Journal of Food Innovation, Nutrition, and Environmental Sciences
September, 2026, Volume 3; Issue 3: 362–371
ISSN No: 3078-5537

ORIGINAL RESEARCH ARTICLE

Phytochemical Profiles and Free Radical-Scavenging Properties of Extracts and Smoke-Water from Amakamo Vessel Fumigation Plants

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DOI: https://doi.org/10.70851/jfines.2026.3(3).362.371

ABSTRACT

Traditional *amakamo* processors in Kiruhura District, Uganda, fumigate milk fermentation vessels with certain plant stem-wood, but information on the phytochemical characteristics of the wood extracts and smoke-derived aqueous preparations remains limited. The study descriptively analysed methanolic, hydroethanolic and smoke-water extracts (SWE) of *Rhus natalensis*, *Combretum molle* and *Gardenia ternifolia*. Three independent stem-wood batches were collected for each species and independently extracted with methanol and 50% ethanol. Three independent SWE preparations were produced using individual smoked gourds. Qualitative phytochemical screening, total phenolic content (TPC), total flavonoid content (TFC), percentage DPPH inhibition and IC_{50} were determined and summarized as mean ±SD (n=3 independent preparations). Extraction yields for methanol ranged from 15.14±0.58% to 23.01±0.10%, while hydroethanolic yields ranged from 16.96±0.12% to 23.76±0.20%. Phenols and flavonoids were detected in all preparations. *Combretum molle* SWE had the highest mean TPC (387.27± 0.00 mg GAE/g) and TFC (7.59 ±0.62 mg RE/g) among all preparations. Hydroethanolic *Combretum molle* exhibited the highest mean % DPPH inhibition (97.33 ±0.58%), whereas methanolic *Combretum molle* had the lowest IC_{50} (0.040 ±0.002 mg/mL). The findings demonstrate that phytochemical and radical-scavenging profiles varied by plant species and preparation method. Because SWE was generated through smoldering, smoke deposition and aqueous recovery rather than direct solvent extraction, its high numerical TPC/TFC values should not be interpreted as evidence of superior extraction efficiency. More studies are required to further characterise the chemical constituents of smoke and evaluate the effects of vessel fumigation on *amakamo* quality and safety.

Article history
Received;
23 July, 2026
Revised;
26 August, 2026
Accepted;
01 September, 2026

Keywords
Combretum molle
Rhus natalensis
Gardenia ternifolia
Fermented milk

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Peer review under responsibility of Journal of Food Innovations, Nutrition, and Environmental Sciences.

A Publication of EcoScribe Publishers company Limited, Uganda. www.ecoscribepublishers.com

1. INTRODUCTION

Amakamo is traditional fermented milk widely consumed by cattle-keeping communities in Kiruhura district, south-western Uganda. During its production, the milk processors use smoke-treated vessels, including gourds and plastic containers, for fermentation. Smoking is achieved by exposing the inner surfaces of these vessels to smoke generated from specific plant species. Among the plants used by processors in Kiruhura district are *Rhus natalensis* Bernh. ex C. Krauss (*R. natalensis*), *Combretum molle* R.Br. ex G. Don (*C. molle*) and *Gardenia ternifolia* (Welw.) Verdc.(*G. ternifolia*), belonging to the Anacardiaceae, Combretaceae and Rubiaceae families, respectively (Gulaita et al., 2025). Beyond their role in milk processing, these plants have been documented for various traditional medicinal and household applications, including the management of infections, wounds, gastrointestinal and respiratory conditions, as well as use as firewood (Anywar et al., 2021; Silén et al., 2023; Tamene et al., 2024). These ethnographic uses suggest longstanding indigenous recognition of their functional properties. Their deliberate selection for smoking *amakamo* fermentation vessels may therefore reflect accumulated indigenous knowledge rather than random selection.

The process of fumigating fermentation vessels was described by Gulaita et al. (2025). During vessel smoking, smouldering wood from the selected plants is placed inside the milk-processing vessel, which is covered for approximately 10 min. to allow smoke to accumulate. The vessel is then opened briefly to release excess smoke. This smoking and aeration cycle is repeated three times to achieve adequate fumigation. Following the final cycle, residual charcoal and burnt plant particles are removed using a small, locally made fish net-like material known as *akakubiiso*. Smoking milk-processing vessels has been associated with reduced microbial contamination, prolonged shelf life and development of the characteristic smoky flavour and aroma of traditionally fermented milk (Karenzi et al., 2013; Wanjala et al., 2016; Wayua et al., 2012). These effects may partly arise from antimicrobial and antioxidant compounds generated during wood combustion and deposited on the vessel surfaces.

Smoke is a complex aerosol comprising water vapour, condensed liquid droplets, solid particles and gas-phase compounds (Zhang et al., 2020). Its chemical profile may include phenols and their derivatives, organic acids, carbonyl compounds such as aldehydes and ketones, alcohols, furans, hydrocarbons and other volatile and semi-volatile compounds, whose relative abundance influences the sensory and functional characteristics of smoked foods (Kwaghvihi et al., 2020; Lingbeck et al., 2014). Smoke composition varies with plant species and smoking conditions, with phenolic compounds, organic acids and carbonyls contributing to smoky flavour and aroma as well as antioxidant and antimicrobial properties (Lingbeck et al., 2014; Nizio et al., 2023; Sokamte tegang et al., 2020). During smouldering, cellulose, hemicellulose and lignin undergo thermal degradation: cellulose and hemicellulose generate compounds including carbonyls, organic acids and furan derivatives, whereas lignin is an important source of phenolic compounds such as guaiacol, syringol and their derivatives. Thus, the chemical and functional characteristics of smoke are influenced by the composition of the plant material from which it is generated.

Phenolic compounds can contribute to the stability of milk and dairy products by scavenging free radicals and chelating pro-oxidant metal ions within the food matrix, thereby limiting lipid peroxidation, undesirable flavour development and loss of oxidation-sensitive nutrients (Granato et al., 2018). Phenolic compounds may also inhibit spoilage and pathogenic microorganisms, while flavonoids have similarly demonstrated antioxidant and antimicrobial activities (Mahmoud, 2023; Parveen et al., 2025; Ullah et al., 2022; Wu et al., 2024). These properties are particularly relevant to traditional *amakamo* processing because the milk does not interact directly with the intact smoking plants, but rather with smoke and smoke-derived compounds deposited on the inner surfaces of fermentation vessels. During smouldering, thermal degradation may destroy or transform existing plant constituents and generate new compounds with properties different from those of the original plant material.

Wood smoke can be characterised directly using analytical techniques such as GC-MS, while smoke-derived compounds can also be captured in aqueous media as smoke-water for evaluation of their chemical and biological properties (Lingbeck et al., 2014; Sokamte tegang et al., 2020). Phenolic and other bioactive compounds identified in wood smoke have been associated with antioxidant, antibacterial and antifungal activities, although their composition and concentrations vary among plant species and smoking conditions (Suryani et al., 2022; Toledo, 2007; Ullah et al., 2022). Smoke-water therefore provides an approach for investigating water-soluble smoke-derived constituents generated during smouldering. Comparison of smoke-water with methanolic and hydroethanolic extracts of the unburnt plant material may provide insight into differences in the recovery of phenolic and flavonoid constituents and their associated free-radical-scavenging activities.

Although the phytochemical and biological properties of *C. molle*, *R. natalensis* and *G. ternifolia* have previously been investigated, limited information is available on the phytochemical and antioxidant characteristics of smoke generated from these plants under conditions relevant to traditional *amakamo* processing. This information is important because these species are among the preferred plants used by milk processors in Kiruhura district for smoking fermentation vessels (Gulaita et al., 2025) , yet the biochemical basis for their selection remains poorly understood. The present study therefore determined the total phenolic content, total flavonoid content and free-radical-scavenging activity of methanolic, hydroethanolic and smoke-water preparations of *R. natalensis*, *C. molle* and *G. ternifolia*. Comparing smoke-water with extracts of the unburnt plant material provides insight into whether phytochemical and antioxidant properties associated with these plants are retained, altered or differently expressed following smouldering, thereby providing scientific evidence for evaluating indigenous plant-selection practices in traditional *amakamo* processing.

2. MATERIALS AND METHODS

2.1 Plant selection and preparation

Plant stem-woods and ready-to-use fermentation gourds were obtained from traditional milk processors in Kiruhura district.

The stem-woods used in this study include those of *Rhus natalensis* Bernh. ex C. Krauss (Voucher GN03), *Combretum molle* R. Br. ex G. Don (GN07) and *Gardenia ternifolia* (Welw.) Verdc. (GN15) belonging to the Anacardiaceae, Combretaceae and Rubiaceae families, respectively. Selection of these plant species was informed by prior field documentation of plants used to smoke milk fermentation vessels (Gulaita et al., 2025). Whole-plant specimens were identified by a senior botanist at the Makerere University Herbarium and voucher specimens deposited there. Traditional processors used dry stem-wood from mature plants. Representative smoking sticks were approximately 1 m long, and diameter of *R. natalensis* (3 cm), *C. molle* (3.5 cm) and *G. ternifolia* (4 cm). Three independent batches of stem-wood were collected for each species. These independent plant batches were collected from Kiruhura Town Council, Rushere Town Council and Kenshunga Sub- County in Nyabushozi County, Kiruhura district in November, 2024.

The dry stem-woods were cleaned, cut manually into approximately 1 cm pieces and further dried in the vacuum air oven (Biogene laboratories) at 40°C±2 for about 6 h to reduce variation in moisture content. The pieces were regularly turned during drying and then milled into fine powders using an electric grinder (DE-500g). The powders were wrapped in aluminum foil and maintained under dry conditions at 40°C±2 before extraction.

2.2 Methanolic and hydroethanolic extraction and extraction yield

Plant powders were extracted using absolute methanol (HPLC grade, Bharat Scientific World, Bengaluru), and 50% ethanol (Analytical grade, Sigma-Aldrich), following Zhang et al. (2018) with modifications. Each of the three independently collected batches of each species was extracted separately with each solvent, giving three independent methanolic and three independent hydroethanolic extracts per species. Approximately 50g of powder was mixed with 250 mL of solvents (1:5 w/v), stirred, covered with aluminum foil and kept in the dark for 72 h with regular shaking. The mixtures were filtered through muslin cloth and then Whatman® No. 4, 125 diameter filter paper. The filtrates were concentrated using a rotary evaporator (Eyela Aspirator-A38, Tokyo Japan), transferred into pre-weighed beakers and dried at 40°C±2. Extraction yield (%) was calculated as mass of dried extract divided by the initial mass of dry powder multiplied by 100. Dried extracts were stored at 4°C until analysis.

2.3 Preparation of smoke-water extracts (SWE)

Smoke-water extracts were prepared following the protocol by Tano-Debrah et al. (2007) with modifications designed to simulate the local smoking practice as described by Gulaita et al. (2025). Ready-to-use gourds obtained from processors, had been cleaned using *Hoslundia opposita Vahl (H. opposita)* and water without soap or synthetic detergent and dried before use. Experimental gourds were cleaned with only *H. opposita*. Gourds of approximately 2-L capacity were used. The exact external dimensions were not recorded; capacity was used as the standardization criterion. Three separate gourds were used to prepare three independent SWE preparations for each plant species.

One end of approximately 1 m dry stem-wood stick was ignited and the visible flame extinguished, leaving the wood smouldering. The smouldering end, rather than the whole stick was introduced into the gourd, which was covered with aluminum foil to retain smoke for 10 min. The gourd was opened to release smoke and the smoking cycle was repeated three times. Wood mass consumed was not quantified because the traditional practice does not suggest a known quantity. Smoke exposure was standardized by the 10 min duration and three repeated cycles. After the final cycle, loose fragments were removed and 100 mL of distilled water added. The gourd was covered and left for 17 h with occasional shaking. This time corresponded to that reported by the traditional processors. Smoke-water extracts were collected and stored at 4°C. Distilled water treated under the same conditions in a cleaned, non-smoked gourd served as the SWE blank during spectrophotometric measurements.

For quantitative phytochemical assays, SWE were lyophilized to obtain a dry residue. The residue was weighed and reconstituted in distilled water to 1 mg/mL, providing a dry-residue basis for total phenolic content (TPC) and total flavonoid content (TFC) determination. Percentage extraction yields relative to starting wood were not calculated for SWE because the mass of wood consumed during smoldering was not quantified.

2.4 Qualitative phytochemical screening of plant extracts

Qualitative phytochemical screening of the extracts was carried out using standard procedures as described by Sorescu et al. (2018). To test for saponins, equal volumes of extract and distilled water were shaken for 15 min in a graduated cylinder and observed for foaming. The size of the foam formed corresponded to the concentration of saponins in the extract. To test for tannins, to 1 mL of extract was added 2 mL of 5% ferric chloride (Alpha Chemika, India). Formation of dark-blue or greenish-black color indicated presence of tannins. Presence of phenolics was determined by placing 0.5 mL of the extract into a clean test tube, followed by addition of 10-fold diluted Folin–Ciocalteu (BDH, UK), reagent and 2 mL of 0.7 M sodium carbonate (BDH, England). A dark blue colour indicated presence of phenolics. For alkaloids, 2 mL of the extract were placed into a test tube and then three drops of a saturated picric acid solution (Hager's reagent, Spectrum Chemical, USA) were added. Formation of a yellow precipitate indicated presence of alkaloids. For flavonoids, three drops of 20% sodium hydroxide (Fischer Scientific, UK), were added to 2 mL of extract in a test tube, a yellow colour indicated their presence.

2.5 Total phenolic content (TPC)

The total phenolic content of the extracts was determined using Folin–Ciocalteu assay as described by Bravo-Monzón et al. (2022). The stock plant extracts were dissolved in corresponding solvents to get a concentration of 1 mg/mL; lyophilized SWE residue was reconstituted in distilled water to the same concentration. From each extract, 0.7 mL was pipetted into Falcon tubes and mixed with 1.5 mL of 10% Folin–Ciocalteu reagent and the mixture vortexed for 10 seconds. The tubes were then covered, incubated at room temperature for 30 min. Thereafter, 5.8 mL of 0.7 M sodium carbonate were added, the tubes were vortexed, covered and incubated for 2 h at room temperature, after which they were centrifuged at 500 rpm (Lab

Junction, India) for 4 min. The supernatant layer which composed of the extract of interest was carefully poured into separate cuvettes in triplicates. The absorbance of the solvent blanks and the corresponding extracts were measured using a UV-Vis spectrophotometer (Thermo Fischer Scientific- US) at 735nm.

To prepare the standard curve, Gallic acid (GA, Sigma-Aldrich, England), was used. Stock GA solution was prepared by dissolving 500mg of GA in one liter of distilled water to obtain a concentration of 500mg/L. The stock solution was serially diluted to obtain 250 mg/L, 200mg/L, 150mg/L, 100 mg/L, 50 mg/L and 25 mg/L. A blank without GA was also prepared and labelled 0 mg/L. The steps for determining the TPC were followed with the standard GA instead of the extracts. A graph of absorbance values against GA standard concentrations (**Figure 1**) was plotted to create a standard curve.

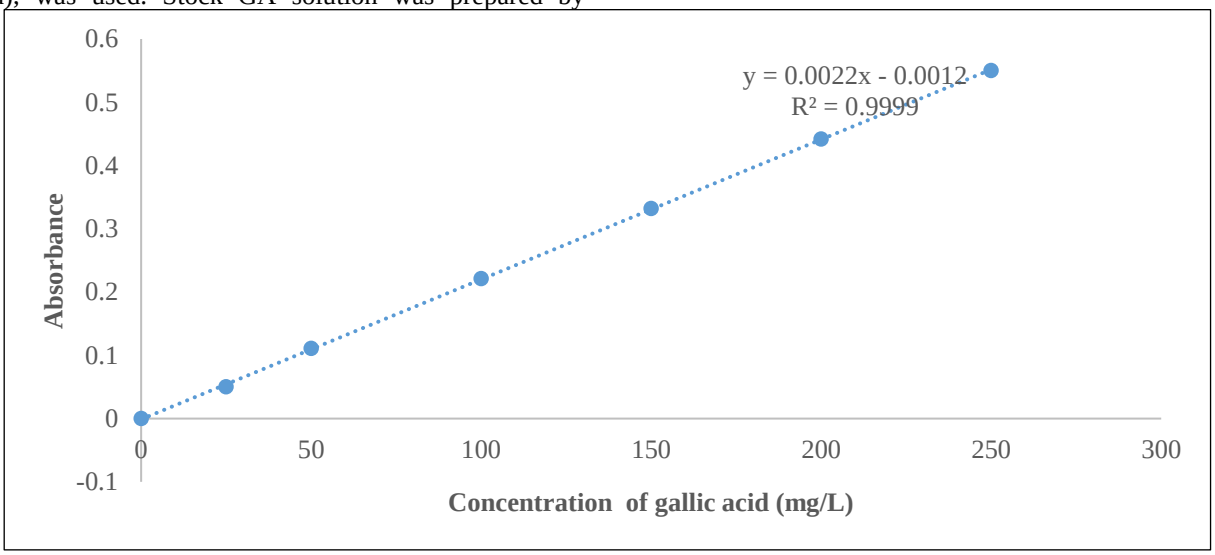

Fig. 1. Standard gallic acid calibration curve

The regression equation of the standard curve (y=0.0022x-0.0012, R^2 =0.9999) was used to calculate the TPC of the plant extract. The results were expressed as milligram of gallic acid equivalents per gram of extract or lyophilized residue (mg GAE/g) after calculation using the formula in "eqn. (1)"

$$TPC = \frac{CV}{M}, \quad \text{(eqn. 1)}$$

Where:

TPC= total phenolic content in mg/g of extract as gallic acid equivalent

C= concentration of Gallic acid established from the calibration curve in mg/mL

V= volume of extract in mL

m= weight of the stock plant extract in g

2.6 Total flavonoid content (TFC)

The total flavonoid content of the extracts was analysed following the method described by Pavun et al. (2018). The stock plant extracts were dissolved in respective solvents to obtain a 1 mg/mL concentration; lyophilized SWE residue was reconstituted in distilled water to the same concentration. For each extract, 0.5 mL were mixed with 1.5 mL of 95% ethanol followed by 0.1 mL 10% aluminum nitrate (Sigma-Aldrich, England), and 0.1 mL of 1 M potassium acetate (Pure-lab chem industries, India), and finally 2.8 mL of deionized water. The mixture was vortexed and incubated at room temperature for 30 min. The absorbance of the solvent blanks and the corresponding extracts were measured using a UV-Vis spectrophotometer at 415 nm. The Rutin standard curve was prepared by dissolving 10 mg of Rutin (Otto Kemi, India) in 10 mL of 80% ethanol to get a concentration of 1mg /mL of stock solution. The stock solution was further diluted in 80% (v/v) ethanol to obtain concentrations of 6.25, 12.5, 25, 50 and 65 µg/mL. A similar procedure described for analyzing total flavonoids in extracts was followed for Rutin and the absorbances at different concentrations were recorded. The standard curve was obtained by plotting absorbance values against the various concentrations of Rutin as shown in **Figure 2**

The regression equation of the standard curve (y=0.0134x + 0.0036, R^2 =0.9999) was used to calculate the total flavonoid content of the plant extract. The results were expressed as TFC milligram of Rutin equivalent per gram of extract or lyophilized residue (mg RE /g) after calculation using the following formula in "eqn. (2)"

$$TFC = \frac{CV}{M} \quad \text{(eqn. 2)}$$

Where:

TFC= total flavonoid content in mg/g of extract as Rutin equivalent (RE)

C= concentration of Rutin established from the calibration curve in mg/mL

V= volume of extract in mL

m= weight of the stock plant extract in g

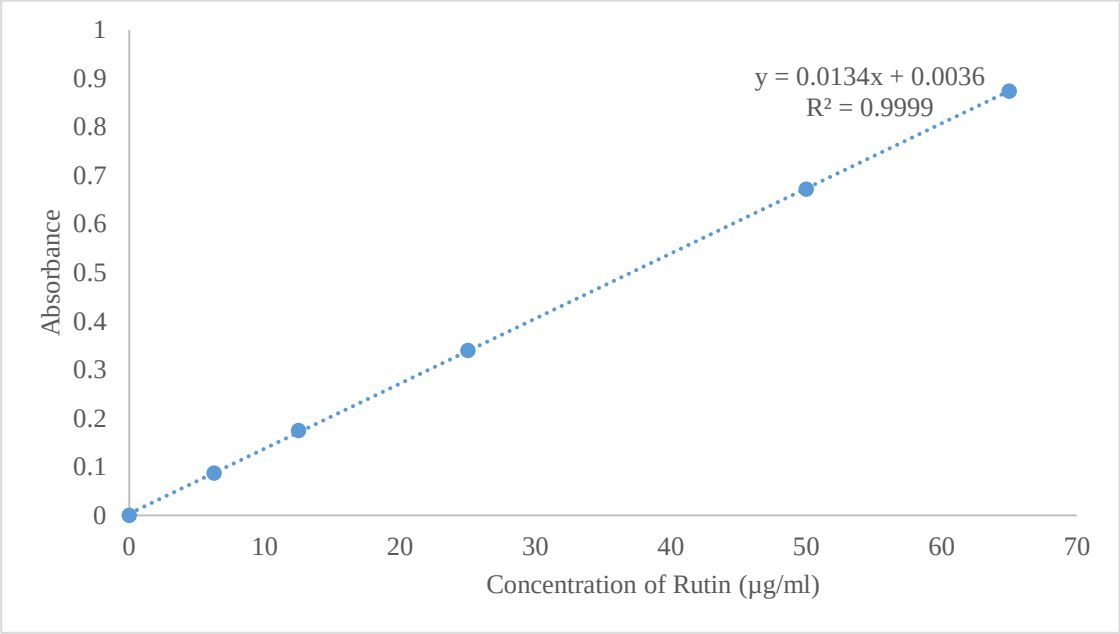

Fig.2. Rutin standard curve

2.7 Radical scavenging activity

Plant and smoke-water extracts were evaluated for radical scavenging capacity using stable DPPH reagent (Alfa Aesar, USA: Lot: R10H030), following the conventional method as employed by Akar et al. (2017) with some slight modifications. The plant extracts and standard L-ascorbic acid (BDH, UK) were dissolved in their respective solvents to obtain a 1mg/mL concentration; lyophilized SWE residue was reconstituted in distilled water to the same concentration. To 750 µL of extracts was added 750µL of 2000µM of methanolic DPPH solution. The contents were vortexed and incubated for 60 min at room temperature in darkness. Absorbance of the samples and control was read at 517 nm using the UV-Vis Spectrophotometer. The absorbance of methanolic DPPH (blank) was also taken and used to calculate the percentage DPPH inhibition using the formula in "eqn. (3)"

$$\% \text{ DPPH inhibition} = \frac{(AO-AC)*100}{AO} \quad (3)$$

Where:
A0= absorbance of control (that is DPPH solution)
AC= absorbance of sample or standard

2.8 Determination of IC_{50}

The IC_{50} of the plant extracts, SWE and standard L-Ascorbic acid, were determined following the protocol described by Akar et al. (2017). The plant extracts, lyophilized SWE and standard L-Ascorbic acid in triplicates were dissolved in their respective solvents to obtain a 2 mg/mL concentration. This concentration was serially reduced from 2, 1, 0.5, 0.25, 0.125 and 0 mg/mL. To 750µL of the extract or standard, 750µL of 2000µM of methanolic DPPH solution was added. The contents were vortexed and incubated for 60 min at room temperature in darkness. Absorbances of the mixtures were read at 517 nm using the UV-Vis Spectrophotometer. The absorbance of methanolic DPPH (blank) was also taken and used to calculate the %DPPH inhibition in "eq. (3)". The % DPPH inhibition values were plotted against concentrations of the extracts and standard. These graphs were then used to calculate the IC_{50}, from the "eqn. (4)"

$$x = \frac{50-b}{a} \quad \text{(eqn. 4)}$$

Where: x= $IC_{50,}$ b = y-intercept, a = slope.
Extracts with the lowest IC_{50} values had the highest radical scavenging activity.

2.9 Data analysis

Qualitative phytochemical screening data was summarized using positive (+), strongly positive (++), or negative (-) reactions based on the observed assay response. Results of the quantitative outcomes from the three independent preparations for each plant species and preparation method were summarized descriptively as means ± standard deviation (SD) using the Statistical Package for Social Sciences (SPSS IBM® Statistics 27) software.

3. RESULTS

3.1 Extraction yield

Extraction yield varied amongst plant species and between solvents. The highest yield in methanolic extracts was observed in *G. ternifolia* (23.01±0.10%) followed by *R. natalensis* (16.40±0.77%). Similarly, hydroethanolic extracts exhibited the highest yield in *G. ternifolia* (23.76±0.20%) followed by *C. molle* (23.34±0.34%) (**Table 1**). The extraction yield of SWE relative to starting wood mass was not calculated.

Table 1. Extraction yields (%) of *R. natalensis*, *C. molle* and *G. ternifolia* stem wood in methanol and hydroethanol

Plant species	Methanolic yield (% mean ±SD, n=3)	Hydroethanolic yield (% mean ±SD, n=3)
R. natalensis	16.40±0.77	16.96±0.12
C. molle	15.14±0.58	23.34±0.34
G. ternifolia	23.01±0.10	23.76±0.20

3.2 Qualitative phytochemical composition

Results of qualitative phytochemical screening of plant extracts and SWE preparations are given in **Table 2**. Phenols and flavonoids were detected in all methanolic, hydroethanolic and SWE preparations. Saponins were detected in all hydroethanolic extracts and SWE preparations, but were not detected in the methanolic extracts. Alkaloids were detected only in the methanolic extracts of *C. molle* and *G. ternifolia*. The deionised water control was negative for all screened phytochemicals.

Table 2. Qualitative phytochemical composition of methanolic, hydroethanolic and SWE preparations of *R. natalensis, C. molle* and *G. ternifolia* used in vessel fumigation

Plant	Preparation	Phenols	Flavonoid	Saponins	Alkaloids
R. natalensis	Methanolic	+	+	-	-
	Hydroethanolic	+	+	++	-
	SWE	++	+	+	-
C. molle	Methanolic	++	++	-	+
	Hydroethanolic	++	++	++	-
	SWE	++	+	++	-
G. ternifolia	Methanolic	++	++	-	+
	Hydroethanolic	++	++	++	-
	SWE	+	+	+	-
Control		-	-	-	-

Key: (+) = positive; (++) =strongly positive; (-) = negative; SWE= Smoke-water extract

3.3 Total phenolic and flavonoid content

The TPC and TFC varied across plant species and preparation methods. Among methanolic preparations, *G. ternifolia* showed the highest mean TPC (265.75±6.39 mg GAE/g), whereas *C. molle* had the highest mean TFC (4.52±0.13 mg RE/g). In the hydroethanolic preparations, *C. molle* had the highest mean TPC (283.35 ± 7.49 mg GAE/g) and TFC (3.34 ±0.10 mg RE/g). Among SWE preparations, *C. molle* had the highest mean TPC (387.27±0.00 mg GAE/g) and TFC (7.59±0.62 mg RE/g) (**Table 3**)

Table 3. Total phenolic and flavonoid contents of *R. natalensis, C. molle* and *G. ternifolia* used in vessel fumigation

Preparation	Plant species	TPC (mg GAE/g)	TFC (mg RE/g)
Methanolic	*R. natalensis*	245.70±6.05	1.12±0.09
	C. molle	235.62±11.12	4.52±0.13
	G. ternifolia	265.75±6.39	4.14±0.11
Hydroethanolic	*R. natalensis*	206.14±7.48	1.62±0.45
	C. molle	283.35±7.49	3.34 ± 10
	G. ternifolia	245.81± 28.49	1.69±0.23
SWE	*R. natalensis*	302.97±2.92	5.83±0.17
	C. molle	387.27±0.00	7.59±0.62
	G. ternifolia	256.00±0.45	2.65±0.19

Key: SWE = smoke water extracts; GAE =gallic acid equivalent; RE =Rutin equivalent. Each value in the table is expressed as Mean ± Standard Deviation (n=3)

3.4 Radical scavenging activity and IC_{50}

The highest mean % DPPH inhibition among the three preparations was observed in hydroethanolic extracts of *C. molle* (97.33±0.58%), whereas the lowest IC_{50} was observed in methanolic extracts of *C. molle* (0.040±0.002 mg/mL). Among the SWE preparations, *C. molle* had the highest mean DPPH inhibition (91.22±0.28%) as well as the lowest IC_{50} (0.391±0.001 mg/mL). The L-ascorbic acid positive control showed 99.23±0.01 % inhibition and an IC_{50} of 0.023 ±0.000 mg/mL. Therefore, the hydroethanolic extracts of *C. molle* and its corresponding methanolic extracts had their % DPPH inhibition and IC_{50} closest to those of L-ascorbic acid (**Table 4**).

Table 4. Radical scavenging activity of *R. natalensis, C. molle* and *G. ternifolia* used in vessel fumigation

Preparation	Plant species	DPPH inhibition (%)	IC_{50} DPPH (mg/mL)
Methanolic	*R. natalensis*	81.67±0.58	0.200 ± 0.003
	C. molle	87.00±0.00	0.040 ± 0.002
	G. ternifolia	82.36±0.58	0.250 ± 0.001
Hydroethanolic	*R. natalensis*	95.67±0.58	0.402 ± 0.001
	C. molle	97.33±0.58	0.200 ± 0.003
	G. ternifolia	88.00±0.02	0.587± 0.001
SWE	*R. natalensis*	76.45±2.05	0.461 ± 0.001
	C. molle	91.22±0.28	0.391 ± 0.001
	G. ternifolia	80.22±1.02	0.611 ± 0.001
Positive control		99.23±0.01	0.023±0.000

Key: SWE = smoke water extracts; Positive control=Standard L-ascorbic acid; Each value in the table is expressed as Mean ± Standard Deviation (n=3).

4. DISCUSSION

The study set out to characterise the phytochemical composition and radical-scavenging activities of *R. natalensis*, *C. molle*, and *G. ternifolia* stem-woods used in the fumigation of fermentation vessels for *amakamo* processing. This involved screening for selected phytochemicals in methanolic, hydroethanolic and SWE preparations. Additionally, total phenolic and flavonoid content; DPPH radical-scavenging activity and IC_{50} were determined for the same preparations. The study showed that phytochemical and radical-scavenging profiles varied based on plant species and preparation method. No single preparation consistently produced the highest values across extraction yield, TPC, TFC, % DPPH inhibition and IC_{50}, indicating preparation-dependent profiles rather than overall superiority of one extraction or traditional treatment.

The fact that phenols and flavonoids were detected in all the three plants and across the three preparations indicates that the stem-woods used in traditional *amakamo* vessel fumigation contain components that are recoverable by the frequently used phytochemical methods. These findings are consistent with other studies that reported presence of phenolic and flavonoid constituents in *R. natalensis*, *C. molle* and *G. ternifolia*. However, our findings cannot be compared numerically directly because of the differences in the plant part, geographical area, extraction conditions and calibration standards used (Alqasoumi et al., 2016; Camara et al., 2023; Ntshanka et al., 2020; Parusnath et al., 2023).

Additionally, the effect of solvent on total extraction yield was dependent on the plant species. Hydroethanol recovered substantially more material than methanol from *C. molle*, while the two solvents gave almost similar yields for *R. natalensis* and *G. ternifolia*. Such patterns may reflect differences in the chemical composition of the wood matrix and the range of the compounds recovered by solvents of different polarity (Dai & Mumper, 2010; Lee et al., 2024). Noteworthy, higher extraction yield did not consistently correspond to higher TPC, TFC or DPPH response. Therefore, extract yield should not be regarded as a measure of phytochemical quantities or radical-scavenging strength.

The SWE preparations of *C. molle* and *R. natalensis* exhibited slightly more average TPC and TFC values. However, much as the SWE was lyophilized, weighed and reconstituted to 1 mg/mL, the quantitative assays of TPC and TFC in the methanolic and hydroethanolic extracts were expressed on a dry-residue basis. The SWE were generated through smoldering, smoke transfer and deposition followed by aqueous recovery, rather than direct solvent extraction of powdered wood. Furthermore, SWE extraction yield relative to the starting wood could not be calculated because the amount of wood consumed during smoking was not quantified. Therefore, the high values of TPC and TFC in some SWE preparations demonstrate recovery of Folin-reactive and flavonoid- reactive constituents from smoke-derived material, but do not establish SWE as a superior extraction method.

The DPPH assay values also showed that no single preparation was consistently highest across all measurements. Hydroethanolic *C. molle* showed the highest average %DPPH inhibition at 1 mg/mL, while methanolic *C. molle* had the lowest IC_{50}. Conversely, *C. molle* had the highest mean %DPPH inhibition at 1 mg/mL and lowest IC_{50} among the three SWE preparations. These patterns show that % DPPH inhibition at 1 mg/mL and IC_{50} should be interpreted as related but with different forms of DPPH radical-scavenging response.

Therefore, caution should be taken against attributing radical-scavenging activity entirely to TPC or TFC. Individual compound composition and interactions among constituents may contribute to the observed response.

The TPC value in the methanolic *C. molle* detected in this study was higher than the values previously reported for methanolic leaf extracts by Ntshanka et al. (2020). On the contrary, the values of TFC were lower than those reported in that study. These differences should be interpreted cautiously because the present study examined stem-woods and expressed TFC as rutin equivalents, whereas other studies may use leaves or bark and different flavonoid standards such as quercetin equivalents. Such differences in methodologies limit exact numerical comparisons across studies.

The present findings provide chemical characterization relevant to understanding traditional fumigation practice, but they do not demonstrate preservation of *amakamo*, antimicrobial effectiveness in milk, extension of product shelf life or safety of smoke exposure. Smoke may contain both potentially functional compounds and undesirable contaminants, including polycyclic aromatic hydrocarbons, depending on the wood type and combustion conditions (Nizio et al., 2023). These outcomes require direct evaluation in the food system before conclusions about preservation efficacy or safety can be made.

5. STUDY LIMITATIONS

Although three independent preparations for each plant species and preparation method were performed, these only allowed characterization of variation among preparations. The smaller number of replicates limits broader statistical generalization. The findings were therefore interpreted descriptively. In addition, extraction yield relative to starting material was determined for methanolic and hydroethanolic preparations but not for SWE because the amount of wood consumed during smoldering was not quantified. The Folin–Ciocalteu, total flavonoid and DPPH assays provide aggregate measures of assay-reactive constituents and radical-scavenging response but do not identify individual compounds. This limitation is particularly relevant to SWE because smoldering thermally transforms the original plant material. The study also did not quantify transfer of smoke-derived compounds into *amakamo*, preservation effects or potentially undesirable smoke-derived contaminants. The findings should therefore be interpreted as baseline exploratory chemical characterization rather than evidence establishing preservation efficacy or safety.

6. CONCLUSION

Methanolic, hydroethanolic and smoke-water preparations of *R. natalensis*, *C. molle* and *G. ternifolia* contained phenolic and flavonoid constituents and showed measurable DPPH radical-scavenging activity. The numerical values varied by species and preparation method. Smoke-water extract preparations of *C. molle* showed the highest mean TPC and TFC among the preparations studied. Hydroethanolic *C. molle* had the highest mean %DPPH inhibition, whereas methanolic *C. molle* exhibited the lowest IC_{50}. These observations demonstrate that no single preparation is superior, because extraction yield, solvent polarity and the different mechanism of SWE preparation affect the nature of the recovered material.

Further studies should be carried out to identify individual smoke-and wood-derived compounds using chromatographic and spectrophotometric techniques such as GC-MS or LC-MS. Direct studies in amakamo are recommended to establish the effect of vessel fumigation on its quality and safety. Additional studies to evaluate the toxigenic effects of smoke from vessel fumigation is necessary.

FUNDING STATEMENT

Data collection for this study was funded by Government of Uganda through the Makerere University Research and Innovation Fund (Mak-RIF 4- PhD track).

CONFLICT OF INTEREST

The authors declare no conflict of interest

ACKNOWLEDGEMENT

The authors appreciate the traditional milk processors who provided information about the plants that were used in this study. We equally thank Mr. William Mukama, Dr. Ambrose Mukisa and Mr. Joseph Owor for the guidance during laboratory analyses.

ETHICAL CONSIDERATIONS

The study was approved by the Makerere University School of Social Sciences Research Ethics Committee (MAKSSREC 08.2024.773). Written informed consent was obtained from all the milk processors before being interviewed. All interviews were conducted in the local language.

DATA AVAILABILITY

The data used to support the findings of this study are available from the corresponding author upon request.

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