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Phytochemical profiling, antioxidant and anti-inflammatory activities of four West African spices incorporated as bioactive ingredients for dermatological formulations

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Research Article

Keywords: African spices, antioxidant activity, anti-inflammatory activity, cold saponification, cosmeceuticals, dermotherapeutic formulations, Xylopia aethiopica, Tetrapleura tetraptera, Monodora myristica, Ricinodendron heudelotii

Posted Date: June 9th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9964544/v1

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Additional Declarations: The authors declare no competing interests.

Abstract

In this study, we evaluated antioxidant and anti-inflammatory activities of four West African culinary spices, *Xylopia aethiopica*, *Tetrapleura tetraptera*, *Monodora myristica*, and *Ricinodendron heudelotii*, and their suitability as bioactive ingredients in cold-process soap formulations. TLC Phytochemical screening revealed the presence of several groups of secondary metabolites, including flavonoids, phenolic acids, tannins, saponins, terpenes, and anthraquinones, which were partially preserved in dermatological formulations after saponification. *Monodora myristica* exhibited the highest total polyphenol content (437.52 ± 28.56 mg GAE/g DW) and the strongest anti-inflammatory activity (IC_{50} = 159.95 ± 72.6 µg/mL), while aqueous extracts of *Xylopia aethiopica* showed the most potent DPPH radical-scavenging activity (IC_{50} = 8.04 ± 4.4 µg/mL). All formulated soap bars met cosmeceutical quality standards for skin-contact products, and foaming performance correlated with species-specific saponin profiles. These findings support the use of these four African spices as ingredients in dermatological formulations.

1. INTRODUCTION

Skin disorders constitute one of the most prevalent categories of health complaints worldwide, spanning a broad clinical spectrum that includes chronic inflammation, oxidative damage, and barrier dysfunction. In sub-Saharan Africa, dermatological conditions are compounded by limited access to modern healthcare, the high cost of pharmaceutical preparations, and the disproportionate burden of infectious skin diseases driven by environmental and socioeconomic factors (WHO 2019; Otimanam et al, 2020). As a result, the management of cutaneous conditions has long relied on plant-based remedies, a tradition that reflects millennia of empirical refinement and represents a significant component of the healthcare infrastructure in the region. The World Health Organization estimates that more than 80% of the global population depends on traditional medicine for primary healthcare needs (WHO, 2019), and phytotherapy for skin disorders occupies a particularly prominent position within this framework (Muthu et al. 2006).

Among the most versatile therapeutic plant resources in Central and West Africa are aromatic spices, which occupy a dual role as culinary flavouring agents and medicinal preparations. *Xylopia aethiopica* (Dunal) A. Rich. (Annonaceae), known as African pepper or alligator pepper, is widely used in Cameroon, Côte d'Ivoire, and the Democratic Republic of the Congo for the treatment of bronchitis, rheumatism, and skin infections (Bouba 2009). *Tetrapleura tetraptera* (Schum. & Thonn.) Taub. (Fabaceae), locally called Aridan or four-sided fruit, is used across West Africa in the management of convulsions, post-partum inflammation, and cutaneous parasitic diseases (Adusei et al. 2019). *Monodora myristica* (Gaertn.) Dunal (Annonaceae), the African nutmeg or calabash nutmeg, is employed as a spice and as a remedy for headache, toothache, and inflammatory conditions of the skin (Kabran et al. 2012). *Ricinodendron heudelotii* (Baill.) Pierre ex Heckel (Euphorbiaceae), known as Njangsa or Bosongo, features in traditional medicinal preparations used for gastrointestinal complaints and skin conditions in Cameroon and the DRC (Odinga Tamuno 2016).

To date, the biological activities of these African spices have been the subject of scattered investigations, but no study has evaluated all four species in parallel within the same experimental framework. Previous reports have characterised individual species: Bouba (2009**)** evaluated the antioxidant activities of *X. aethiopica* and related spices; Kabran et al. (2012) quantified polyphenols in *M. myristica*. Adusei et al. (2019) investigated the antioxidant properties of *T. tetraptera*. These studies, however, employed different extraction methods, assay conditions, and reference standards, making direct comparison difficult and leaving the relative bioactivity profiles of the four species unclear. Specifically, a particularly underexplored avenue is the incorporation of these spices into topical dermatological formulations. Yet, soap formulations remain among the most accessible and universally used personal hygiene products and constitute an effective vehicle for the topical delivery of plant bioactive compounds. Dermotherapeutic soaps enriched with plant extracts have also attracted growing interest as affordable, accessible alternatives to synthetic dermatological preparations, particularly in resource-limited settings such as in sub-Saharan Africa (Otimanam et al. 2020; Elaka et al. 2020). Additionally, cold-process saponification, conducted at temperatures between 30 and 40°C, is known to be well suited to preserve thermolabile plant phytochemicals that would otherwise be degraded during hot-process manufacturing. Here, we report for the first time a comparative evaluation of *X. aethiopica*, *T. tetraptera*, *M. myristica*, and *R. heudelotii* within a unified experimental framework. To better characterize the four plants investigated, we first examined their microscopical features for botanical authentication. Additionally, we determined their chromatographic fingerprints and quantified polyphenol and flavonoid contents. Furthermore, we evaluated antioxidant and anti-inflammatory activities of aqueous and organic extracts. Lastly, we formulated dermatological soap bars using cold saponification and assessed their quality control (pH, moisture content, foaming capacity, and total mass).

2. RESULTS AND DISCUSSION

2.1. Microscopic features of powders

The four plant materials were purchased at the Sandaga market, Douala, Cameroon (Fig. 1). Micrographic examination with Steinmetz reagent revealed species-specific histological elements that confirmed the botanical identity of each material (Table 1; **Figures S1–S8** of the Supporting Information). In *X. aethiopica*, diagnostic features included fibre fragments, starch grains, oil droplets, tannin cells, and distinct, orange-colored droplets. *T. tetraptera* was characterised by abundant fibres, starch grains clustered in aggregates, and oil droplets of varying size, alongside unique structural elements requiring further elucidation. *M. myristica* showed spherical starch grains, fibre fragments, and trichomes. *R. heudelotii* was distinguished from the other three species by the presence of urchin-like calcium oxalate crystal aggregates, in addition to starch grains, oil droplets, and fibre fragments. These novel species-specific microscopic elements present promising avenues for future characterization.

The structural elements identified in each species were consistent with expected anatomical features. The tannin-containing cells observed in *X. aethiopica* are an expected feature of a species with a well-documented polyphenolic richness (Bouba 2009), while the urchin-like calcium oxalate crystals detected in *R. heudelotii* are a recognised taxonomic characteristic of the Euphorbiaceae (Odinga Tamuno 2016). The trichomes observed in *M. myristica* are consistent with earlier anatomical descriptions of Annonaceae fruit organs (Liyongo Inkoto et al. 2018; Tshilanda et al. 2019). These features support the authenticity of the plant materials collected and constitute a useful botanic and pharmacognostic reference for the quality control of these raw materials in future cosmeceutical applications (Mukherjee 2019).

Table 1
Micrographic features of the four plant powders observed at 10× and 40× magnification using the Steinmetz reagent.

Species	Plant Part	Micrographic features
Xylopia aethiopica	Fruit	Fiber fragments, starch grains, oil droplets, tannin cells
Tetrapleura tetraptera	Fruit	Abundant fibers, clustered starch grains, oil droplets
Monodora myristica	Fruit	Spherical starch grains, fiber fragments, trichomes
Ricinodendron heudelotii	Fruit	Urchin-like calcium oxalate crystal aggregates, starch grains, oil droplets, fiber fragments

2.2. Phytochemical screening by thin-layer chromatography

TLC screening revealed a rich and diverse secondary metabolite profile across the four species and the corresponding soap fractions (Table 2; Figs. 2–4). Flavonoids and phenolic acids were most prominent in the powder extracts of *X. aethiopica* and *T. tetraptera*, identified by blue, fluorescent spots (phenolic acids) and yellow to green, fluorescent spots (flavonoids) at 366 nm after Neu reagent staining. Saponins were mostly pronounced in *T. tetraptera*, with a faint signal in *X. aethiopica* and negligible presence in the remaining two species. Terpenes were detected across all species in varying intensities. Tannins and anthraquinones were present in most species, while coumarins and iridoids showed a more restricted distribution. No alkaloids were detected under the conditions applied. The TLC profiles of the four species are broadly consistent with previously reported phytochemistry: the detection of phenolic acids and flavonoids in *X. aethiopica* is consistent with its known composition of hydroxy-cinnamic acid derivatives and C-glycosyl flavones (Bouba 2009); the strong saponin signal in *T. tetraptera* aligns with its characteristic aridanin and related saponins (Adusei et al. 2019; Houmènou et al. 2018); and the near-absence of saponins and the weak flavonoid profile in *R. heudelotii* are consistent with the limited phytochemical literature for this species (Odinga Tamuno 2016).

The phytochemical profiles of soap extracts were also generally consistent with those of the corresponding plant powders, though with attenuated TLC band intensities, indicating partial but measurable retention of secondary metabolites through cold saponification. The reduction in band intensity is expected, as the alkaline soap matrix partially hydrolyses ester-linked phenolic compounds and dilutes the extract concentration. The persistence of flavonoids, tannins, and saponins in the finished soap products justifies the choice of cold saponification over hot-process methods for this application.

Table 2
TLC screening of secondary metabolites in plant powder extracts and soap extracts. ++ = strongly present; + = present; +/− = trace; − = absent. XP, TP, MP, RP: powder extracts of *X. aethiopica*, *T. tetraptera*, *M. myristica*, and *R. heudelotii*, respectively; S: soap formulated with dry extract; L: soap formulated with aqueous macerate.

Sample	Iridoids	Anthocyanins	Anthraquinones	Tannins	Coumarins	Flavonoids	Phenolic acids	Saponins	Terpenes
XP	+	+	++	++	+	++	++	+	++
TP	+	++	++	++	−	+	+	++	+/−
MP	++	+	++	++	+	+	+	+/−	+
RP	+/−	−	−	−	−	−	+/−	+/−	+/−
XS	+	+	+	+	+	+	+	+	+
TS	+	+	+	+	−	+	+	+	+
MS	+	+/−	+	+	+	+	+	+/−	+
RS	+/−	−	−	−	−	+/−	+/−	+/−	+/−
XL	−	+	+	+	+	+	+	+	+
TL	−	+	+	+	−	+	+	+	+/−
ML	+	−	+	+	+	+/−	+	−	+
RL	−	−	−	−	−	−	−	−	−

2.3. Total polyphenol and flavonoid contents

Results of total polyphenol and flavonoid contents for all four species are summarized in Table 3. *Monodora myristica* exhibited the highest total polyphenol content (437.52 ± 28.56 mg GAE/g DW), followed by *X. aethiopica* (351.84 ± 5.71), *T. tetraptera* (332.80 ± 3.30), and *R. heudelotii* (257.60 ± 14.28 mg GAE/g DW). Total flavonoid contents were more comparable across species, ranging from 10.48 ± 0.22 mg QE/g DW in *R. heudelotii* to 14.91 ± 0.48 mg QE/g DW in *T. tetraptera*. The comparatively narrow range of flavonoid contents across the four species (10.48 to 14.91 mg QE/g DW) suggests that the non-flavonoid fraction, including hydrolysable tannins, phenolic acids, and related compounds, likely drives the differences observed in total polyphenol content, particularly the high value recorded for *M. myristica*. Polyphenols are recognized as potent antioxidants, and their radical scavenging capacity has been studied experimentally and computationally confirmed for plants rich in benzoic acid derivatives (Isamura et al. 2023). The total polyphenol contents of these studied spices are consistent with their antiradical activity against ABTS, in which *M. myristica* aqueous extracts showed reproducible antioxidant activity despite a flavonoid content similar to that of the other species (Salem et al. 2018).

Table 3
Total flavonoid and polyphenol contents of the four plant extracts (mean ± SD, n = 3). QE: quercetin equivalents; GAE: gallic acid equivalents; DW: dry weight.

Species	Total flavonoids (mg QE/g DW)	Total polyphenols (mg GAE/g DW)
Xylopia aethiopica	14.54 ± 0.39	351.84 ± 5.71
Tetrapleura tetraptera	14.91 ± 0.48	332.80 ± 3.30
Monodora myristica	12.22 ± 0.25	437.52 ± 28.56
Ricinodendron heudelotii	10.48 ± 0.22	257.60 ± 14.28

2.4. Antioxidant activity

The IC_{50} values for DPPH and ABTS radical scavenging activity are presented in Table 4 and Fig. 5. The aqueous extract of *X. aethiopica* showed the strongest DPPH scavenging activity (IC_{50} = 8.04 ± 4.4 µg/mL), similar to quercetin, the reference standard (IC_{50} = 3.67 ± 0.49 µg/mL). The organic extract of *X. aethiopica* exhibited moderate-to-low DPPH scavenging activity (IC_{50} = 405.51 ± 68.2 µg/mL). Against the ABTS radical, aqueous extracts of *X. aethiopica* and *T. tetraptera* produced the lowest IC_{50} values (30.97 ± 2.1 and 50.23 ± 1.9 µg/mL,

respectively). Across all four species, aqueous extracts outperformed organic extracts in both assays. Statistical differences between aqueous and organic fractions were significant for *X. aethiopica* ($p < 0.0001$ for ABTS; $p > 0.9999$ for DPPH), *T. tetraptera* ($p < 0.0001$ for ABTS), and *M. myristica* ($p < 0.0001$ for ABTS). *R. heudelotii* showed the weakest antioxidant activity across both assays (DPPH IC_{50} = 3288.52 ± 6 µg/mL for the aqueous extract; $p = 0.0943$ between extract types for DPPH). The IC_{50} of the organic extract of *R. heudelotii* could not be determined for ABTS within the concentration range tested.

Table 4
IC_{50} values (µg/mL) for DPPH and ABTS radical scavenging by aqueous and organic extracts (mean ± SD, n = 4). ND: not determined within the tested concentration range.

Species	**Part**	**Extract**	**ABTS IC_{50} (µg/mL)**	**DPPH IC_{50} (µg/mL)**
Xylopia aethiopica	Fruit	Aqueous	30.97 ± 2.1	8.04 ± 4.4
		Organic ($MeOH/CH_2Cl_2$)	187.50 ± 11.7	405.51 ± 68.2
Tetrapleura tetraptera	Fruit	Aqueous	50.23 ± 1.9	92.90 ± 4.6
		Organic	120.78 ± 10.4	82.41 ± 4.6
Monodora myristica	Fruit	Aqueous	103.99 ± 3.1	429.54 ± 63.9
		Organic	437.52 ± 94.8	677.64 ± 354.1
Ricinodendron heudelotii	Fruit	Aqueous	74.99 ± 6.9	3288.52 ± 6.0
		Organic	ND	250.03 ± 51.4
Quercetin (standard)			2.55 ± 0.06	3.67 ± 0.49

The strong DPPH scavenging activity of the aqueous extract of *X. aethiopica* is relatively similar to the value of 17.44 ± 0.2 µg/mL reported by Bouba (2009**)** for the same species. The obtained IC_{50} value of 8.04 ± 4.4 µg/mL could be due to enhanced extraction of polar antioxidants, particularly hydroxycinnamic acid derivatives, through an extended 24-hour aqueous maceration used in the present study. The ABTS assay is performed using a mixed aqueous-organic medium which allows the capture of both hydrophilic and lipophilic antioxidants (Floegel et al. 2011). For this reason, the ABTS assay yielded lower IC_{50} values than DPPH across the aqueous extracts, suggesting that the antioxidant potency of these four species may be conferred mainly by polar, water-soluble phytochemical compounds. Additionally, the differences in plant origin, harvest season, drying methods, and solvent polarity are well-established factors leading to inter-study variability in phytochemical screening research (Fraenkel 1959; Kubola and Siriamornpun 2008; Elaka et al. 2020; Muipata et al. 2025). The superiority of *in vitro* antioxidant activities of aqueous over organic extracts across all species in both assays provides practical advantages of using aqueous macerates: aqueous macerates are eco-friendly, less costly to prepare, and can also be incorporated directly into cold-process soap formulations without additional solvent removal.

2.5. Anti-inflammatory activity

The anti-inflammatory activity evaluated by inhibition of thermal egg albumin denaturation in aqueous extracts is presented in Table 5. *Monodora myristica* exhibited the strongest activity (IC_{50} = 159.95 ± 72.6 µg/mL), followed by *Xylopia aethiopica* (IC_{50} = 184.50 ± 60.3 µg/mL) and *T. tetraptera* (IC_{50} = 537.03 ± 156.3 µg/mL). *R. heudelotii* was essentially inactive.

The thermal denaturation of proteins is a well-validated *in vitro* model for the assessment of anti-inflammatory activity, as the conformational disruption of proteins is one of the molecular hallmarks of tissue inflammation and is a mechanism by which anti-inflammatory agents exert protective effects (Masengo et al. 2023; Mostefaoui et al. 2022; Fokou et al. 2013). The IC_{50} values obtained for *M. myristica* and *X. aethiopica* suggest potent anti-inflammatory activity of these botanicals. To the best of our knowledge, this study is the first to report evaluation of the anti-inflammatory activity of *M. myristica* using the thermal protein denaturation method. Given the potent anti-inflammatory capacity and the high polyphenol content, *M. myristica* emerges as the most promising candidate among the four species for further *in vivo* biological and mechanistic studies, including evaluation of cyclooxygenase inhibition and cytokine modulation. Although extracts of *R. heudelotii* have been shown to be inactive, fractionation studies are needed to determine the active fractions or whether essential oil extracts could reveal anti-inflammatory compounds that are present but inaccessible under aqueous maceration conditions.

Table 5
IC_{50} values (µg/mL) for inhibition of thermal albumin denaturation by aqueous extracts (mean ± SD, n = 3). Sodium diclofenac was used as the positive control.

Plant	*X. aethiopica*	*T. tetraptera*	*M. myristica*	*R. heudelotii*	Diclofenac
IC^{50} (µg/mL)	184.50 ± 60.3	537.03 ± 156.3	159.95 ± 72.6	44,771 ± 40.6	28.45 ± 4.8

2.6. Soap formulation and quality control

The successful formulation of 40 soap bars across eight distinct formulations, representing four species in two extract types each, yielded cosmeceutical products that satisfy all physicochemical requirements for safe dermotherapeutic use. The quality control data reported in **Tables S1–S2** of the Supporting Information constitute a proof of concept for the development of plant-derived cosmeceutical soaps from West African culinary spices.

All measured pH values (10.23 to 10.76) fell within the well-established optimal range of 9 to 11 for cold-process soaps intended for skin contact (Abbane et al. 2024), as summarized in Table 6. This mildly alkaline environment does not disrupt the natural acid mantle of the skin (pH 4.5 to 6.5) but is sufficient to confer a passive bacteriostatic environment through the inherent antimicrobial properties of the alkaline soap matrix (Babeker 2023). It is worth noting that the aqueous formulations of *X. aethiopica* recorded the highest pH values (10.76), which may reflect a higher residual mineral content from the aqueous macerate, while *R. heudelotii* formulations recorded the lowest values (10.23 and 10.26 for powder and aqueous, respectively), consistent with the minimal secondary metabolite content of this species.

Table 6
pH, moisture content (%), and mass (g) of formulated soap bars. Full foaming power data across all water types is provided in Table S2 of the Supporting Information.

Parameter	Extract Type	*X. aethiopica*	*T. tetraptera*	*M. myristica*	*R. heudelotii*
pH	Powder	10.35	10.33	10.30	10.23
	Aqueous	10.76	10.40	10.41	10.26
Moisture (%)	Powder	5.67	9.78	5.19	9.81
	Aqueous	3.30	5.98	4.47	5.36
Mass (g)	Powder	110	110	106	106
	Aqueous	106	106	110	110

Moisture content, determined by the ISO 672–1978 gravimetric method (Babeker 2023), ranged from 3.30% to 9.81%, values that are uniformly well within the ISO threshold of 13 to 16%. Low moisture content is a critical cosmeceutical quality indicator: it correlates with greater physical hardness and longer bar life during consumer use, while simultaneously reducing the risk of microbial contamination within the product during extended storage. The dry-extract formulations of *T. tetraptera* and *R. heudelotii* showed the highest moisture values among the powder-type soaps (9.78% and 9.81%, respectively), likely because the particulate plant powder creates micro-spaces within the saponified matrix that transiently retain water during curing.

The foaming power results yield the most striking evidence for bioactivity-formulation concordance in the present study (Fig. 9; **Figures S11–S14**). *T. tetraptera*-enriched soaps achieved foam column heights of 4.0 cm in distilled and tap water, while *M. myristica* powder soaps reached the overall maximum of 5.0 cm in tap water. This performance reflects the saponin profiles observed by TLC phytochemical screening, where *T. tetraptera* showed greater saponin bands among all four species, and saponins are known to be the primary phytochemical class responsible for foam formation through their surface-active amphiphilic structure. In resource-limited settings such as Central and West Africa, where these spices are already culturally embedded in traditional skin-care practice, this herbal-based formulation approach could offer a scientifically validated, low-cost, and scalable pathway to affordable cosmeceutical products.

3. EXPERIMENTAL

3.1. Chemicals and reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), potassium persulfate, and aluminium trichloride were purchased from Sigma-Aldrich (Bornem, Belgium). Folin–Ciocalteu reagent, sodium carbonate, and all TLC detection

reagents (Neu reagent, ferric chloride, Dragendorff reagent, sulfuric anisaldehyde, phosphoric vanillin) were of analytical grade. Gallic acid (purity ≥ 97%) and quercetin (purity ≥ 98.5%) were used as reference standards. For TLC derivatization, the Neu reagent was prepared as a 1% (w/v) solution by dissolving 1.0 g of 2-aminoethyl diphenylborinate in 100 mL of methanol, which was paired with a 5% (w/v) Polyethylene Glycol 4000 solution in absolute ethanol as a fluorescence stabilizer. All solvents used for extraction and TLC were of analytical grade.All solvents used for extraction and TLC were of analytical grade.

3.2. Plant material

Dry mature fruits of *T. tetraptera*, *M. myristica*, *R. heudelotii*, and *X. aethiopica* were purchased from the Sandaga market, Douala, Cameroon, in February 2024. All four plant materials were authenticated by Mr. Boniface Nlandu of the Institut National d'Études et de Recherches Agronomiques (INERA), Kinshasa, Democratic Republic of Congo. Voucher specimens were deposited at the CESNOV herbarium, Faculty of Pharmaceutical Sciences, University of Kinshasa, under the following voucher numbers: *T. tetraptera* N. EP001; *M. myristica* N. EP002; *R. heudelotii* N. EP003; *X. aethiopica* N. EP004. The fruits were oven-dried and finely ground into powder using a laboratory mill and kept in a dry and desinfected storage box at room temperature.

3.3. Preparation of extracts

Aqueous extracts were prepared by macerating 10 g of each plant powder in 100 mL of distilled water for 24 hours, followed by 30 to 60 minutes in an ultrasonic bath (Ahmed and Adrar 2022). The mixture was filtered through Whatman No. 1 paper, and the filtrate was centrifuged; the supernatant was dried in an oven and stored at 4 to 8°C. Organic extracts were prepared by percolation of 10 g of plant powder with methanol/dichloromethane ($MeOH/CH_2Cl_2$, 50:50 v/v) following the methods of Elaka et al. (2020) and Muipata et al. (2025). After maceration for 24 to 48 hours, the percolate was collected dropwise until exhaustion of the plant material (minimum 100 mL). The solvent was evaporated at 45°C, and the dry residue was stored at 4 to 8°C. Extract yields were calculated using gravimetry.

3.4. Micrographic characterization of powders

Observations were made using the Steinmetz reagent following the method of Bahati et al. (2017). The reagent consisted of 2.0% (w/v) mercuric chloride, 0.02% (w/v) acid fuchsin, 0.1% (w/v) iodine, and 0.2% (w/v) potassium iodide dissolved in 90% lactic acid. Two to three drops of Steinmetz reagent were deposited on a glass slide; a small quantity of each plant powder was added and covered with a coverslip. Observations were carried out at 10× and 40× magnification using a Novex BBPPH 86.375 optical microscope.

3.5. Phytochemical screening by thin-layer chromatography

TLC analysis was performed on Silica gel 60 F254 plates (Merck) using 10 µL of 10 mg/mL solutions of methanol and ethyl acetate extracts, following the methods of Wagner and Bauer (2016**) and** Wagner et al. (2017). Separate mobile phases were applied for each metabolite class: flavonoids and phenolic acids (dichloromethane/formic acid/acetone, 80:8:20, and ethyl acetate/formic acid/glacial acetic acid/water, 100:11:11:26); saponins (toluene/diethyl ether, 1:1, saturated with 10% acetic acid); terpenes (toluene/ethyl acetate, 93:7); tannins (ethyl acetate/methanol/water, 40:8:5) (Diouf et al. 2015); anthraquinones (ethyl acetate/methanol/water, 100:13.5:10); iridoids (ethyl acetate/methanol/water, 100:10:35); alkaloids (dichloromethane/methanol/25% ammonia, 8:2:0.5); and coumarins (toluene/diethyl ether, 1:1, saturated with 10% acetic acid). Detection was performed under UV at 254 and 366 nm, followed by spraying with the appropriate reagent for each class (Wagner and Bauer 2016; Wagner et al. 2017).

3.6. Total polyphenol and flavonoid quantification

Total polyphenol content was determined in triplicate using the Folin–Ciocalteu method (Quettier-Deleu et al. 2000; Mbaiogaou et al. 2022) and expressed as mg gallic acid equivalents per gram of dry weight (mg GAE/g DW), using the calibration equation $y = 2.100x + 0.0487$ ($R^2 = 0.9893$). Total flavonoid content was determined by the aluminium trichloride spectrophotometric method (Mbaiogaou et al. 2022) and expressed as mg quercetin equivalents per gram of dry weight (mg QE/g DW). Absorbances were read at 725 nm (polyphenols) and 415 nm (flavonoids) using a BIOBASE UV-vis spectrophotometer.

3.7. Antioxidant activity

Antioxidant activity was evaluated by DPPH and ABTS radical scavenging assays following the method of Kapepula et al. (2018) and Nkakapen (2022**)**. For the DPPH assay, 3.2 mg DPPH was dissolved in 100 mL of 80% methanol and equilibrated for 1 hour in the dark. Twenty microliters of each extract concentration were added to 1980 µL of DPPH solution, incubated in the dark for 30 minutes, and absorbance was read at 517 nm. For the ABTS assay, the $ABTS^{\bullet+}$ radical was generated by reacting equal volumes of ABTS and potassium persulfate solutions in water, stored in the dark for 12 to 16 hours, and diluted to an absorbance of 0.6 to 0.8 at 734 nm. Absorbance was read at 734 nm following 30 minutes of incubation in the dark. In both assays, percentage inhibition was calculated as

% inhibition = [1 − (A_x/A_c)] × 100, where A_x is the absorbance in the presence of the extract, and A_c is the absorbance of the control. IC_{50} values were determined by non-linear regression using Microsoft Excel 2016. Quercetin was used as the positive control.

3.8. Anti-inflammatory activity

Anti-inflammatory activity was assessed by the thermal egg albumin denaturation method (Masengo et al. 2023; Mostefaoui et al. 2022). The reaction mixture contained 200 µL egg albumin, 1600 µL phosphate-buffered saline (PBS, pH 6.8), and 1000 µL of aqueous extract at concentrations of 500, 750, 1000, and 2000 µg/mL. After incubation at 37°C for 15 minutes followed by heating to 70°C for 5 minutes, absorbance was measured at 660 nm. Sodium diclofenac (250 µg/mL) was used as a positive control and distilled water as the negative control. Percentage inhibition of protein denaturation was calculated as % inhibition = [1 − (D/C)] × 100, where D is the absorbance of the extract or positive control, and C is the absorbance of the negative control (Fokou et al. 2013). IC_{50} values were determined from dose–response curves. All determinations were performed in triplicate.

3.9. Soap formulation

Soaps were prepared by cold saponification, a method selected for its simplicity, low cost, and proven ability to preserve heat-sensitive plant constituents. Refined palm oil and coconut oil were each weighed separately and melted in a water bath, then combined. Sodium hydroxide (NaOH) was dissolved slowly in distilled water to produce the lye solution, to which sodium chloride (NaCl) was added. Both the lye solution and the oil blend were equilibrated to 30 to 40°C as measured by a digital cosmetic thermometer. The lye solution was added slowly to the oils with continuous mixing using a stick blender until a trace was observed. Plant extracts, either dry powder extract or aqueous macerate depending on the formulation, were incorporated at this stage. The soap mass was poured into oil-coated silicone molds, hardened for 24 to 48 hours, then demolded and cured for 3 to 4 weeks in a dry location. A total of 40 soaps were produced: 20 formulated with dry extract and 20 with aqueous macerate, with each of the four species represented in both forms.

3.10. Soap quality control

pH was measured after calibrating a pH meter against buffer solutions at pH 4.01 and 7.00, and determined directly from 1% (w/v) aqueous soap solutions at room temperature. Moisture content was determined by drying 1 g of each soap at 105°C to constant mass in accordance with ISO standard 672–1978 (Babeker 2023). Foaming power was assessed by dissolving 1 g of soap in 6 mL of each of four water types (distilled, demineralized, tap, and mineral), agitating for 2 minutes, then transferring 3 mL to a test tube, bringing to 6 mL with the same water type, agitating vigorously for 10 to 15 seconds, and measuring foam height after 1 minute. Mass was recorded for each of the 40 soap bars using an electronic analytical balance.

3.11. Statistical analysis

Each concentration was tested in triplicate in each assay, and at least 3 different assays were performed. All results were expressed as mean values ± standard deviation (SD). The statistical analysis was performed with GraphPad Prism 10.4.1 (GraphPad Software, San Diego, California, USA). Two-way analysis of variance (ANOVA) and Student's paired t-test were used; multiple comparisons of all data were performed using the "Tukey" Multiple Comparisons Test, and the level of statistical significance was set at $p < 0.05$. The IC_{50} values were calculated with GraphPad Prism 10.4.1 under application of the function "log (inhibitor) vs. normalized response-variable slope" after converting the concentrations into their decimal logarithm.

4. CONCLUSION

In this study, four West African culinary spices, *Xylopia aethiopica*, *Tetrapleura tetraptera*, *Monodora myristica*, and *Ricinodendron heudelotii*, were evaluated for *in vitro* antioxidant and anti-inflammatory activities and their suitability as bioactive ingredients in cold-process soap formulations. Phytochemical analysis of all four plants revealed the presence of diverse and complementary groups of known secondary metabolites, including polyphenols, flavonoids, terpenes, and saponins, potentially contributing to their overall bioactivity. Among the four species, *X. aethiopica* stood out for its antioxidant activity with a DPPH IC_{50} value comparable to quercetin, the reference standard while *M. myristica* demonstrated the most potent and promising anti-inflammatory activity in the thermal protein denaturation assay. Cold saponification preserved a measurable fraction of these bioactive compounds from all four species in the finished soap formulations. Our findings provide a rationale for the traditional use of these four African spices in skin care and constitute a proof-of-concept for their use as bioactive ingredients in dermatological formulations. Further research should focus on isolating and characterizing the active phytochemicals and assessing the safety and skin tolerability of these herbal soap formulations through *in vitro* and *in vivo* studies.

Declarations

DISCLOSURE STATEMENT

The authors declare they have no competing interests.

AUTHOR CONTRIBUTIONS

CRediT: Estelle Poungam: Investigation, Data Curation, Methodology. **Messie Muipata:** Methodology, Data Curation, Writing – review & editing. **Isaac Kaba:** Formal analysis, Writing – original draft, Writing – review & editing, Visualization. **Jeancy Boy:** Methodology, Writing – review & editing. **Carmel Kandhe:** Formal analysis, Writing – review & editing. **Jonathan Mukanya:** Methodology, Writing – review & editing. **Paulin Mutwale:** Conceptualization, Supervision, Writing – review & editing. **Nadège Ngombe:** Conceptualization, Supervision, Resources.

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Figures

Figure 1

Plant materials purchased at the Sandaga market, Douala, Cameroon (February 2024): *Tetrapleura tetraptera* (A), *Monodora myristica* (B), *Ricinodendron heudelotii*(C) and *Xylopia aethiopica* (D).

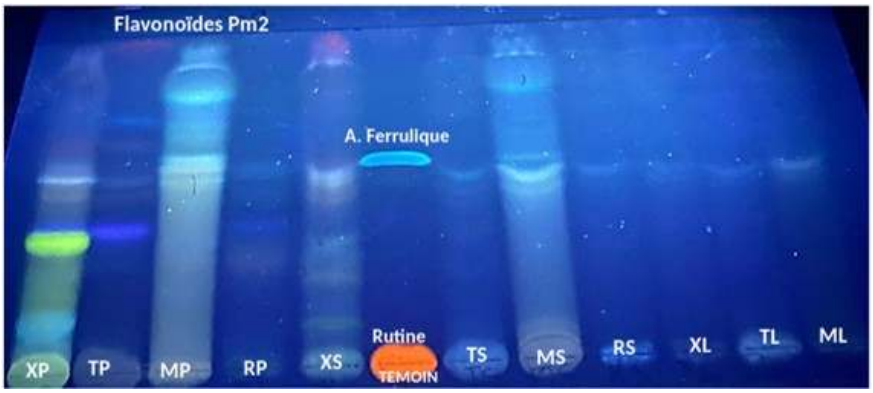

Figure 2

TLC chromatograms for saponin detection at 366 nm (Silica gel F254; toluene/diethyl ether, 1:1, saturated with 10% acetic acid; 10% sulfuric alcohol). XP, TP, MP, RP: plant powder extracts; XS, TS, MS, RS: soaps with dry extract; XL, TL, ML: soaps with aqueous macerate.

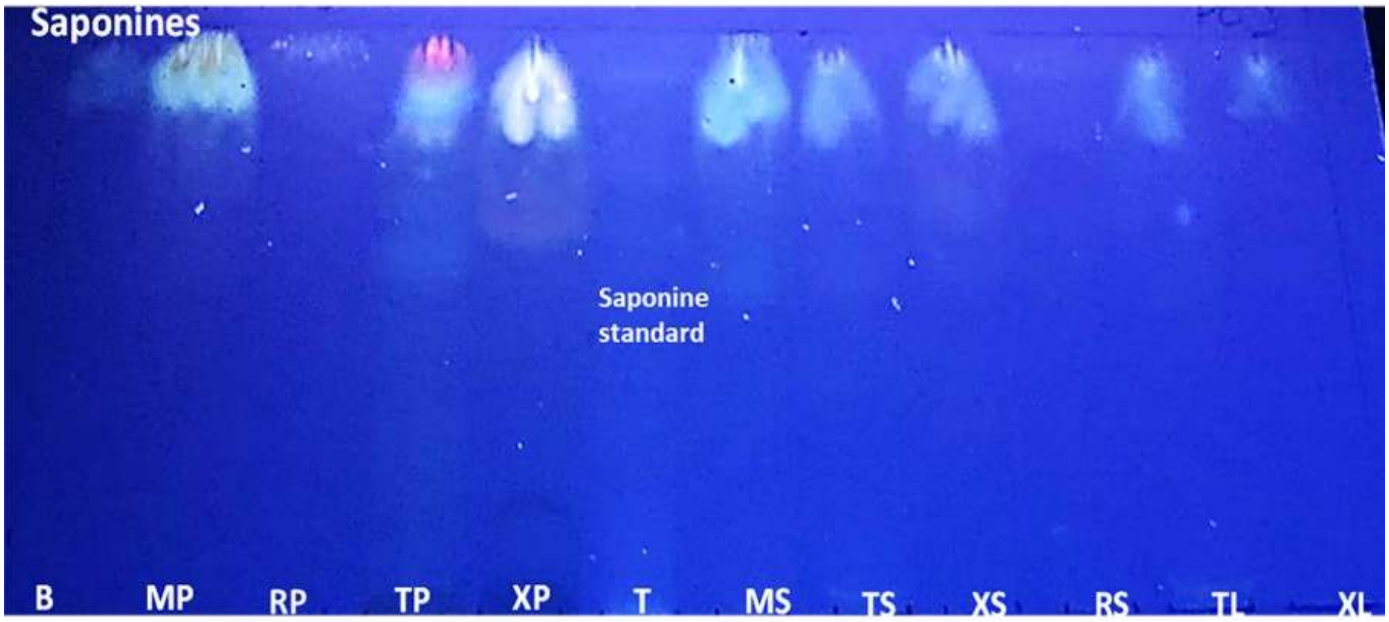

Figure 3

TLC chromatograms of methanolic extracts and corresponding soap extracts, for the detection of flavonoids and phenolic acids at 366 nm (Silica gel F254; DCM/formic acid/acetone, 80:8:20; Neu reagent). XP, TP, MP, RP: plant powder extracts; XS, TS, MS, RS: soaps with dry extract; XL, TL, ML: soaps with aqueous macerate.

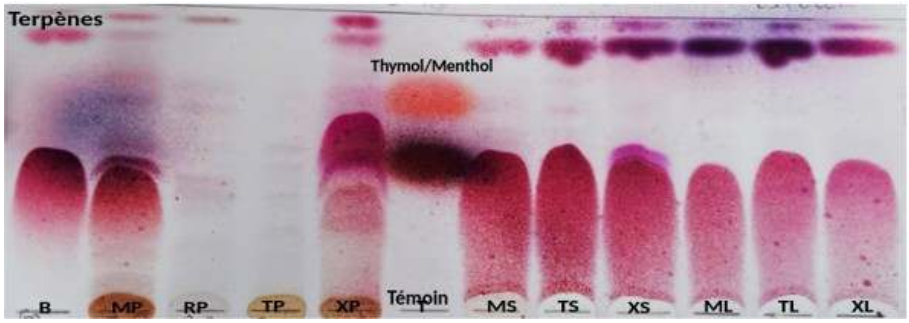

Figure 4

TLC chromatograms for terpene detection in visible light (Silica gel F254; toluene/ethyl acetate, 93:7 v/v; sulfuric anisaldehyde). XP, TP, MP, RP: plant powder extracts; XS, TS, MS, RS: soaps with dry extract; XL, TL, ML: soaps with aqueous macerate.

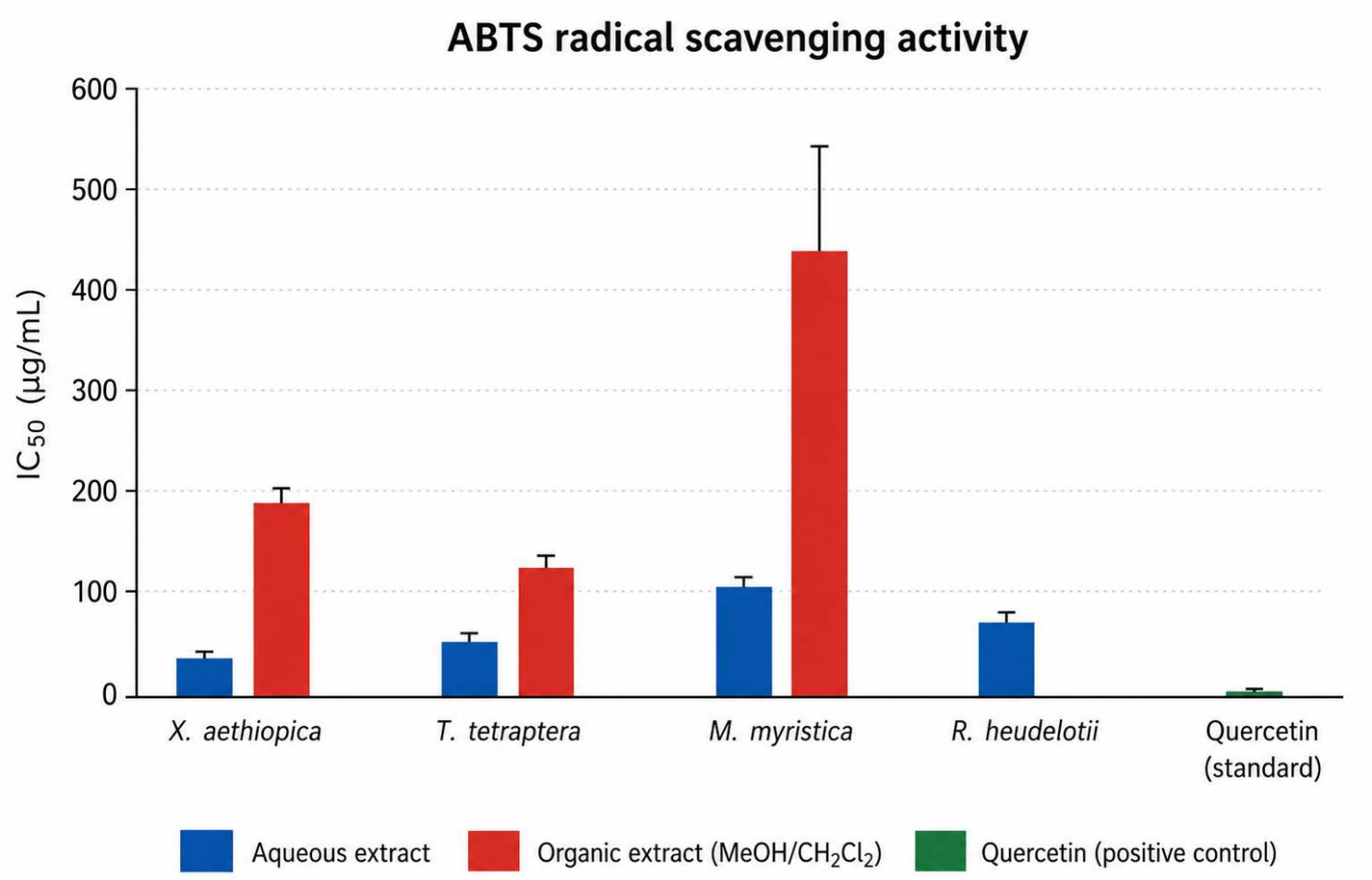

Figure 5

ABTS radical scavenging activity of aqueous and organic extracts of four West African culinary spices.IC_{50} values (µg/mL) for $ABTS^{\bullet+}$ radical scavenging by aqueous and organic extracts of *Xylopia aethiopica*, *Tetrapleura tetraptera*, *Monodora myristica*, and *Ricinodendron heudelotii*. Quercetin was used as the positive control. Bars represent mean ± SD (*n* = 4).

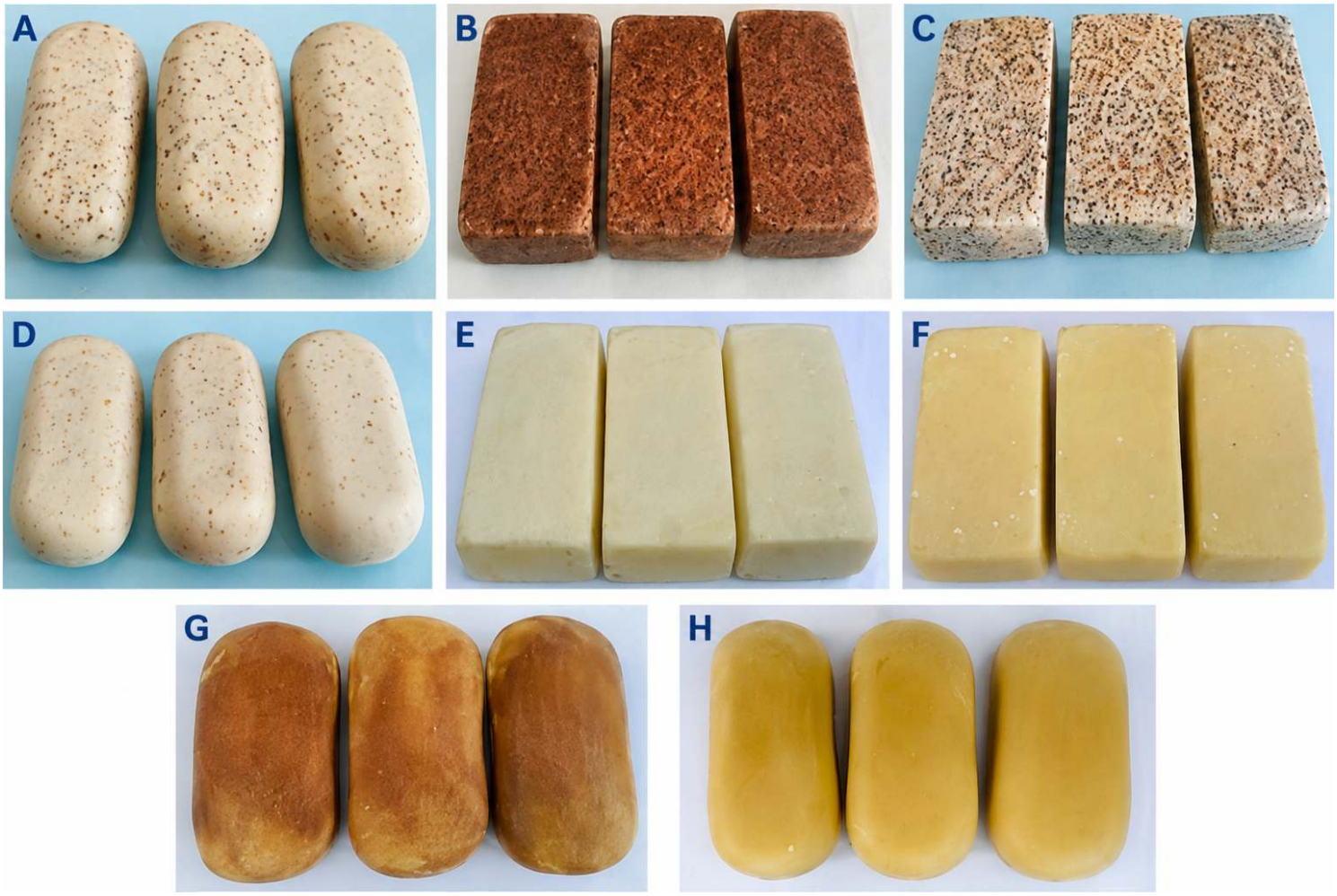

Figure 6

Visual appearance of soap bars formulated by cold saponification using different plant-derived treatments. Powder-enriched formulations: (A) *Monodora myristica*, (B) *Tetrapleura tetraptera*, (C) *Xylopia aethiopica*, and (D) *Ricinodendron heudelotii*. (E) Control formulation: Blank soap base (no plant extract; pH 10.44; moisture content 6.38%). Aqueous extract formulations: (F) *Monodora myristica*, (G) *Tetrapleura tetraptera*, (H) *Xylopia aethiopica*, and (I) *Ricinodendron heudelotii*.

Supplementary Files

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