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Original Research Article

Effects of Oven-Drying on the Phytochemical and Phenolic Acid Contents of Ethanol Extracts of the Root, Stalk and Leaves of *Cymbopogon citratus*

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ABSTRACT

Drying techniques play vital roles in the post-harvest processing of medicinal plants because they control microbial growth and enzymatic breakdown of plant nutrients. *Cymbopogon citratus* possesses diverse medicinal values in different cultures. However, little is known about the effect of drying methods on the phytochemical and phenolic acid content of lemongrass. The study comparatively evaluated the effect of drying methods on phytochemical and phenolic acids in the stalk, root and leaf extract of *Cymbopogon citratus*. The phytochemical test was done by standard techniques while the phenolic acids were analyzed by the high-performance liquid chromatographic (HPLC) method. The presence of flavonoids, glycosides, alkaloids, saponins, phenols, and tannins was detected via qualitative phytochemical analyses. The results of the quantitative phytochemical analysis showed that the concentrations of terpenoi ds (2.63 to 2.00 mgLI/g extract) and saponins (4.60 to 3.79 mg DE/g extract) were significantly ($p < 0.05$) lower in all oven-dried samples. There was a significant difference ($p < 0.05$) in the alkaloids, saponins and terpenoids contents of shade- and oven-dried samples. The HPLC analysis of phenolic acids revealed higher concentrations of total phenolic (0.09 vs 25.35 mg GAE/g extract), caffeic (1.0×10^{-4} vs 4.5×10^{-4} mg/g extract) and coumaric (1.9×10^{-4} vs 4.5×10^{-4} mg/g extract) acids in the oven-dried compared with shade-dried samples. The study showed that the variations in the phytochemical and phenolic acids constituents of shade- and oven-dried tissues of *C. citratus* may provide useful data germane for determining lead compounds during post-harvest processing.

***Keywords*:** Drying methods, High-performance liquid chromatography, Lemon grass, Medicinal plants, Phenolic compounds.

Introduction

The advantages of phytomedicines cannot be overstated, and medicinal plants currently have a prominent role in plant research and medicine.[1] The bioactive phytochemical elements of plants are the source of the plants' therapeutic efficacy.[2] Alkaloids, tannins, flavonoids, phenolics, phlobatannins, saponins, and cardiac glycosides are the most important bioactive phytochemicals.[3] Unfortunately, most of these plants with sound therapeutic properties are scarcely available all year round, especially in urban areas.[4] Given that, most researchers, ethnomedical experts and consumers have adopted different preservation techniques to ensure their availability at all times. Drying and powdering methods have been recommended for the preservation and improvement of product quality such as shelf life, prevention of product spoilage and retention of aromatic compounds.[5] Undesirably, drying methods have been reported to affect plant microstructure, colour profile and volatile compounds.[5]

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Cymbopogon citratus (DC) Stapf, a plant that is frequently used as a spice and belongs to the Poaceae family is known to contain phenolic compounds, which are potent antioxidants.[9, 10] Previous researchers have extracted various important chemicals; triterpenoids, flavones, citral α, citral β, geraniol, nerol, geranyl acetate, citronellal, terpinolene, terpinol methylheptenone and myrcene from different parts of lemon grass.[11] Recent investigations have shown a strong association between the antioxidant activities of plants and the functions of phytochemicals such as polyphenols, carotenoids, flavonoids, alkaloids, lignins, terpenoids and vitamins. Polyphenols are vital in antioxidant activity, scavenging of reactive oxygen species and consequently in combat oxidative stress.[11] Study on the antioxidant potentials of lemon grass indicated that its essential oil efficiently scavenged free radicals and could be a potential antioxidant.[12] The pharmacological profiles of lemon grass include cytotoxicity, neurobehavioral effects, anti-diabetic, antitrypanosomal, chemopreventive activity, anti-inflammations, insecticidal and larvicidal activity.[13]

Cymbopogon citratus is a medicinal herb frequently dried to suppress microbial growth and improve shelf life. Drying is a critical phase in post-harvest handling because it helps prevent enzymatic failure and microbial growth while retaining the beneficial characteristics of the dried plants.[14] It has the benefit of lowering shipping and storage costs, but it can also cause scent and visual changes, which can degrade plant quality. Plant bioactive molecules that may have had beneficial health-promoting qualities, such as antioxidant capabilities, have been reported to be reduced by drying.[15] The effects of drying methods on the phytochemical composition, bioactivities, chlorophyll, oleoresin, essential oil and moisture contents and other quality

parameters of ginger powder, bamboo leaves and *C. flexuosus* have been reported .[14,16, 17]

Studies revealed that the morphological structure, colour profile and citral concentration of *C. citratus* were significantly impacted by different drying methods, unlike the moisture contents.[5] The influence of natural drying techniques (sun, shade and cabinet) on the phytochemical compositions of lemon grass has also been elucidated.[4] Though several studies have reported on the phytochemical constituents, antioxidant activities, and total phenolic content of leaves of lemon grass, the impact of drying techniques on the amounts of phenolic acids and phytochemicals in lemon grass is poorly understood, particularly when using artificial techniques such as oven-drying, freeze-drying, solar-drying and microwave-drying. [16, 17] Therefore, this study evaluated the effect of drying methods on the phytochemical and phenolic acid contents of ethanolic extracts of the root, stalk and leaves of *C. citratus*.

Materials and Methods

Plant collection and identification

Leaves, stems and roots of *Cymbopogon citratus* were collected, in May, 2023, from the Botanic Garden, University of Nigeria Nsukka, Nigeria (N 6° 51'55.7892, E 7° 24'42.92028). They were identified using the voucher specimen number TN6228 by the taxonomist at the Herbarium of the Department of Plant Science and Biotechnology, University of Nigeria Nsukka.

Preparation of plant material

The collected sample of *C. citratus* was separated into leaves, stalks and roots and divided into two batches each. The first batch was air-dried at 25 °C for 14 days, while the second batch was oven-dried at 70 °C (Heraeus T 5050, Belgium) and weighed repeatedly at 2 h intervals until a constant weight after three measurements was attained across all parts and a 15% moisture was obtained measured using a moisture meter (EXTECH, MO260, United States). The plant materials were ground to a fine powder with a laboratory mill (MacsaLab 200, South Africa). The powdered samples were then stored in an air-tight amber container in the dark at room temperature until needed.

Extraction of plant materials

The powdered plant materials were extracted using cold-maceration of 5 g of each sample in 500 mL of methanol (80%v/v) for 48 h as previously described.[18] The mixtures were agitated intermittently for 48 h and filtered using Whatman filter paper. The filtrates were separately concentrated and dried using a vacuum rotary evaporator at 40 °C. The extracts were then stored in an air-tight amber container in the dark at room temperature until needed.

Qualitative phytochemical screening

Qualitative phytochemical screening of the extracts of *C. citratus* for the presence of alkaloids, terpenoids, saponins, glycosides, flavonoids and phenolic compounds was done following the standard methods adopted by previous studies.[19]

Quantitative phytochemical screening

Estimation of alkaloids

Approximately 5 mL buffer solution (pH 4.7) was added to 1 mL of extract sample before adding bromocresol green (BCG) solution (5 mL) and chloroform (4 mL) and then shaking the mixture. A 10 mL volumetric flask was used to collect the extract and then diluted with chloroform to adjust the volume. The absorbance of the solution in chloroform was read at 470 nm against a blank prepared without the extract using a UV spectrophotometer (UV-1280, Shimadzu, Japan). The concentration of alkaloids was expressed as atropine equivalents.[20]

Estimation of saponins

Total saponin was determined by the vanillin sulphuric acid colorimetric reaction.[21] To the 50 mL of sample extract, 250 µL of vanillin reagent was added. Then 2.5 mL of H_2SO_4 (72%) was added before thoroughly mixing the solution. The mixed solution was heated in a water bath maintained at 60 °C for 10 minutes. The solution was subsequently cooled in ice-cold water before reading the absorbance at 544 nm using a UV spectrophotometer. The values were derived from the standard curve as equivalents of diosgenin (mg DE/g extract).

Estimation of terpenoids

The total terpenoid in the sample was extracted using petroleum ether in the separatory funnel. The extract was evaporated to dryness and then dissolved in 95% methanol (5 mL). The mixtures were sonicated for 15 minutes, followed by incubation in the dark at 25 °C for 48 h. The mixture was filtered after incubation and 3 ml of chloroform was added to 1 mL of the extract. The solution was vortexed and cooled in ice packs before the addition of 400 mL of conc. H_2SO_4, and then incubated again at 25 °C for 48 h. A reddish brown colour was observed at the bottom of the tubes and the supernatant was decanted and the coloured part was made up to 4 mL with 95% methanol. A UV spectrophotometer was used to measure the absorbance at 538 nm, while 95% methanol was used as the blank. The standard curve was prepared as the equivalent of linalool.[21]

Estimation of glycosides

Glycoside composition was determined following the method adopted by the previous researcher.[20, 22] A freshly prepared Baljet's reagent (10 mL) was added to 1 mL of extract and the mixture was incubated for 1 h. The solution was then diluted with 20 ml of distilled water before reading the absorbance at 495 nm using UV spectrophotometer. Total glycosides were expressed as mg of securidaside standard per g of dried extracts.

High-performance Liquid Chromatographic (HPLC) analysis

The total phenolic, tannins, flavonoids and acids (coumaric, phenolic, caffeic, ferulic and sinapic) contents of the extracts was carried out using HPLC (LC-20AB, Shimadzu, Japan) analysis. The HPLC system (Waters, Singapore) was made up of a photodiode array detector (W2998), temperature control module II (TC2 waters), dual pump system (515 waters), a system controller (EMOAA01712), a pump control module (PC2 waters) and a reverse phase HPLC analytical column water (a 5 μm particle size Spherisorb C8, 4.6 × 100 mm,). The flow rate was adjusted to 1.2 mL/min; the detector was set at 220, 240, 260, 270, and 280 nm at 1.2 nm resolution with the mobile phase 0.1% methanol: phosphoric acid (50: 50 v/v, isocratic mode). The active constituent of maximum potential energy extract was dissolved in a mixture of methanol and water (6: 4 v/v) and identified by comparison of the retention time in chromatogram with standard phenolic acids (Sigma Chemical Co, St. Louis, USA).

Statistical analyses

Data obtained from the phytochemical and total phenolic content were subjected to multivariate analysis of variance, and significant means were separated using the least significant difference test (LSD) using IBM Statistical Product and Service Solution (SPSS) version 23.

Results and Discussion

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tannins and flavonoids. Similar observations were reported in previous research except for the case of saponins that was absent in the leaves of lemon grass.[4] Interestingly, there was a reduction in total phenolic content in the roots after oven-drying (Table 1).

The quantitative investigation of the phytochemicals showed that the total glycosides present in both oven and shade-dried extracts of *C. citratus* have the highest total concentration when compared to other phytochemicals present in the ethanolic extracts of plants (Table 2). More so, the glycosides contained in lemon grass parts dried with the

two drying methods did not show any significant variations. (Table 2). The highest total glycosides content observed in *C. citratus* ethanolic extract is in agreement with the report obtained from *Moringa oleifera* ethanolic root and leave extract as reported by Furo *et al*.[24] Inactive glycosides are a type of chemical that plants store and can be activated by hydrolyzing enzymes. which breaks down the sugar component of the molecule, allowing it to be used in medication manufacture.[25] Saponins and terpenoids showed an appreciable minimum total concentration in both oven and shade-dried respectively (Table 2). However, saponins and terpenoids were significantly higher in ethanolic extracts obtained from shade-dried lemongrass than the oven-dried (Table 2). The minimum total concentration of saponins and terpenoids conformed to the study of Praditvarn and Samhandharaksa,[26] who also reported the minimum concentration of the aforementioned phytochemicals in lemon grass extracts. Terpenoids are involved in the defense against insects and environmental stress, as well as wound healing and restoration.[27] The presence of these phytochemicals has suggested the use of *C. citratus* as a lead plant for the production of drugs for the management of several diseases. Alkaloids were significantly higher in the extracts of oven-dried than in the shade-dried lemon grass (Table 2). Alkaloids also showed the least total concentration in both oven and shade-dried and were also present in the roots only (Table 2). This could be a pointer to the potency of the utilization of the roots for medicinal purposes. Alkaloids exhibit a variety of characteristics, including anti-inflammatory, anticancer, analgesic, local anesthetic and pain relief. More functions include neuropharmacological, antibacterial, antifungal, and other properties are all demonstrated by alkaloids.[4] They can be used as diet items, supplements, and medications, as well as in medical and other human applications. Alkaloids are also essential substances in organic synthesis for the development of novel semi-synthetic and synthetic drugs with potentially higher biological activity than their parent compounds.[28] In general, the different drying methods affected the concentration of phytochemicals analyzed. A similar observation was reported by Thot et al.[4] when they recorded significant differences in the phytochemicals of *C. citratus* leaves dried with different drying methods.

Table 1: Qualitative phytochemical content of *C. citratus* leaf, stalk and root

Parameters	Shade-dried			Oven-dried		
	Leaf	Stalk	Root	Leaf	Stalk	Root
Alkaloids	-	-	+	-	-	+
Saponins	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+
Phenolic compound	+	+	+	+	+	+
Tannin	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+

(-) = absent; (+) = present

Table 2: Quantitative phytochemical content of *C. citratus* leaf, stalk and root

Plant parts	Drying method		Plant parts total
	Shade-dried	Oven-dried	
	Alkaloids (mg AT/g extract)		
Leaf	-1.67±0.00^{c}	-1.67±0.00^{c}	-1.67±0.00^{b†}
Root	3.67±0.33^{b}	5.33±0.00^{a}	4.50±0.50^{a†}
Stalk	-1.67±0.00^{c}	-1.67±0.00^{c}	-1.67±0.00^{b†}
Drying methods total	0.11±1.13^{b‡}	0.67±1.48^{a‡}	
	Saponins (mg DE/g extract)		
Leaf	2.88±0.17^{b}	1.77±0.28^{c}	2.32±0.35^{b†}
Root	7.71±0.11^{a}	6.60±0.11^{a}	7.16±0.33^{a†}
Stalk	3.21±0.06^{b}	2.99±0.06^{b}	3.10±0.07^{b†}
Drying methods total	4.60±0.99^{a‡}	3.79±0.92^{b‡}	
	Terpenoids (mg LI/g extract)		
Leaf	4.05±0.06^{a}	3.13±0.02^{b}	3.59±0.27^{a†}
Root	2.42±0.02^{c}	1.55±0.01^{d}	1.99±0.25^{b†}
Stalk	1.42±0.01^{e}	1.32±0.00^{f}	1.37±0.03^{c†}
Drying methods total	2.63±0.48^{a‡}	2.00±0.36^{b‡}	
	Glycosides (mg SE/g extract)		
Leaf	2.20±0.15	2.49±0.09	2.35±0.11^{c†}
Root	8.57±0.22	8.86±0.07	8.72±0.12^{a†}
Stalk	4.66±0.04	4.64±0.02	4.65±0.02^{b†}
Drying methods total	5.15±1.18^{a‡}	5.33±1.18^{a‡}	

Data are presented as mean ± SE; average content along the last column†; average content along the last row‡ of each parameter; ^{a,b,c,d} different alphabets represent significant means separated using LSD test at $p < 0.05$.

The results as presented in Figures 1A-C showed the standard curves used in the determination of total phenolic, total tannin and total flavonoid content in the root extract of *C. citratus*. The gallic acid, tannic acid and quercetin standard curves all showed a strong correlation between concentration and absorbance. The regression equation for the determination of total phenolic, tannin and flavonoid contents was $y = 0.002x + 0.031$ ($R^2 = 0.97$), $y = 0.003x + 0.046$ ($R^2 = 0.98$) and $y = 0.003x + 0.016$ ($R^2 = 0.99$) respectively (Fig 1A-C). The results as presented in Table 3 showed a significant reduction in total phenolic, tannin and flavonoid content in oven-dried samples as compared to the shade-dried. This finding corroborates the report of Barimah *et al*.[29] who similarly reported a reduction in total phenolic content of dandelion leaves using oven-drying. According to Ketharin *et al*.,[30] majority of phenolics are soluble in water and unstable at high temperatures. During the extraction procedure, the heat process may disintegrate the cell wall or break down insoluble phenolic compounds, resulting in the release of bound phenolic acids.[31] More so, the leaves contained significantly higher total phenolic and flavonoid content while the root had significantly higher tannin and saponin contents. This is an indication of the usefulness of the leaves and roots for medicinal purposes.[32]

As shown in the HPLC chromatograms of different parts of *C. citratus* at shade- and oven-dried temperatures (Figures 2A-G), Figure 2A represents the chromatogram standard while Figures 2B – G represent the chromatograms of the shade- and oven-dried samples of the root, stalk and leaves of the plant. The chromatograms showed various peaks signaling the presence of different phenolic acids. The HPLC result presented in Table 4 shows the phenolic acid content of extracts of lemon grass dried at room temperature and in the oven. Caffeic and coumaric acids were the prominent phenolic acid compounds in the lemon grass in both shade- and oven-dried extract respectively. However, sinapic and ferulic acids were observed to be absent. The observed phenolic acids were higher in concentration with the oven-dried samples. Caffeic acid was also reported as the main phenolic acid compound present in lemon grass infusion.[33] More so, the phenolic acids were more concentrated in the leaves than the stalk, while none was observed in the root. This finding corroborates the report of Costa *et al*. who earlier opined that harvest region and seasons might account for the differences in profile and content of phenolic compounds in plants.[34, 35]

Table 3: Quantitative total phenolic content of extract of *C. citratus* leaf, stalk and root

Plant parts	Drying method		Plant parts total
	Shade-dried	Oven-dried	
	Total phenolic content (mgGAE/g)		
Leaf	18.58±0.05^{a}	15.00±0.12^{b}	16.79±1.03^{a†}
Root	12.32±0.01^{c}	11.12±0.12^{d}	11.72±0.35^{b†}
Stalk	6.85±0.02^{e}	6.22±0.04^{f}	6.53±0.18^{c†}
Drying methods total	12.58±2.14^{a‡}	10.78±1.61^{b‡}	
	Total tannins content (mgTAE/g)		
Leaf	7.49±0.04^{c}	6.63±0.00^{d}	7.06±0.25^{b†}
Root	8.91±0.03^{a}	8.75±0.03^{b}	8.83±0.05^{a†}
Stalk	4.34±0.10^{e}	3.66±0.01^{f}	4.00±0.20^{c†}
Drying methods total	6.91±0.85^{a‡}	6.35±0.93^{b‡}	
	Total flavonoids content (mgQE/g)		
Leaf	10.18±0.06^{a}	9.45±0.03^{b}	9.81±0.21^{a†}
Root	6.00±0.05^{c}	3.67±0.02^{d}	4.83±0.67^{b†}
Stalk	1.95±0.02^{e}	1.31±0.03^{f}	1.63±0.19^{c†}
Drying methods total	6.04±1.50^{a‡}	4.81±1.53^{b‡}	

Data are presented with mean ± SE; average content along the last column†; average content along the last row‡ of each parameter; ^{a,b,c,d} different alphabets represent significant means separated using LSD test at $p < 0.05$.

Table 4: Quantitative phenolic acids contents of extract of *C. citratus* leaf, stalk and root

Plant parts	Drying method		Plant parts total
	Shade-dried	Oven-dried	
	Coumaric acid (x 10^{-4} mg COA/g extract)		
Leaf	5.70±0.07^{c}	6.50±0.10^{b}	6.10±0.19^{a†}
Root	-	-	-
Stalk	-	7.10±0.07^{a}	3.50±1.58^{b†}
Drying methods total	1.90±0.94^{b‡}	4.50±1.13^{a‡}	
	Caffeic acid (x 10^{-4} mg CAA/g extract)		
Leaf	3.10±0.07^{b}	1.03±0.33^{a}	6.70±1.63^{a†}
Root	-	-	-
Stalk	-	3.10±0.13^{b}	1.60±0.70^{b†}
Drying methods total	1.0±0.51^{b‡}	4.50±1.53^{a‡}	

	Total phenolic acid (mg GAE/g extract)		
Leaf	0.27±0.14	55.41±27.83	27.84±17.52^{a†}
Root	-	-	-
Stalk	-	20.63±10.36	10.32±6.54^{b†}
Drying methods total	0.09±0.06^{b‡}	25.35±11.78^{a‡}	

Sinapic and ferulic acids were not detected in all the samples; Gallic acid equivalent (GAE), Coumaric acid COA), caffeic acid (CAA), Data are presented with mean ± SE; average content along the last column†; average content along the last row‡ of each parameter; ^{a,b,c,d} different alphabets represent significant means separated using LSD test at $p < 0.05$

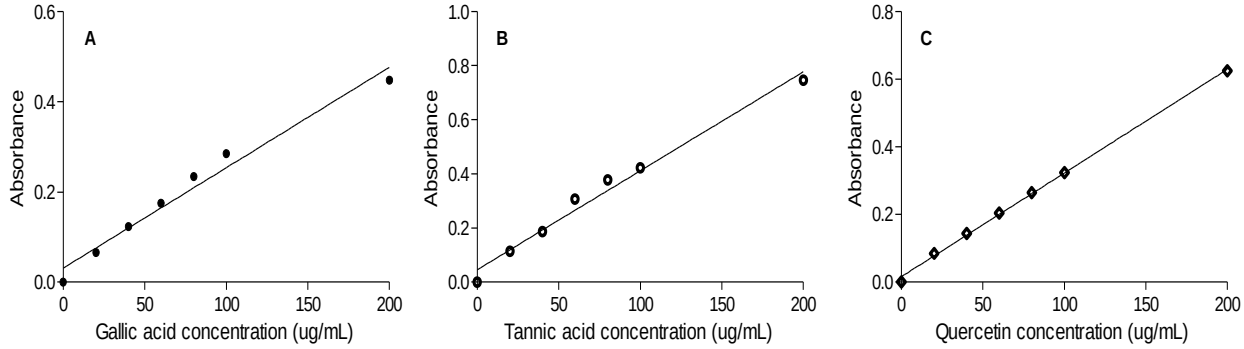

Figure 1: Standard calibration curves: Gallic acid for phenolic content (A), tannic acid for tannin content (B) and quercetin for flavonoids content (C) in extract of *C. citratus* leaf, stalk and root

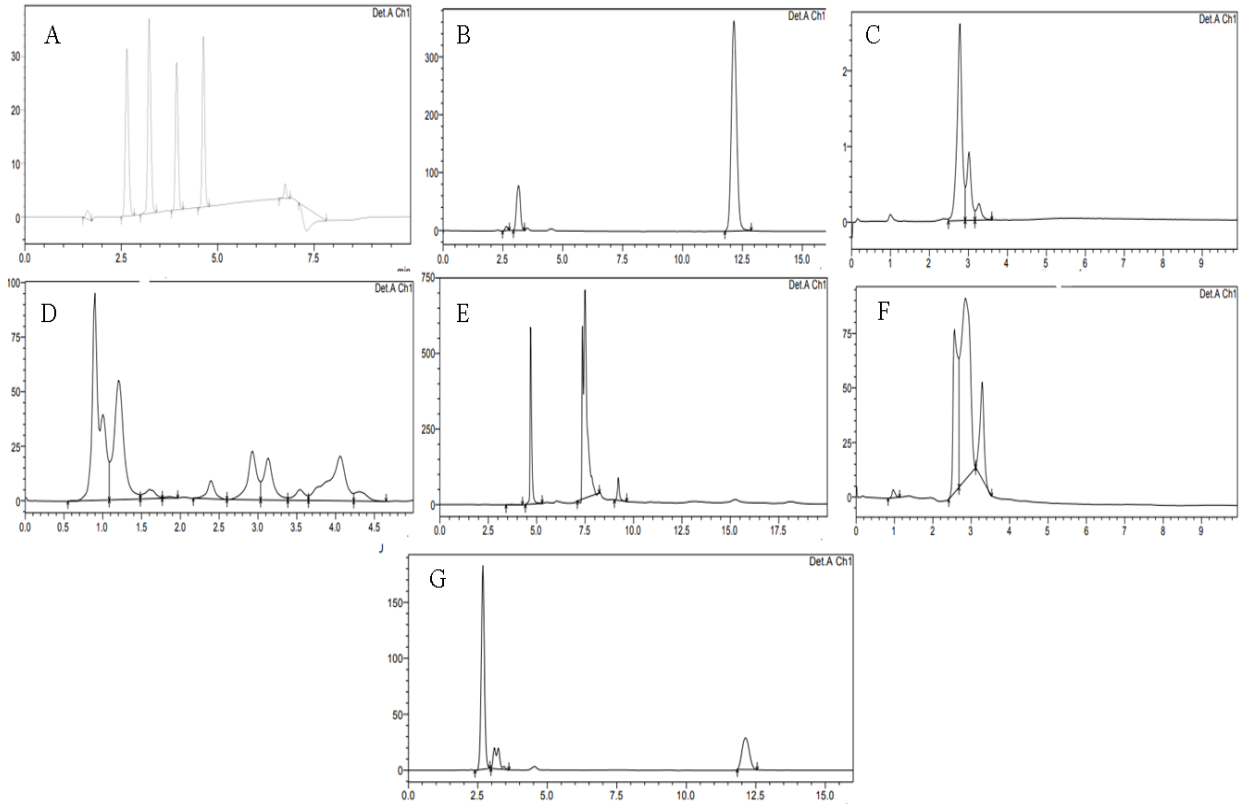

Figure 2: HPLC chromatograms of phenolic acids standard (A), shade-dried leaf extract (B), oven-dried leaf extract (C), shade-dried root extract (D), oven-dried root extract (E), oven-dried stalk extract (F) and oven-dried stalk extract (G)

Conclusion

The current study compared the phytochemical and phenolic acids found in *C. citratus* extracts from the stalk, roots, and leaves. Sufficient levels of phenolic acids, such as caffeic and coumaric acid, were present in the ethanolic extracts of *C. citratus* that have been shade- and oven-dried. Higher alkaloids found in the root sample can be used to create effective analgesics, anti-inflammatory, anti-cancer, neuropharmacological, antibacterial, and antifungal medications. The leaves had higher levels of phenolic acids, which can be used to create lead medications to treat oxidative illnesses. The oven-dried approach may be preferred to get a higher concentration of phenolic acids since the amount of phenolic acid observed in the dried extract was higher than that of the shade-dried extract. Nevertheless, other phytochemicals such as terpenoids, alkaloids, flavonoids, and saponins

were not well preserved by oven-drying. Thus, more research should be done to determine the ideal drying time, which would increase the plant's ability to produce a higher concentration of phytochemicals.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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