

Full Length Research Paper

Nutritional composition of cinnamon and clove powder and the evaluation of the antimicrobial properties of their extracts: A comparison between Ghana and other countries

Gamaliel Seth Tawiah Jnr. Lartey^{1*}, Josiah Wilson Tachie-Menson² and Stephen Adu¹

¹Animal Science Department, College of Agriculture and Natural Science, University of Cape-Coast, Ghana.

²Crop Science Department, College of Agriculture and Natural Science, University of Cape-Coast, Ghana.

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Current trends have suggested that the continuous use of chemical additives has carcinogenic effects on consumers. This has instigated the research into more organic ingredients such as plant parts which could serve as potential replacement for these additives in food. This study was therefore conducted to compare the proximate and mineral compositions of the powder of cinnamon bark and clove buds collected from Cape Coast-Ghana, and the effects of their extracts on the growth of selected food spoilage micro-organisms. The proximate analysis revealed both cinnamon and clove as rich carbohydrate sources (61.63 and 36.02%, respectively) with protein as their least components (3.44%). Mineral analysis of the samples revealed potassium as predominant in both cinnamon and clove powder (714.8 and 1296.2 mg/100 g) as both samples were found to be very low in magnesium (0.16 and 0.27 mg/100 g, respectively). The Agar well diffusion method was used to ascertain the inhibitory activity of cinnamon and clove oils on some common food spoilage microorganisms. The analysis revealed cinnamon as potent against *Escherichia coli* and *Salmonella Typhi* while clove showed a significant inhibitory activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Shigella*. In conclusion, the powder of cinnamon bark and clove buds could serve as good carbohydrate and potassium supplements in food while they contribute to increased shelf-life via inhibiting food-borne bacteria.

Key words: Proximate, cinnamaldehyde, eugenol, spices, palatability.

INTRODUCTION

Many plant parts have been used as spices for centuries. Spices refer to dry, aromatic parts of plants, which may include the seed, fruit, root, and bark, serving the purpose of colouring and flavour in foods (Savić et al.,

2019). As regards their specific uses, they vary from one country and culture to another due to their different uses in perfumery, religious rituals, medicine, cosmetics, and foods (Bouba et al., 2012). As there is an increasing

*Corresponding author. E-mail: lateyseth00@gmail.com.

global demand for safer foods, spices have become a more immediate refuge, especially, not only satisfying the safety demands of consumers, but also contributing to the enhancement of flavour and the nutritional quality of diets, and a potential replacement for food ingredients such as salt, sugar and added saturated fat which are less desirable in recipes (Ponzo et al., 2021). Spices have been particularly known for their role in modifying food texture (Mohammadi-Moghaddam et al., 2021), their use as prevention and cure for cardiovascular diseases (Rastogi et al., 2017), cancer and diabetes mellitus among other diseases, and their ability to enhance digestion (Peterson et al., 2019). These features and several others certainly have won spices their popularity in pharmacology and local medicinal practices including the food industry (Khanal et al., 2021). They have become very important in food preparation, not only for their flavour enhancement, but also their potential to replace already known chemical additives. For several decades, synthetic additives have been adopted in foods, having successfully slowed down or inhibit completely the activity of food spoilage micro-organisms, improved colour and flavour, and eventually extending shelf-life. The excessive or continuous use of these additives has been associated with allergic reactions, cancer and cardiovascular diseases (Juul et al., 2021). Over the years, several studies have been conducted to assess the nutritional, microbial and antioxidant properties of some plant parts and their potential to extend the shelf-life of food (Calo et al., 2015; Jayasena and Jo, 2013, 2014; Van Haute et al., 2016). Clove, a plant whose flower buds are normally used as spice has attracted quite a lot of attention lately, following its tremendous performance in terms of antioxidant property, compared with other spices (El-Saber Batiha et al., 2020). Having an 8 to 12 m sized tree, it is known to grow more frequently along the coastal areas at approximately 200 m above sea level. Studies have revealed that for a long time, it has been used for suppressing nausea and the urge to vomit, relieving pain and as an antiseptic (Batiha et al., 2020). Mbaveng and Kuete (2017) reported clove to be potent against gastro-intestinal spasm, stomach distension and flatulence. Besides its pharmaceutical essence, studies conducted have indicated that cloves have very good nutritional composition (Kumar Dey et al., 2021). Similar to clove, cinnamon has been reported to contain cinnamaldehyde, eugenol, cinnamic acid, cinnamyl alcohol, and coumarin, and has been beneficial to the pharmaceutical industry due to their antimicrobial and antioxidant properties. Kumar et al. (2019) revealed that cinnamon is proven to be potent in the management and cure of rheumatism, frigidity, vaginitis, impotence, neuralgia, dyspnoea, eye inflammations, leukorrhoea, and toothaches. For many years, it has also been an important part of the food industry, having been used as a flavouring agent in alcoholic beverages in Europe and a condiment in the preparation of chocolate in Mexico (Haddi et al., 2017). In Africa, particularly in Ghana,

cinnamon and clove are very popular plants whose parts (bark and buds, respectively) are used on a daily basis as flavour enhancers in food, performance of traditional rituals and massage therapies. This is due to its wide availability and cheap cost.

Therefore, this study investigated the nutritional and mineral composition of cinnamon collected from Ghana. The antimicrobial effects of cinnamon were also examined against *Shigella*, *Staphylococcus aureus*, *Salmonella* Typhi, *Pseudomonas* species, and *Escherichia coli*.

MATERIALS AND METHODS

Literature review

An extensive review of relevant literature on the nutritional composition and antimicrobial properties of cinnamon and clove was carried out for the purpose of comparison to the current study. Literature on similar studies conducted in at least five places around the globe (Sudan, Pakistan, India, Nigeria, Sokoto and Jordan) were acquired using various databases, including Science Direct (<http://www.sciencedirect.com>), Google Scholar (<http://www.scholar.google.com>), Scopus (<http://www.scopus.com>), and PubMed (<http://www.ncbi.nlm.nih.gov/pubmed>).

Sample collection and preparation

Dry samples of the bark of cinnamon tree and clove buds were obtained from the open market in Cape-Coast, Ghana. They were ground and stored in air-tight plastic containers in a cool dry place for subsequent use.

Proximate analysis

The assessment of proximate parameters of the plant materials were done to determine their storability and wholesomeness, and subsequently, food products treated with these plant materials. The proximate composition of the samples was determined using the methods of Thiex (2009) as the following.

Moisture content analysis

Ground sample (1.0 g) was weighed and placed in washed, dried and pre-weighed crucible. This was placed in an oven and dried at 105°C for 3 h. The sample was allowed to cool in a desiccator and then re-weighed. The percentage moisture content was calculated as the ratio of loss in weight on drying to the initial weight of the sample used and expressed as a percentage.

$$\% \text{ Moisture content (MC)} = \frac{W_o}{W_i} \times 100\% \quad (1)$$

where W_o = loss in weight (g) on drying and W_i = initial weight of sample (g).

Crude protein content

Determination of crude protein was done using the AOAC standard methods (Thiex, 2009). The total organic nitrogen was then measured using the following formula:

$$\% N = \frac{TV \times NE \times TVd}{Ms \times Vd} \quad (2)$$

where *TV* = Titre value, *NE* = mg nitrogen equivalent to molarity of acid, *TVd* = total volume to which digest was diluted, *Ms* = mass of sample (g), and *Vd* = volume of digest distilled.

$$\text{Percentage crude protein (CP)} = \% N \times 6.25 \quad (3)$$

Ether extract

Determination of ether extract of the samples was done using the Soxhlet apparatus, using petroleum ether (boiling range 400 to 600°C) as solvent. 3.0 g of the dried sample was weighed in triplicate and secured in the extraction thimble of the Soxhlet. The thimble was then put into the 20 cm³ capacity extractor compartment of the apparatus. A washed and oven-dried 100 cm³ round-bottomed flask was weighed and approximately 60 cm³ of the 40 to 600°C boiling range petroleum ether was poured into it. The flask was then mounted on the heating mantle and connected to the extractor (with condenser). The condenser and heating mantle were then activated and extraction carried on for 4 h. The solvent was evaporated when extraction was complete and the flask was dried in the oven (at 600°C). The flask was then cooled and reweighed. The percentage crude lipid was calculated using the formula:

$$\% CL = \frac{Mex}{Ms} \times 100\% \quad (4)$$

where *Mex* = mass of extract and *Ms* = Mass of sample used.

Carbohydrate

Total carbohydrate content of each sample was estimated by 'difference'. The sum of the percentages of all the other proximate components was subtracted from 100%, that is, total carbohydrate (%) = 100 – (% crude protein + % crude lipid + % ash + % crude fibre).

Mineral analysis

Sodium (Na), Potassium (K), Iron (Fe), Phosphorus (P), Magnesium (Mg), Calcium (Ca), Zinc (Zn), and Copper (Cu) in cinnamon and clove samples were determined using atomic absorption spectrophotometer (AA 6300, Shimadzo, Japan) equipped with flame and graphite furnace. Metal contents were determined with Air-acetylene flame. The following conditions were ensured in the operation of the instrument: acetylene run at 1.8 L/min while air flow was maintained at 15 L/min. Inert argon gas flow and the temperature parameters were followed as recommended by manufacturer.

Antimicrobial activity of cinnamon and clove

The Agar well diffusion method was used to ascertain the inhibitory activity of cinnamon and clove oils on some common food spoilage micro-organisms (*Shigella*, *Staphylococcus aureus*, *Salmonella* Typhi, *Pseudomonas* species, *Escherichia coli*). The microorganisms were collected from the Microbiology and Biotechnology Department of the College of Agriculture and Natural Science, University of Cape Coast, Ghana. Bacterial cultures were grown on MacConkey agar at 37°C for 24 h in an incubator

suspensions were prepared by introducing 3 to 5 distinct colonies of each test organism from their culture into 5 ml of sterile saline water aseptically. They were then vortexed (2 to 6 h) until a turbidity comparable to 0.5 McFarland standard solution was achieved ($\approx 1 \times 10^8$ cfu/ml). Wells (7.0 mm) were made in Petri dishes containing solidified Mueller-Hinton agar. The dry agar plates were seeded by streaking a sterile cotton swab dipped into the suspensions (Wayne, 2011). The plant ether extracts obtained as described earlier using petroleum ether and Soxhlet Apparatus were diluted in 2% dimethyl sulphoxide (DMSO) to constitute different concentrations of cinnamon and clove (25, 50, 75 and 100%). 40 µl of each concentration was added to the wells and plates were incubated for 24 h. 2% DMSO was used as the negative control while Ciprofloxacin (2 µg/ml water) served as positive control. The diameters of the inhibition zones were measured from the edge of the zone to the centre of the well with a ruler. Inhibition zones <12 mm were interpreted as microbial resistance, 12 to 16 mm as intermediate microbial susceptibility and >16 mm as complete microbial susceptibility.

Data analysis

All experiments were performed in triplicates and data collected were analysed using the Analysis of Variance (ANOVA) tool of GenStat (12th edition).

RESULTS AND DISCUSSION

Nutritional composition of cinnamon and clove

The study revealed very low protein composition in cinnamon, while largely consisting of carbohydrate. Similar to cinnamon, clove was also found to be high in carbohydrate but lowest in ash content. Table 1 provides the details of proximate composition cinnamon and clove powder.

Carbohydrates in spices have been suggested to be very essential in suppressing the negative effects of saturated fatty acids and cholesterol in diets, especially ones prepared for diabetics (Xue et al., 2017). Generally, almost all the widely used parts of cinnamon have been established as carbohydrate rich due to the high records of carbohydrate composition percentages relative to the rest of the proximate parameters.

The story was not different in this study. However, comparing this value (61.63%) to similar studies conducted, it was found to be higher than the 3.5% reported by Gul and Safdar (2009) and the range of 4 to 11% found by Haider et al. (2018), but lower than the percentages (80.59%) documented by Goel and Mishra (2020) and Sana et al. (2019).

Table 1 further showed clove carbohydrate composition as 36.02% which is lower than the value (68.6%) obtained by Ogunka-Nnoka and Mepba (2008) and 77.18% obtained from studies by Umar et al. (2006). However, it was slightly higher but comparable to the 31% obtained by Erefej et al. (2015) and 32.1% by Kaur et al. (2019). Protein composition of cinnamon (3.44%) in this study compares with that which was reported recently (Goel and Mishra, 2020; Sana et al., 2019).

Table 1. Proximate composition of cinnamon and clove.

Extract	Ether extract	Carbohydrate	Crude fibre	Crude protein	Moisture	Citation
Cinnamon	5.12±0.00	61.63±0.14	12.21± 0.13	3.44±0.03	14.07±0.04	Current Study
	4.5	52	33	3.5	5.1	Gul and Safdar (2009)
	1.72±0.22	55.13±0.04 ^b	65.55±0.52	4.09±0.07	10.8±0.15	Haider et al. (2018)
	1.24	80.59	-	3.99	-	Goel and Mishra (2020)
	2.99±0.05	70.3±2.53	17.14±1.01	3.01±0.03	4.55±0.0	Sana et al. (2019)
1.36 ± 0.02	84.98 ± 0.24	10.88 ± 0.53	6.31 ± 0.05	5.22 ± 0.20	Ng and Wan Rosli (2014)	
Clove	16.33±0.00	36.02±0.24	10.57±0.22	7.24±0.12	24.56±0.07	Current study
	9.3±0.1	68.6±0.2	1.1±0.1	7.8±0.2	12.1±0.3	Ogunka-Nnoka and Mepba (2008)
	5.5±1.00	77.18±0.01	4.00±1.50	2.32±1.16	36.00±8.5	Umar et al. (2006)
	4.3	31	31.2	9.3	-	Ereifej et al. (2015)
	5.86±0.03	32.1±0.05	14.37±0.04	6.91±0.37	29.47±0.08	Kaur et al. (2019)

Percentage Mean ± standard error of difference (S. E. D); number of replicates (n)=3.

Source: Authors

However, Ng and Wan Rosli (2014) and Haider et al. (2018) recorded slightly higher protein values. Several reports have indicated very low levels of protein in clove (less than 10%) with some as low as 1.2% (Sulieman et al., 2007). This study revealed a 7.24% protein composition, which was not different from the amounts documented by Kaur et al. (2019) and Ereifej et al. (2015) by much. It was however very similar to that of the 7.8% reported by Ogunka-Nnoka and Mepba (2008). Further in this study, cinnamon lipid composition was higher than most of the values from previous studies (Gul and Safdar, 2009). Dietary fat is established to be very important in ensuring the absorption and retention of flavour, thereby increasing palatability (Antia et al., 2006). On the other hand, very high levels have also been associated with cardiovascular complications.

Over the years, various studies have shown clove to contain varying percentages of fat. Though studies have shown values less than 10%, which is significantly lower than the 16.33% recorded in this study, Shafique et al. (2010) and Sulieman et al. (2007) also documented values which were above 10%. The relatively high levels of fat could be very important in enhancing palatability of food. However, this could also be a recipe for the quick onset of oxidative reactions which may result in rancidity in the spice. The sample of cinnamon used in this study was also found to be higher in moisture level (14.07%) than the range of values (4.5 - 11.9%) which were revealed elsewhere (Haider et al., 2018; Sana et al., 2019). Moisture levels in dry plant parts used for domestic purposes are known to play an important role in their storability. Though high levels have been suggested to help in maintaining the contents of cell protoplasm (Gbadamosi et al., 2011), levels above 10% have been established to expose food products to early spoilage (Ajuru et al., 2017). The relatively higher level of moisture in the sample from this study establishes more

vulnerability to microbial invasion over time if not stored properly. The moisture content of clove (24.56%) was lower than the percentage of moisture reported in studies previously conducted by Umar et al. (2006) and Kaur et al. (2019), while Shafique et al. (2010) and Sulieman et al. (2007) observed significantly low outcomes.

The differences seen in the percentage composition of the parameters between different studies is suspected to be due to length of drying time and the variation of cinnamon and clove due to differences in varieties, growth conditions, harvesting times, soil properties, climate, origin, environmental conditions and geographic parameters. Due to this, researchers have recorded a wide range of percentage composition, hence, and not specific standards.

Mineral composition of cinnamon and clove

Results from the mineral analysis of cinnamon revealed that the highest mineral composition was potassium, as was the case for clove. Interestingly, both plants had their lowest composition to be magnesium. The rest of the details are presented in Table 2.

The results from the mineral analysis revealed potassium as the most predominant inorganic constituent in both cinnamon and clove (714.8 g/100 g and 1296 g/100 g, respectively), while they both had the least values in magnesium composition (0.1641 g/100 g and 0.2722 g/100 g, respectively). This agrees with the findings of Sana et al. (2019) in which potassium constituted the majority of the mineral composition of cinnamon whereas Umar et al. (2006) and Kaur et al. (2019) previously revealed potassium percentage composition as the highest among other minerals in clove. Potassium has been documented as characteristically highly present in most plant foods and

Table 2. Mineral composition of cinnamon and clove.

Extract	K	Na	P	Fe	Cu	Zn	Ca	Mg	Citation
Cinnamon	714.8±2.93	45.28±0.08	127.3±2.89	23.85±0.22	15.1±0.40	22.8±0.11	1.16±0.01	0.16±0.00	Current Study
	134.7	0.0	42.4	7.0	-	2.6	83.8	85.5	Gul and Safdar (2009)
	272.00±2.65	5.60±0.16	40.00±2.65	1.72±0.10	0.35±0.19	0.48±0.23	594.00±2.65	39.60±0.43	Haider et al. (2018)
	431	-	60	8.32	0.339	1.83	1002	60	Goel and Mishra (2020)
Clove	122.5±9.21	1.2±0.02	-	6.0±0.01	-	2.01±0.01	75±4.10	-	Sana et al. (2019)
	1296±11.13	291.1±1.07	174.1±0.57	34.61±0.09	22.7±0.65	23.57±0.23	1.51±0.01	0.27±0.01	Current study
	275.10±0.10	19.50±0.18	3.51±0.48	9.45±2.25	2.00±0.12	23.89±2.80	5.28±0.03	180.88±2.43	Umar et al. (2006)
	111.6	61.6	1.6	8.3	0.4	1.4	117.5	196.8	Ereifej et al. (2015)

Results are presented in mg/100 g; number of replicates (n)=3.
Source: Authors

is essential for the maintenance of balance in water, electrolyte, acid, and base in the body while ensuring proper nerve and muscle functioning as well (Tazoe et al., 2007). The second highest occurring mineral component in cinnamon was phosphorus. This was corroborative to Goel and Mishra (2020), whose work revealed phosphorus as following calcium in the order of percentage composition. Its occurrence in such a quantity makes cinnamon a good addition to food for the maintenance of regular heart contraction, osteosynthesis, general body cell growth and regulation of blood sugar level as indicated for the role of phosphorus (Indrayan et al., 2005). Sodium was identified as the second most predominant mineral in clove. In similar studies conducted earlier, the levels of sodium have been revealed to be present at very significant levels.

Both cinnamon and clove exhibited a deficiency in calcium and magnesium, having had the lowest composition levels compared to the other mineral components in the analysis of the two plant samples. The nutritional composition of plant materials varied across geographical locations due to the differences in climate and growth

conditions. As a result, there are no established standard amounts of the nutritional components expected as they are strictly dependent on a combined parameters of growth conditions, species and the part of the plant. Additionally, plant parts used as spicing agents are seldom expected to be major sources of nutrients. They largely serve as flavour enhancers, shelf life extending agents.

Antimicrobial evaluation of cinnamon and cloves at different concentrations

The potential of cinnamon and clove extracts to inhibit microbial growth was evaluated. *E. coli* was the most susceptible to all the concentrations of the extracts used while *Shigella* was the most resistant. Details of results are presented in Table 3.

E. coli is known to be one of the most occurring bacteria in the gastro-intestinal tract of humans. In small numbers they have been found to be commensals and useful in the reduction of the numbers of pathogenic microbes. This however

could change and make them the causative organisms for a number of infections when their load increases. In this study, a quite significant positive result was achieved as the growth of *E. coli* was totally inhibited by all cinnamon concentrations applied. It was observed that the activity of cinnamon extract on the organism was similar to that of the standard antimicrobial agent, ciprofloxacin used as a positive control. This is in agreement with Muthuswamy et al. (2008) who achieved a 94% *E. coli* inhibition using 2% w/v ethanol extract. Similar, all except the concentrated extract (100%) of the cloves significantly inhibited the growth of *E. coli*, revealing the plant extract as equally potent as ciprofloxacin. While cinnamon could not inhibit the growth of *P. aeruginosa*, clove exhibited a good antimicrobial potential against the organism, except for the least concentration used (25 %). Ethanol extract of clove has been previously proven to be effective against a number of food related pathogens. The study further revealed cinnamon as equally potent against *S. aureus* as ciprofloxacin, with an inhibition zone of 13.5 mm for the extract at 100% concentration and 12.0

Table 3. Antimicrobial activity of ethanol extract of cinnamon and clove (inhibition zones measured in mm.).

Extract	Percentage Conc.	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>S. Typhi</i>	<i>Shigella</i>	F. Pr.
Cinnamon	100	30.00±0.00	6.50±0.50	13.50±0.50	12.50±0.50	8.00±1.00	<.001
	75	30.00±0.00	6.50±6.50	11.00±0.00	14.00±3.00	8.00±1.00	0.019
	50	30.00±0.00	10.00±3.00	11.00±0.00	16.50±0.50	8.5±2.50	0.002
	25	30.00±0.00	3.50±3.50	12.00±3.00	9.50±0.50	5.00±1.00	0.002
	Pos. cont.	30.00±0.00	3.50±3.5	12.00±3.00	9.50±0.50	5.00±1.00	0.002
	Neg. cont.		R	R	R	R	R
Clove	100	9.5±0.5	12±4.5	18.5±3.0	10±3.0	10±0.0	0.191
	75	18.5±3.5	15±3.0	22.5±0.5	7±0.0	18±1.0	0.025
	50	22.5±5.5	18.0±0.0	12±4.0	9±2.0	17.5±1.5	0.153
	25	18±6.0	9±0.0	8.5±2.5	2±2.0	9±0.0	0.101
	Pos. cont.	18.5±0.5	11.5±0.5	12.5±0.5	27.5±0.5	14.5	<.001
	Neg. cont.		R	R	R	R	R

Means ± standard error of difference (S.E.D); number of replicates (n)=3; R = Resistant; Inhibition zones measured in mm; F. Pr. = Frequency probability; Conc.=concentration.

Source: Authors

mm for 25% concentration as against 12.0 mm for ciprofloxacin. Similarly, clove exhibited a significant *S. aureus* growth inhibition, with the first three concentrations (100, 75 and 50%) recording from moderate to high inhibitory activity. *S. typhi* was susceptible to the first three concentrations of cinnamon (100, 75 and 50%) while at 25%, the extract could not inhibit its growth. Meanwhile, the organism was resistant to clove. *Shigella* was found to be highly resistant to cinnamon at all concentrations, with inhibition zone measurements of less than 9 mm. Clove on the other hand exhibited a good inhibitory effect at 75 and 50% concentrations against *Shigella*.

Gram-negative bacteria are generally known to be more resistant to the components of plant extracts than their Gram-positive counterparts due to the complex nature of their cell membrane (Kowalska et al., 2021). The cell membranes of Gram-negative bacteria contain a thick layer of lipopolysaccharide outer membrane covering the cell wall. This makes them impermeable to hydrophobic substances such as essential oils. However, recent studies into the mechanism of action of plant extracts on bacteria suggest a by-pass to the factors that contribute to their resistance. A study by Zhang et al. (2016) reported *E. coli* resistance to the treatment of cinnamon at minimum inhibitory concentrations, but as the concentration increased, the relative electric conductivity (an index for determining the permeability of bacterial cell membrane to materials) increased, basically suggesting an electrolyte leakage caused by a disruption in the cell membrane. Further studies suggest that the disruption of the cell membrane of bacteria is greatly enhanced by the presence of significantly high levels of phenolic compounds present in some essential oils, as they are able to easily penetrate the phospholipid bilayer

of the bacterial cell wall, enabling them to bind with and suppress the action of the proteins that enhance cellular activity (Vasconcelos et al., 2018). The resistance of some organisms to cinnamon and clove extracts could be attributed to evidence produced by Torrens et al. (2019), whose research revealed that some Gram-negative bacteria have an added advantage of a more conserved peptidoglycan cell wall structure than others; making them more adamant to changes, which could be forced by external conditions such as antibiotic treatment.

Conclusion

Apart from the documented pharmaceutical importance of cinnamon and clove due to their essential oil constituents, the current study revealed significant levels of nutritional parameters such as carbohydrates and fibre which support several other studies suggesting high levels of carbohydrate in these plant parts. This makes them suitable in supplementing meals while simultaneously improving their flavour to enhance palatability.

The study also revealed both cinnamon and clove as very good sources of both major and minor elements which play significant roles in bone, blood and enzyme activities.

Findings from this study proved a strong additional preliminary scientific endorsement of cinnamon and clove as antibiotics, and a possible replacement for chemical additives in food, helping to alleviate the fear and concerns of their effects by consumers.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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