices in Nigeria as a justification for increased domestication; 2013.
18. Wongsa P, Chaiwarit J, Zamaludien A. In vitro screening of phenolic compounds, potential inhibition against α-amylase and α-glucosidase of culinary herbs in Thailand. Food Chemistry. 2012;131(3):964-71.
19. Maizura M, Aminah A, Wan Aida W. Total phenolic content and antioxidant activity of kesum (*Polygonum minus*), ginger (Zingiber officinale) and turmeric (*Curcuma longa*) extract. International Food Research Journal. 2011;18(2).
20. Mishra N, Tripathi R, Khan Z. Physicochemical and Antioxidant Potential of Garlic: Heat Processing Effects. J Agric Eng Food Technol. 2017;4:127-33.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/84466