REVIEW ARTICLE

A Review on the Extraction of Antibacterial Bioactive Compounds from Tanzanian Medicinal Plants Using CO_2-Expanded Liquid Technique

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Abstract: The threat of antimicrobial resistance has resulted in the need to search for alternative cures from medicinal plants. This review examines the relevance and suitability of using the carbon dioxide expanded liquid extraction (CXLE) method in extracting bioactive compounds from selected medicinal plants in Tanzania. The study focuses on their potential as antimicrobial agents against common pathogenic bacteria. It highlights the advantages of using CXLE, including higher purity of bioactive compounds, preservation of thermally sensitive compounds, and lower organic solvent use. The review also discusses the selection of medicinal plants based on their traditional applications and the scientific evidence supporting their antimicrobial properties. To gain a broader insight and sufficient overview, the review has considered previous studies that focused on similar plant species or extraction techniques. Furthermore, it examines the influence of extraction parameters on the yield and quality of extracts, as well as how these factors affect antibacterial efficacy. The review concludes with suggestions for further research to optimise the CXLE process for optimal yield of bioactive compounds, to evaluate their antibacterial activity, and to combine with chromatographic techniques to separate compounds in a single step. This review emphasises the integration of traditional knowledge with modern extraction techniques to explore the medicinal potential of Tanzanian plants.

Keywords: CO_2 -Expanded Liquid Extraction; Medicinal Plants; Bioactive Compounds; Antibacterial; Selective Extraction

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Introduction

Bacteria are known to cause numerous health problems worldwide and are associated with 56.2% of sepsis deaths globally, with the top five bacteria accounting for 54.9% mortality [1]. These bacteria include: *Escherichia coli*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*. Increasing antibiotic resistance in bacteria is a major concern in healthcare. 4.71 million annual deaths worldwide are linked to antimicrobial resistance (AMR), with 1.14 million deaths directly caused by bacterial AMR in 2021 [2]. The deaths associated with AMR are projected to reach 8.22 million deaths annually by 2050 if no immediate measures are taken [2]. A search for alternative cures to combat AMR

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has been directed to medicinal plants (MP) [3, 4]. Medicinal plants have a proven ability to inhibit bacteria resistant to antibiotics, as their extracts have been observed to inhibit the growth of methicillin-resistant *Staphylococcus aureus* [5, 6]. Additionally, MPs offer further advantages since they contain numerous bioactive compounds, and some possess nutraceutical potential that strengthens human health [3]. Unlike synthetic drugs, MPs are regarded as having significant therapeutic potential against various skin diseases with relatively low side effects and costs [7].

Traditional herbs derived from plants have been utilised since ancient times, but have recently gained recognition in modern health care [8]. About 80% of the population in developing countries relies on Traditional Medicines (TM) [9]. In Tanzania, TM plays a vital role, with an estimated 60 - 79% of the population relying on it [10]. Tanzania has over 10,000 known plant species, of which 25% are wild medicines [11]. Many Tanzanian MPs remain underexplored in terms of antimicrobial potential, despite their ethnopharmacological significance. For instance, a review of studies on respiratory diseases involving 133 plant species found that only 16 exhibited a Minimum Inhibitory Concentration (MIC) of ≤ 50 µg/mL. Structural compounds were identified for 49 species; only 7 species had a MIC of ≤ 5 µg/mL [12], which may be related to the extraction techniques used. When exploiting the full potential of natural products from MPs, scalability, solubility, toxicity, and bioavailability remain major challenges [13].

Plant leaves are the most utilised part of the plant, accounting for 51.42% of herbal medicines derived from plants [14]. Antimalarial extracts are primarily prepared using the decoction method (67%), with infusions being the next most common (17%). They are also less frequently prepared by powdering (7%), juicing (4%), steaming (3%), burning (1%), and concoction (1%) [15]. Although decoction is popular, it involves boiling, which causes decarboxylation, hydrolysis, and dehydration of heat-sensitive compounds, resulting in a loss of bioactivity [16]. Additionally, it concentrates impurities by dissolving all water-soluble compounds at around 100 °C, thereby reducing the concentration of bioactive compounds and leading to decreased activity.

The choice of extraction method influences the efficient recovery of bioactive compounds from MP. Conventional methods like maceration, steam distillation, Soxhlet extraction and mechanical pressing are popular due to their simplicity. However, these techniques are time-consuming; Soxhlet and maceration also require larger solvent volumes [17]. Meanwhile, hydro-distillation and Soxhlet expose heat-sensitive compounds to prolonged thermal stress, which can cause oxidative deactivation of bioactive compounds [18]. This process has been observed to decrease the total phenolic content at temperatures exceeding 40 ºC [19]. Steam distillation, operating at around 100 ºC, remains the most common method, representing 93% of essential oils extraction techniques [20]. The use of modern dryers in herb processing necessitates low temperature regimes to preserve the activity of compounds, such as vitamin C (50 - 60 ºC), polyphenols (55 - 60 ºC), flavonoids (60 - 70 ºC), glycosides (45 - 50 ºC) and volatile compounds (40 -50 ºC) [21].

Extraction using a mechanical press is considered inexpensive and easier to operate, even on a small scale, to extract oil from oilseeds [22]. However, this technique is unable to expel target bioactive compounds from the complex matrix of the plant [23]. Mechanical presses yield extracts with contaminants, thus requiring refining [24]. Additionally, it lowers the concentration of bioactive compounds in the extracts and affects the quality of output [25].

To address these technical limitations of conventional extraction methods, modern extraction methods such as Microwave-Assisted Extraction (MAE) and Ultrasound-Assisted Extraction (UAE) are used. UAE enhances mass transfer through cavitation, disrupts cell walls, and improves solvent permeability. This process reduces solvent consumption and shortens extraction time compared to conventional techniques [26]. Conversely, MAE uses microwave energy to heat the plant-solvent matrix, generating localised pressure that speeds up the release of bioactive compounds [27]. Both MAE and UAE are regarded as green extraction methods, but due to their operational nature, their scalability and ability to selectively extract bioactive compounds with diverse polarities remain challenging.

The use of Carbon Dioxide Expanded Liquid Extraction (CXLE) can address the limitations of conventional and other modern extraction techniques. CXLE provides a distinct advantage due to its ability to tune polarity and its reduced solvent consumption, making it a green extraction method [17]. The compressed and subcooled CO_2 gas is dissolved into the solvent to form a carbon dioxide expanded liquid (CXL) with improved thermodynamic and transport properties. These properties enhance diffusivity into the plant matrix and improve transport properties, such as reducing viscosity [28], which in turn enhances extraction kinetics. As illustrated in Fig. 1, increasing pressure in CXLE increases the dissolution of CO_2 into the liquid phase and thus reduces its viscosity and improves diffusivity. More importantly, to increase selectivity, the solvent's polarity can be adjusted to match that of the target compound by modifying thermodynamic parameters like CO_2 mole fraction,

temperature and pressure. Moreover, CXLE utilises the extraction pressure to push the extract through narrow pores of sintered stainless-steel filters (0.5 μm), producing high-quality extracts.

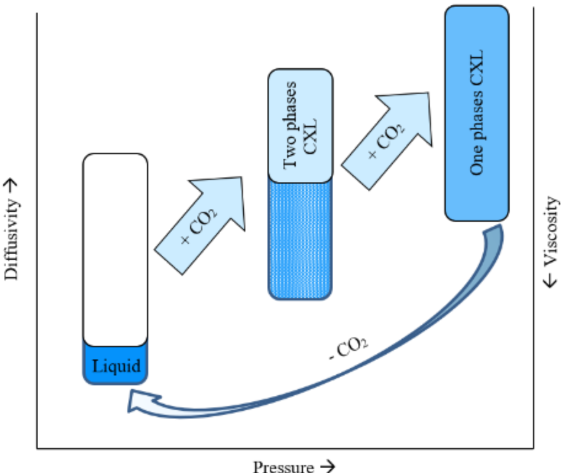

Fig. 1: Transition of pure liquid solvent to CXL and improved physicochemical properties

Recent advancements in high-pressure solvent extraction include supercritical fluid extraction (SFE) and CXLE. In extracting bioactive compounds, the SFE method utilises CO_2, methane or propane at high pressures, typically ranging from 10 to 65.5 MPa, with optimal extracts obtained at higher pressures [29]. On the other hand, CXLE operates at lower pressures, usually below 8 MPa [28]. Another drawback of SFE is its limited solvation capacity for certain compounds due to CO_2's limited polarity as a solvent [17]. The use of cosolvents improves solubility; the ratio between CO_2 and cosolvent functions as a switch to target specific compounds [17, 30]. The cosolvent is chosen based on the solubility parameters of the target compound. Ethanol, methanol, ethyl acetate, acetone, hexane, and acetonitrile are often used as cosolvents in CXLE.

Recent studies have examined CXLE for extracting high-quality oil from rice bran husks and sunflower cake, specifically for biofuel production [31, 32]. However, in a developing country like Tanzania, the economic feasibility of using CXLE for biofuel is challenging, as the oils must compete with conventional fuels, such as diesel, gasoline and natural gas. CXLE utilises energy-intensive chillers, high-pressure CO_2 and solvent pumps. Therefore, CXLE is more suitable for extracting high-value medicinal products, which are produced in small quantities, highly concentrated and of high quality. The concentration and fineness of extracts influence pharmacokinetics by improving bioavailability and delivery of phytochemicals to the targeted area of the human body. This may serve as the basis for standardisation and formulation of herbal bioactive compounds.

In reference to Tanzanian medicinal plants with diverse antibacterial bioactive compounds, CXLE presents a promising alternative for the extraction of phytochemicals from these plants, including alkaloids, phenolics, flavonoids and terpenoids. These bioactive compounds have a wide range of polarities, necessitating flexible extraction strategies. CXLE can adjust the solvent properties, which provides better interaction with target compounds, thus producing extracts enriched with antibacterial compounds. In addition to that, its compatibility with food-grade CO_2 and relatively mild operating conditions enhances its scalability and supports the principles of green chemistry.

Comparative Evaluation of Bioactive Compounds Extraction Methods

In the diverse range of extraction methods for bioactive compounds from medicinal plants, it is essential to evaluate their relative limitations and strengths. As described in the introduction, different extraction methods vary significantly in selectivity, efficiency, solvent usage, and costs. To move beyond description, it is essential to compare these techniques based on their capabilities. Table 1 presents a Pugh matrix evaluating conventional methods (Maceration, Soxhlet, steam distillation, cold pressing) and emerging techniques (MAE, UAE, SC-CO_2, CXLE) against key criteria with assigned priorities (weights), such as selectivity (4), preservation of bioactive compounds (5), yield (3), throughput (2), capital expenditure (2), operating expenditure (3) and extraction time (2). Three qualitative indicators -1, 0, +1 represent poor, moderate, and best performance, respectively, and are multiplied by their corresponding weights. The weighted scores for each method are calculated as the sum of these products. The results are then interpreted, with the highest score indicating the most suitable method for

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extracting bioactive compounds from medicinal plants. Regarding set criteria, CXLE stands out as the best performing method, achieving a total score of 16 out of 21, followed by SC-CO_2 with a score of 11

Table 1: Pugh chart analysis of different extraction methods of bioactive compounds from medicinal plants

Criteria	Method Weight	Steam Distillation	Cold Pressing	Solvent Extraction	MAE	UAE	CXLE	SC-CO_2
Selectivity	4	-1	-1	-1	-1	-1	+1	+1
Preservation of bioactive compounds	5	-1	+1	+1	0	+1	+1	+1
Yield	3	0	-1	+1	+1	+1	+1	+1
Throughput	2	+1	+1	0	+1	+1	+1	+1
Capital expenditures	2	+1	+1	+1	+1	+1	0	-1
Operating expenditures	3	+1	+1	0	0	0	0	-1
Extraction time	2	-1	0	-1	+1	+1	+1	+1
Weighted Scores		-4	5	4	5	10	16	11

Where; -1 = poor, 0 = moderate, +1 = best, CXLE = carbon dioxide expanded liquid extraction, SC-CO_2 = Supercritical CO_2, MAE = microwave-assisted extraction, UAE = ultrasonic-assisted extraction

Selected Tanzanian Medicinal Plants: Antimicrobial Properties, Bioactive Compounds, and Future Prospects of CXLE Technique

The medicinal plants considered in this review were selected based on their antimicrobial potential. The antibacterial properties of the chosen medicinal plants from Tanzania have been widely explored using conventional extraction methods such as maceration, steam distillation, and Soxhlet extraction, as summarised in Table 3. These studies show variations in extract potency, often influenced by the solvent system used. Polar solvents generally produce higher antibacterial activity, but pose a risk of co-extracting unwanted compounds such as pigments, waxes, and toxic lectins. According to the studies summarised in Table 3, there is limited focus on productivity, yield, selectivity, and extraction conditions. Most research concentrated on bioassays, with less information on processes relevant to industrial production, which stands out as a knowledge gap. Phytochemicals evaluated in Table 4 of the selected medicinal plants have been identified as various classes of bioactive compounds such as terpenoids, flavonoids, phenolics, alkaloids, and fatty acids, along with their reported mechanism of action on bacterial cells, such as inhibiting cell wall synthesis, preventing biofilm formation, inhibiting cell membrane, and disrupting quorum sensing. CXLE offers a promising alternative for extracting these compounds by enabling the tuning of solvent polarity through the adjustment of thermodynamic factors. This solubility turning allows for the selective concentration of target bioactive compounds under mild operating conditions. The potential of CXLE to improve yield, reduce co-extraction of unwanted compounds and produce extracts with consistent phytochemical profiles makes it suitable for scalable production and pharmacological formulations of plant extracts. The selected medicinal plants include *Abrus precatorius*, *Aleurites moluccanus*, *Garcinia buchananii*, *Bidens pilosa*, *Ocimum canum*, *Vernonia amygdalina*, *Plectranthus barbatus*, and *Tetradenia urticifolia*.

The *Abrus precatorius*, also known as rosary pea, or mtipitipi in Kiswahili or kaligaligo in the Haya language of Tanzania, belongs to the family Fabaceae. As shown in Fig. 2, *A. precatorius* is a perennial climber that twines around trees. The plant's leaves are traditionally used to treat cough, cancer sores, and swollen tonsils [33]. As shown in Table 3, across studies, leaf extracts of *A. precatorius* in Tanzania have minimum inhibitory concentration (MIC) values ranging from 0.078 - 1.25 mg/mL against fungal strains and Inhibition Zone Diameter (IZD) values ranging from 10 - 28 mm against bacterial strains. All parts of the plant contain active compounds, including alkaloids, tannins, flavonoids, saponins, terpenoids, phenols, and steroids [34]. These compounds are known for their antibacterial properties, with different mechanisms highlighted in Table 4. Since these compounds are polar or intermediately polar, they can be selectively recovered using CXLE with polar or intermediately polar cosolvents [17, 35]. Additionally, the plant *A. precatorius* contains toxic lectin, abrin, mainly found in its seeds [36]. By adjusting solvent polarity and lowering the temperature in CXLE, it can be selectively excluded from the extracts.

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Fig. 2: ***Abrus precatorius***

The *Aleurites moluccanus*, also known as the candlenut tree, kukui in the Maui language, and ekinyobwa in the Haya language of Tanzania, belongs to the family Euphorbiaceae. As shown in Fig. 3, *A. moluccanus* is a tree that produces oil-rich nuts mainly composed of Polyunsaturated Fatty Acids (PUFA). The oil of A. moluccanus is traditionally used to treat skin disorders such as eczema, chapped and dry skin, and wounds [37]. The kernel of *A. moluccanus* contains oil content ranging from 55 - 66 %w/w [38-40], comprising linoleic (40.3 - 43.7 mg/100mg), ∝-linolenic (30.2 - 34.1 mg/100mg), oleic (15.5 - 17.9 mg/100mg), stearic (2.4 - 2.63 mg/100mg) and palmitic (5.51 - 6.21 mg/100mg) [41]. Studies show that these compounds exhibit antibacterial properties through different mechanisms of action highlighted in Table 4 and exhibit minimum inhibitory concentrations ranging from 8 μg/mL to 1,000 μg/mL [42, 43]. The extract obtained by the conventional method from this plant was found to contain cardiac glycoside, neriifolin [44], which contributes to its mild toxicity. Previous studies have shown that CXLE demonstrates selectivity in extracting fatty acids and suppressing contaminants [31, 32, 45]; therefore, it can be used to extract these beneficial bioactive compounds from this plant.

Fig. 3: ***Aleurites moluccanus*** **tree**

The *Bidens pilosa* is also known as black jack or mshonanguo in the Kiswahili language, belonging to the family Asteraceae. As shown in Fig. 4, *B. pilosa* is a herbaceous flowering plant. The plant is traditionally used as an anti-malarial, antibacterial, antiviral, and wound healing agent [46]. *B. pilosa* extracts from Tanzania exhibit antibacterial properties with MIC values ranging from 78 μg/mL to 1,250 μg/mL, as highlighted in Table 3. The variation in activity efficacies is influenced by the solvent used. As shown in Table 4, *B. pilosa* contains various bioactive compounds that act on bacterial cells through different mechanisms. The compounds identified in Tanzanian *B. pilosa* include fatty acids, steroids, and flavonoids. CXLE is suitable for extracting these classes of compounds due to its tunable polarity, and it was used to extract similar compounds, as shown in Table 2.

Table 2: Literature Summary on CXLE Performance Across Different Plants and Target Compounds

Plant species (matrix)	Target compound(s) (class of compound)	Solvent system (assessed range)	Optimal conditions (CO_2 mole fraction, pressure, temperature)	Key findings (yield, selectivity, bioactivity)	Ref.
Citrus reticulata (mandarin peels)	D-Limonene (1) Essential oil (monoterpenes)	CO_2 + hexane (5 - 6.5 MPa, 25 ºC, 0.7 - 0.82 CO_2 mole fraction)	0.7 mole fraction, 6.5 MPa, 25 ºC	Yield: 0.155 g EO/g dry peel with 51% D-limonene, which is 13% higher than that obtained with hexane maceration and 300% higher than with hydro-distillation. The TPC is 27% lower than that of hexane maceration and 172% higher than that of hydro-distillation. The antioxidant activity is the same for CXH and hexane maceration, and is 125% higher than that of hydro-distillation.	[47]
Cydonia oblonga (fruit)	Quercetin (2) (flavonoid)	CO_2+ethanol+H_2O (15 - 30 MPa, 65.9 - 80 ºC, 0.1 CO_2 volumetric fraction)	10/81/9 (v/v/v), 66 °C, 22.3 MPa, at 3 mL/min, took 30 minutes	The amount of quercetin recovered is 294.37 ng/ g sample compared to 1.12 µg/g present in the plant. The drying method is reported to reduce the amount recovered. No activity was tested, and there is no comparison with conventional extraction methods.	[17]
Silybum marianum (seeds)	Flavolignan (fraction) Silymarin (6), taxifolin (3) (flavonoid compound)	CO_2 + EtOH + H_2O (9 MPa, 40 ºC, 0.25 - 0.75 CO_2 mole fraction)	For silymarin, a solvent ratio of 25:60:15 at 40 ºC and 9 MPa in 160 minutes. For taxifolin, a solvent ratio of 75:5:20 at 40 °C and 9 MPa.	The silymarin yield is 55.97%. It exhibits antioxidant and anti-inflammatory activities, with IC_{50} values of 8.80 µg/mL and 28.5 µg/mL, respectively. The concentration of silymarin compounds in the extract is 59.6%. The yield of silymarin was found to be 82% higher than SC-CO_2. Regarding Taxifolin, the amount is 12.273 mg/g of extract, which is 18% higher than that obtained by maceration.	[48]
Boswellia sacra (tree resin)	α-pinene (4), cis-verbenol (5) (monoterpenes)	CO_2+ethanol (6 - 10 MPa, 40 - 80 ºC, 0.1 - 0.5 CO_2 mole fraction)	0.31 mol, 40 ºC, 9.3 MPa	Yield of 23.9 mg/g for α-pinene, which is 7% higher than that of SC-CO_2 and 60% higher than that of maceration. Yield of 4.9 mg/g for cis-verbenol, which exceeds that of SC-CO_2 by 17% that of maceration by 28%. The CXE method demonstrated a faster initial extraction rate, up to 10 times quicker than SC-CO_2 at the highest tested flow rate of 3 mL/min	[49]
Olea europaea (Olive seed by-products)	Cholesterol-lowering compounds (phenolic compounds and phytosterols such as β-sitosterol (7))	CO_2 + ethyl acetate (8 - 25 MPa, 40 - 80 ºC, 0.15 - 0.55 CO_2 mole fraction)	Yield at 0.15 mole fraction at 40 ºC, 8 MPa. Higher amount of β-sitosterol and higher RMCS (62%) activity at 0.55 mole fraction, 62 °C, 25 MPa	The highest yield is 50.7 wt%. The maximum reduction in micellar cholesterol solubility (RMCS) of 62% was achieved at maximum values of β-sitosterol recovered.	[35]
Oryza sativa (rice bran)	Fatty acids, targeted to lower phospholipids and free fatty acids (FFA)	CO_2 + acetone (3.3 - 6.7 MPa, 16.6 - 33.4 ºC, 0.66 - 0.86 CO_2 mole fraction)	25 ℃, 5.0 MPa, and 0.76 CO2 mole fraction yielded	Yield 0.237 g- oil/g-sample, which is 25% higher than obtained with maceration using pure acetone. The FFA was 9.60 wt%, which is 23.2% lower than with maceration using pure acetone. Phospholipid concentration was 4.5 ppm, 91% lower than with maceration using pure acetone.	[31]
Helianthus annuus (seeds)	Fatty acids, targeted to lower phospholipids and free fatty acids (FFA)	CO_2 + hexane (3.8 - 7.2 MPa, 16.6 - 33.4 ºC, 0.64 - 0.94 CO_2 mole fraction)	0.82 mole fraction, 5.0 MPa, 25 ℃.	Yield: 0.379 g-oil/g-sample, which is 18% higher than maceration with liquid hexane. Free fatty acid of 1.91 wt%, which is 9.5% less than that obtained by liquid hexane. The phosphorus concentrations were 7.2 µL/L, which is 37.4% lower than that of maceration with liquid hexane.	[32]
Malus domestica (fruit)	Total phenolic contents	CO_2 + ethanol + water (10 - 30 MPa, 30 - 80 ºC, 0.1 - 0.7 CO_2 volumetric fraction)	(34/53.8/12.2; v/v/v), 12 MPa, 75 ºC	Yielded phenolic content of 2442 µg/g, which was 9.7% higher than that of ultrasonic extraction (UAE).	[50]
Gardenia jasminoides (fruit pulps)	Natural Pigments Compounds (crocin-1 and crocin-2)	CO_2 + ethanol + water + Direct sonication (5 - 25 ºC, 8 - 14 MPa)	20/80 (v/v) 25 ºC, 10 MPa	For crocin-1, a yield of 13.63 ± 0.5 µg/mL, which is 30% higher than that obtained by maceration, and for crocin-2, 0.51 ± 0.05 µg/mL, which is 37.8% higher than that obtained by maceration.	[51]

Whereby EO is the essential oils, TPC is the total phenolic contents, CXH is CO_2-expanded hexane, SC-CO_2 is supercritical CO_2, CXE is CO_2-expanded ethanol, RMCS is the micellar cholesterol solubility, and FFA is free fatty acid.

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Fig. 4: ***Bidens pilosa***

The *Garcinia buchananii* is also known as Omusharazi in the Haya language of Tanzania, belongs to the family Clusiaceae, and it is native to sub-Saharan Africa and is widely distributed in tropical regions. As shown in Fig. 5, *G. buchananii* is a tree that produces edible fruits. The plant is traditionally used to treat chronic diarrhoea, tuberculosis, ulcers, herpes zoster, herpes simplex and skin rashes [52]. As highlighted in Table 3, extracts of *G. buchananii* exhibit antibacterial properties with a MIC value of 1.5625 mg/mL and anti- protozoa properties with inhibitory concentration (IC_{50}) values ranging from 2.8 to 23.57 µg/mL. Smeathxanthone A and smeathxanthone B, isolated from the related species *Garcinia smeathmannii* (Oliver), have demonstrated antibacterial activity with MICs between 156.25 and 625 µg/mL against bacterial strains (such as *E. coli*, *K. pneumoniae*, *P. vulgaris*, *S. typhi*, *S. aureus*, and *S. faecalis*) [53]. As detailed in Table 4, *G. buchananii* contains phenolic and flavonoid bioactive compounds that act differently on bacterial cells. Due to the nature and composition of *G. buchananii* barks, if the CXLE is applied to this plant, it will be able to diffuse through its matrix and extract the targeted compounds, similar to what was achieved in Table 2.

Fig. 5: A tree of ***Garcinia buchananii***

Ocimum species belong to the family Lamiaceae and are widely distributed in tropical and subtropical regions, including Tanzania. As shown in Fig. 6, the aerial part of *Ocimum canum* is used traditionally in managing diabetes [54] and gastrointestinal issues [55]. Other Ocimum species include *Ocimum africanum*, *Ocimum kilimandscharicum*, *Ocimum basilicum*, *Ocimum gratissimum*, *Ocimum campechianum*, *Ocimum sanctum*, *Ocimum tenuiflorum* and *Ocimum suave*. All

these species contain similar bioactive compounds in different ratios, known for their antimicrobial effects. Extracts from Ocimum species demonstrate antibacterial activity with MICs ranging from 50 µg/mL to 6,250 µg/mL, as highlighted in Table 3. These activity differences depend on the extraction method, with some using maceration with methanol, or ethyl acetate or ethanol and others employing hydro-distillation. As shown in Table 4, Ocimum species mainly contain terpenoid compounds, which can be efficiently extracted with CXLE, as previous studies have demonstrated, as highlighted in Table 2 [47, 49].

Table 3: Extraction methods and antibacterial activity of crude extracts and some isolated compounds from selected Tanzanian medicinal plants

Plant specie	Crude/ compound	Extraction method and plant part	Solvents (material/solvent ratio)	Micro-organism tested	Reported activity (MIC/ inhibition zone/IC_{50})	Ref.
Abrus precatorius	Crude extract	Maceration of dried leaves for 72 hours	Methanol (1:3 w/v) (g/mL)	*K. pneumoniae*, *S. enterica var. Typhi*, *P. aeruginosa*, *S. aureus* and *S. pneumoniae*	Disk diffusion: at a concentration of 100 mg/mL. The zone of inhibition ranged from 11 - 13 mm.	[56]
	Crude extract	Soxhlet extraction of leaves, stem and seeds.	Methanol (at 78 ºC), water (at 100 ºC) and petroleum ether (at 60 - 80 ºC)	*S. aureus, S. epidermidis, B. subtilis, P. mirabilis, P. aeruginosa*	Leaves aqueous extract: MIC, 256-512 µg/mL; leaves methanol extract: MIC, 16-512 µg/mL; stem aqueous extract: MIC, 64-512 µg/mL; stem methanol extract: MIC, 128-512 µg/mL; seeds aqueous extract: MIC, 32-64 µg/mL; seeds methanolic extract: MIC, 32-128 µg/mL.	[57]*
Bidens pilosa	Crude extract	Maceration of dried leaves for 48 hours	70% methanol, room temperature	*E. coli* and *S. aureus*	Agar well diffusion: the concentration of 200 mg/mL yielded zones of inhibition ranging from 10 to 28 mm	[46]
			Dichloromethane and Ethanol	*C. albicans, C. neoformans, A. Niger*	The leaves dichloromethane extract MIC range: 0.078 - 0.313 mg/mL, leaves ethanol extract MIC: 0.469 - 1.25 mg/mL	[58]
Garcinia buchananii	Crude extract	Maceration of dried barks for 5 days (yield of 9.4%)	70% ethanol ratio of 1:6 w/v	*S. pneumoniae*	MIC of 1.5625 mg/mL	[59]*
	Crude extract	Maceration of dried barks	CH_2Cl_2: MeOH (1:1) (hexane, ethyl acetate fractions)	*P. falciparum* (both chloroquine susceptible and resistant)	Inhibitory concentrations of protozoa, IC_{50} range: 7.50±0.60 to 23.57±0.59 µg/mL	[60]
	Isolated compound (Isogarcinol and guttiferone)	Extracted by maceration, then isolated by column chromatography	Crude CH_2Cl_2: MeOH (1:1) Isolation (hexane & ethyl acetate) by increasing polarity from 90:10 to 0:100	*P. falciparum* (both chloroquine susceptible and resistant)	Inhibitory concentrations of protozoa, IC_{50} range: 2.8±0.90 to 10.64±4.50 µg/mL	[60]
Plectranthus barbatus	Crude extract	Maceration of the stem for 48 hours	Chloroform, methanol, ethyl acetate (sample/solvent ratio not specified)	*P. mirabilis*, *K. pneumoniae*, *P. aeruginosa*, *S. typhi*, *K. oxytoca*, *E. coli* and *S. kisarawe*	Broth microdilution. Chloroform extract: MIC 12.5 mg/mL, methanolic extract: 3.12-12.5 mg/mL, ethyl acetate extract: 3.12-12.5 mg/mL.	[61]
	Crude extract	Steam distillation of fresh leaves. Yield 0.08% (v/w)	Steam (95 - 100 ºC)	*Candida albicans*, *Aspergillus niger*, *Candida tropicalis*, *Candida krusei*, *Candida glabrata*, *Trichophyton interdigitale*, *Trichophyton rubrum*, and *Microsporum gypseum*	Agar well diffusion: 5 µL of essential oils in each well. Zone of inhibition range: 11 - 27 mm	[62]
Vernonia amygdalina	Crude extract	Maceration of dried leaves for 72 hours	70% ethanol (1:5 w/v) (g/mL)	*K. pneumoniae*, *S. enterica var. Typhi*, *P. aeruginosa*, *S. aureus* and *S. pneumoniae*	Disk diffusion: at a concentration of 100mg/mL. Zone of inhibition range: 15 - 20 mm	[56]*
	Crude extract	Maceration of dried leaves for 24 hours	Aqueous, 80% Ethanol (138g in 250 mL)	*Bacillus subtilis, Vibrio cholerae, Staphylococcus aureus*	Broth microdilution assay, aqueous extract MIC range: 3.906 - 15.625 mg/mL, ethanolic extract MIC: 3.906 - 31.25 mg/mL	[63]
	Crude extract	Maceration of dried leaves for 72 hours	Ethanol or ethyl acetate (50g in 250 mL)	*E. coli and S. aureus*	Disk diffusion: at a concentration of 100 mg/mL. Zone of inhibition 14 ± 1.9 - 26.67 ± 1.25 mm.	[64]*
Aleurites moluccanus	Crude extract	Soxhlet extraction of husks, leaves and stem barks	Acetonitrile, methanol, water	*Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Klebsiella pneumoniae*	Husks acetonitrile extract MIC: 1,562 - 6,250 µg/mL, husks methanolic extract MIC: 12,500 µg/mL, husks aqueous extract MIC: 25,000 µg/mL, leaves methanolic extract MIC: 12,500 µg/mL, stem bark acetonitrile extract MIC: 195 - 25,000 µg/mL, stem bark methanolic extract MIC: 98 - 390 µg/mL, stem bark aqueous extract MIC: 50,000 µg/mL.	[65]*

	Crude extract	*A. moluccanus* oil extract		*Staphylococcus aureus, E. coli*	Zone of inhibition *S. aureus*, 8.73±1.76 mm, *E. coli*, 0.00 mm.	[38] *
	Isolated compounds	Fatty acid derived from *A. molucanus* oil		*Staphylococcus aureus, E. coli*	1% oil in ethanol zone of inhibition 8.75 - 11.02 mm, negative control (ethanol) 6.25 - 6.75 mm.	[66] *
Tetradenia riparia	Crude extract	Maceration of leaf, stem bark, and root bark for 48 hours	95% methanol, ethanol, n-hexane, distilled water	*Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*	Leaves ethanolic extract range: 2.5 - 5 mg/mL, leaves methanolic extract 2.5 - 5 mg/mL, leaves hexane extract 5 mg/mL, leaves aqueous extract 2.5 mg/mL, root ethanolic extract 1.25 - 5 mg/mL, root methanolic extract 1.25 - 5 mg/mL, root hexane extract 5 mg/mL, root aqueous extract 2.5 - 5 mg/mL.	[67]
Ocimum sp.	Crude extract	Maceration of *O. graticimum* leaves for 48 hours	methanol, ethyl acetate	*P. mirabilis*, *K. pneumoniae*, *P. aeruginosa*, *S. typhii K. oxytoca*, *E. coli* and *S. kisarawe*	Methanolic extract: MIC of 1.56 - 25 mg/mL, ethyl acetate extract: 6.25 - 12.5 mg/mL and > 25 mg/mL for *P. aeruginosa*	[61]
	Crude extract	Hydro-distillation of leaves and flowering tops for 3 hours of *O. basilicum*, *O. kilimandscharicum*, *O. lamiifolium*, and *O. suave*	Boiled water	*S.aureus, S. epidermidis, P. aeruginosa, E. cloacae, K. pneumoniae, E. coli.*	*O. basilicum* extract MIC: 3.14 - 12.5 mg/mL, *O. kilimandscharicum* MIC: 1.55 - 3.35 mg/mL, *O. lamiifolium* MIC: 1.75 - 4.90 mg/mL, *O. suave* MIC: 0.05 - 3.10 mg/mL	[68]
	Crude extract	Soxhlet extraction of seeds from *Ocimum canum*	Methanol	*S. aureus, E. coli, B. cereus, S. typhi*	MIC range: 2.5 - 3.5 mg/mL	[69] *

* Dedicated antimicrobial studies in Tanzania that assessed MIC for the pointed plant species are limited; thus, studies from other parts of Africa were referred.
MIC is the minimum inhibitory concentration

Fig. 6: ***Ocimum canum***

Vernonia amygdalina, also known as bitter leaf or Omubilizi in the Haya language of Tanzania, is a perennial shrub belonging to the family Asteraceae. Shown in Fig. 7 is a shrub of *V. amygdalina*, which is traditionally used to treat malaria, gastrointestinal disorders and skin infections [70]. Across studies, *V. amygdalina* extracts exhibit antibacterial properties with zones of inhibition ranging from 14 to 26.67 mm, as highlighted in Table 3. As shown in Table 4, *V. amygdalina* contains sesquiterpene lactones, flavonoids and steroids with different mechanisms of action on bacterial cells. CXLE can be tailored to extract these classes of compounds due to its polarity tunability, and it was used to extract similar compounds, as shown in Table 2.

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Table 4: Bioactive compounds from selected Tanzanian medicinal plants, their chemical classes, and the mechanism of action on a bacterial cell

Medicinal plant	Isolated compound name	Structure of bioactive compound	Formula	Compound class	Mechanism of action on the bacterial cell	Mechanism Ref.	Occurrence Ref.
Bidens pilosa	Stigmasterol		$C_{29}H_{48}O_1$	Steroids	Inhibit the surface protein of a bacterial cell, 'sortase', thus preventing transpeptidation.	[71]	[72, 73]
	β-Sitosterol		$C_{29}H_{50}O_1$	Steroids	Inhibit the biosynthesis of peptidoglycan, thus preventing bacterial cell wall formation by inhibiting SrtA and MurA enzymes.	[74]	[72, 73]
	Myristic acid	$CH_3(CH_2)_{12}CO_2H$	$C_{14}H_{28}O_2$	Fatty acids	Target the cell membrane by disrupting the electron transport chain and oxidative phosphorylation.	[75]	[72, 73]
	1-phenylhepta-1,3,5-triyne		$C_{13}H_8$	Polyacetylenes	Deform the bacterial cell membrane by forming pores. Biofilm inhibition	[76, 77]	[72, 73]
	α-linolenic acid		$C_{18}H_{30}O_2$	Fatty acids	Target the cell membrane by disrupting the electron transport chain and oxidative phosphorylation.	[75]	[72, 73]
	Oleic acid		$C_{18}H_{34}O_2$	Fatty acids	Target the cell membrane by disrupting the electron transport chain and oxidative phosphorylation.	[75]	[72, 73]
	Apigenin		$C_{15}H_{10}O_5$	flavonoids	Attack the bacterial cell membrane, leading to cell shrinkage and leakage of intracellular contents. Inhibit the activity of the *mcr*-1 protein. Activates cellular oxidative pathways dependent on the production and	[78-80]	[72, 73]

					accumulation of RNS/ROS, leading to bacterial apoptosis.		
	Apigenin 7-*O*glucopyranoside		$C_{21}H_{20}O_{10}$	Flavonoid glycosides	Inhibit quorum sensing (QS), exopolysaccharides (EPS) and cell surface hydrophobicity (CSH), thereby inhibiting biofilm formation.	[81]	[72, 73]
	Luteolin		$C_{15}H_{10}O_6$	flavonoids	Inhibit biofilm formation, disrupt the integrity of the cell wall and cell membrane, influence the expression of proteins, inhibit nucleic acid synthesis, and interfere with energy metabolism.	[82, 83]	[72, 73]
	Luteolin 7-*O*-*β* *D*glucopyranoside		$C_{21}H_{20}O_{11}$	Flavonoid glycosides	Modulate reactive oxygen species (ROS) levels and inhibit topoisomerases I and II in bacterial cells.	[84]	[72, 73]
Garcinia buchanani i	Isogarcinol		$C_{38}H_{50}O_6$	Benzophenones	Inhibit bacterial biofilm formation	[85]	[86]
	Garcinol		$C_{38}H_{50}O_6$	Benzophenones	Inhibit bacterial biofilm formation	[85]	[86]
	Taxifolin		$C_{15}H_{12}O_7$	Flavonoids	Inhibit membrane-bound cysteine transpeptidase, sortase A (SrtA). Inhibit biofilm formation. Induces oxidative stress that disrupts the intracellular free radical metabolic balance.	[87, 88]	[89]

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1,5-dimethoxyajacareubin		$C_{20}H_{20}O_6$	Phenolics	Disrupts the cell wall/membrane, affects the cell membrane potential, and interferes with energy metabolism.	[90]	[86]
Depsidone garcinisidone-G		$C_{19}H_{18}O_7$	Phenolics	Disrupts the cell wall/membrane, affects the cell membrane potential, and interferes with energy metabolism.	[90]	[86]
Euxanthone		$C_{13}H_8O_4$	Phenolics	Disrupt the cell wall by interacting with lipoteichoic acid or lipopolysaccharides, and suppress the DNA synthesis.	[91]	[86]
(2R,3S,2″S)-buchananiflavanone		$C_{30}H_{22}O_{12}$	Flavonoids	Inhibits DNA, proteins, and cell envelope biosynthesis, causing damage to the cell membrane.	[92]	[89]
(2R,3R,2″R,3″R)-naringenin-C-3/C-8″dihydroquercetin		$C_{30}H_{22}O_{12}$	Flavonoids	Inhibits DNA, proteins, and cell envelope biosynthesis, causing damage to the cell membrane.	[92]	[89]
2R,3S,2″R,3″R)-manniflavanone		$C_{30}H_{22}O_{13}$	Flavonoids	Inhibits DNA, proteins, and cell envelope biosynthesis, causing damage to the cell membrane.	[92]	[89]
(2R,3R)-taxifolin-6-C-β-D-glucopyranoside		$C_{21}H_{22}O_{12}$	Flavonoid glycosides	Inhibit nucleic acid synthesis, energy metabolism, cytoplasmic membrane function, biofilm formation, porin	[93]	[89]

	(2R,3R)-aromadendrin-6-C-β-D-glucopyranoside		$C_{21}H_{21}O_{11}$	Flavonoid glycosides	on the cell membrane and alteration of the membrane permeability.		
Plectranthus barbatus	α-Pinene		$C_{10}H_{16}$-D2	Monoterpenes	Destruction of the cell membrane by releasing nucleic acid and protein out of the membrane, and heat shock is induced by direct contact with the DnaKJE-σ32 complex.	[94]	[62]
	Borneol		$C_{10}H_{18}O$	Monoterpenoids	Disrupt the bacterial cell wall, causing leakage of nucleic acids and proteins, and ultimately bacterial death.	[95]	[62]
	Camphene		$C_{10}H_{16}$-E1	Monoterpenes	No activity reported yet	[96]	[62]
	Viridiflorene		$C_{15}H_{24}$	Sesquiterpenes	Disruption of quorum sensing in bacterial cells	[97]	[98]
	Germacrene-D		$C_{15}H_{24}$	Sesquiterpenes	Infiltrate the cells and interact with the cellular metabolic mechanisms	[99]	[98]
	Forskolin		$C_{22}H_{34}O_7$	Diterpenoids	Disrupt the quorum-sensing of the bacterial cell	[97]	[100]
	Barbatusin		$C_{24}H_{30}O_8$	Diterpenoids	Disrupt the quorum-sensing of the bacterial cell	[97]	[100]

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	Coleon C		$C_{20}H_{24}O_5$	Diterpenoids	Disrupt the quorum-sensing of the bacterial cell	[97]	[100]
	Barbatusol		$C_{20}H_{28}O_2$	Diterpenoids	Disrupt the quorum-sensing of the bacterial cell	[97]	[100]
	Abieta triene		$C_{20}H_{30}$	Diterpenes	Disrupt the quorum-sensing of the bacterial cell	[97]	[101]
Abrus precatorius	Abrusogenin		$C_{30}H_{44}O_5$	Triterpenoids	Inhibit biofilm formation and quorum-sensing in the bacterial cell.	[102]	[103]
	Trigonelline		$C_7H_7NO_2$	Alkaloids	Inhibit biofilm formation and quorum-sensing of the bacterial cell.	[104]	[105]
	Abrusoside A		$C_{36}H_{54}O_{10}$	Triterpenoid saponins	Destroy the bacterial cell membrane	[106]	[107]
	Abrine (N-methyltryptophan)		$C_{12}H_{14}N_2O_2$	Alkaloids	Limited studies on its antibacterial mechanism		[108]
	Hypaphorine		$C_{14}H_{18}N_2O_2$	Alkaloids	Limited studies on its antibacterial mechanism		[105]

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	Precatorine		$C_{14}H_{11}NO_6$	Alkaloids	Limited studies on its antibacterial mechanism		[105]
Vernonia amygdalina	Vernodalinol.		$C_{19}H_{22}O_8$	Sesquiterpene lactone	Disrupt the membrane and interfere with essential metabolic pathways	[109]	[110]
	Vernodalol		$C_{20}H_{24}O_8$	Sesquiterpene lactone	Disrupt the membrane and interfere with essential metabolic pathways	[109]	[111]
	Vernolide		$C_{19}H_{22}O_7$	Sesquiterpenoids	Disrupt the membrane and interfere with essential metabolic pathways	[109]	[112]
	Isorhamnetin		$C_{16}H_{12}O_7$	flavonoids	Causes bacterial cell lysis and disrupts the cytoplasmic membrane	[113]	[112]
	Luteolin		$C_{15}H_{10}O_6$	flavonoids	Inhibit biofilm formation, destroy the integrity of the cell wall and cell membrane, influence the expression of proteins, inhibit nucleic acid synthesis, and interfere with energy metabolism.	[82, 83]	[112]
	Venolepine		$C_{15}H_{16}O_5$	Sesquiterpenoids	Disrupt the membrane and interfere with essential metabolic pathways of a bacterial cell	[109]	[112]

	β-Sitosterol		$C_{29}H_{50}O_1$	Steroids	Inhibit the biosynthesis of peptidoglycan, thus preventing bacterial cell wall formation by inhibiting SrtA and MurA enzymes.	[74]	[114]
	Vernonioside B1		$C_{35}H_{52}O_{10}$	Steroid saponins	Disrupt the cell membrane, leading to the leakage of bacterial cell contents.	[115]	[116]
Aleurites moluccanus	Linoleic acid		$C_{18}H_{32}O_2$	Fatty acids	Target the cell membrane by disrupting the electron transport chain and oxidative phosphorylation.	[75]	[41]
	α-linolenic acid		$C_{18}H_{30}O_2$	Fatty acids	Target the cell membrane by disrupting the electron transport chain and oxidative phosphorylation.	[75]	[41]
	Oleic acid		$C_{18}H_{34}O_2$	Fatty acids	Target the cell membrane by disrupting the electron transport chain and oxidative phosphorylation.	[75]	[41]
Tetradenia riparia	Fenchone		$C_{10}H_{16}O$	Monoterpenoids	No antibacterial activity reported yet	[117]	[118]
	α-terpineol		$C_{10}H_{18}O$	Monoterpenoids	Ruptures the cell wall and cell membrane, reducing the nucleus and cytoplasm. Disrupt the proton motive force, causing the leakage of ATP. Increases the membrane gelation and decreases the membrane fluidity.	[119, 120]	[118]

	α-Cadinol	$C_{15}H_{26}O$	Sesquiterpenoids	Its electrostatic interaction affects the bacterial zeta potential and cell permeability	[121]	[118]
	β-Caryophyllene	$C_{15}H_{24}$	Sesquiterpenes	Causes alteration of bacterial cell membrane permeability and integrity, leading to membrane damage and intracellular content leakage	[122]	[118]
	δ-Cadinene	$C_{15}H_{24}$	Sesquiterpenes	Its electrostatic interaction affects the bacterial zeta potential and cell permeability	[121]	[118]
	6,7-Dehydroroyleanone	$C_{20}H_{26}O_3$	Diterpenoids	Penetrates the cell membrane and cell wall, thereby affecting their metabolic pathways	[123]	[118]
	9β,13β-epoxy-7-abietene	$C_{20}H_{32}O$	Diterpenoids	Causes oxidative damage of macromolecules in cells, such as peroxidation of membrane lipids, oxidative damage of nucleic acids and other sulfuric groups in proteins	[124]	[118]
Ocimum canum	Camphor	$C_{10}H_{16}O$	Monoterpenoids	Inhibit the bacterial cell quorum-sensing	[125]	[126]
	D-limonene	$C_{10}H_{16}$-D1	Monoterpenes	Disrupts the cell membrane by releasing nucleic acid and protein out of the membrane, and heat shock is induced by direct contact with the DnaKJE-σ32 complex.	[94]	[126]
	Linalool	$C_{10}H_{18}O$	Monoterpenoids	Destroy the membrane integrity and the respiratory chain of bacteria. Affects the activity of the key enzymes, such as pyruvate kinase (PK), succinate	[127, 128]	[126]

				dehydrogenase (SDH), ATPase, and respiratory chain dehydrogenase.		
Camphene		$C_{10}H_{16}$-E1	Monoterpenes	No antibacterial activity reported yet	[96]	[126]
α-Pinene		$C_{10}H_{16}$-D2	Monoterpenes	Destructs the cell membrane by releasing nucleic acid and protein out of the membrane, and heat shock is induced by direct contact with the DnaKJE-σ32 complex.	[94]	[126]

The *Plectranthus barbatus*, or synonym *Coleus barbatus*, also known as mzugwa in Kiswahili or ekijeera in the Haya language of Tanzania, is a perennial herb belonging to the family Lamiaceae. Fig. 8 shows the aerial part of *Plectranthus barbatus*, whose roots are rich in labdane diterpene (forskolin), used as a potential drug for eczema, hypertension, painful urination, colic, congestive heart failure, insomnia, respiratory disorders, and convulsions [129]. Traditionally, *P. barbatus* is used to treat skin and urinary tract infections, eczema and gastrointestinal disorders [100]. As highlighted in Table 3, across various studies, *P. barbatus* extracts exhibit antifungal properties with zones of inhibition ranging from 11 to 27 mm, and antibacterial properties with MIC values ranging from 3.12 to 12.5 mg/mL. The variation in performance between extracts is due to differences in the solvent system used. *P. barbatus* mainly contains terpenoids (C10, C15 and C20) as shown in Table 4. CXLE can extract these compounds with antibacterial properties due to its flexibility.

Tetradenia urticifolia belongs to the family Lamiaceae and is called Omushunshu in the Haya language of Tanzania. Fig. 9 shows the aerial part of *T. urticifolia*. A closely related species is *Tetradenia riparia*. Extracts from these species have various ethnomedicinal applications and are currently utilised in Tanzania for their antibacterial, antifungal, and antiviral properties [130]. Traditionally, the leaves are used to treat insect bites, wounds, malaria, tonsillitis, and ulcers [131]. The plant leaves are also used to repel insects, such as fire ants, mosquitoes, and fleas [132], and their scent produced by the leaves is known to deter bee swarms that invade residential houses (Misbahu Katakweba, personal conversation, 12 January 2025, Bukoba, Tanzania)

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Fig. 7: A shrub of *Vernonia amygdalina*

Fig. 8: *Plectranthus barbatus*

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Fig. 9: ***Tetradenia urticifolia***

Apparently, no study has reported on the antibacterial activity of extracts from *T. urticifolia*. Instead, the closely related species in the same genus, *Tetradenia riparia*, is reported to have antibacterial potential. The methanolic extract from the roots of *Tetradenia riparia* inhibited bacterial growth with an inhibition zone diameter ranging from 20.0±1.0 mm to 29.33±0.88 mm and minimum inhibitory concentrations of 1.25 mg/L to 5 mg/L [67]. As shown in Table 4, this plant mainly contains monoterpenoids, sesquiterpenoids and diterpenoids, which contribute to its antibacterial activity through different mechanisms. Due to the nature of these compounds, they can be extracted by CXLE using polar solvents at high CO_2 mole fractions or by intermediate polar solvents at intermediate CO_2 mole fractions, as similar compounds were extracted as shown in Table 2.

CO_2-Expanded Liquid Application in Bioactive Compounds Extraction

Before performing extraction with the CXLE method, it is useful to conduct cosolvent screening following the steps outlined in Fig. 12. The target compound should first be identified, followed by the determination of solubility parameters. These parameters are used to estimate the minimum energy interaction difference between the target compound and the solvent system. The Hansen solubility parameters (HSPs) approach [133] is commonly used to screen suitable solvents for extracting the target compound. The reference Hansen solubility parameters at 25 ºC and 1 atm, for some compounds from selected Tanzanian medicinal plants listed in Table 4, are available in the literature, while others are not. Therefore, the reference solubility parameters at 25 ºC and 1 atm for some compounds in Table 4 can be calculated using group contribution methods [134, 135] or software such as Hansen Solubility Parameters in Practice (HSPiP) [35] or conductor-like screening model for real solvents (COSMO-RS) [136] can be used. However, extraction in CXLE is conducted at different temperatures, pressures and CO_2 mole fractions; therefore, these factors need to be taken into account.

Effect of Temperature and Pressure in CXLE

In most cases, the CXLE operates under varying extraction conditions; therefore, the effect of temperature on the solubility parameters of a target compound is estimated by using Equation (1) [137, 138]. The thermophysical properties, such as critical temperature (T_c) and boiling point (T_b), can be obtained from the ThermoData Engine (TDE), provided by the National Institute of Standards and Technology (NIST), accessed through Aspen Plus software [138].

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$$\frac{\delta_2}{\delta_1} = \left(\frac{1-T_{r,2}}{1-T_{r,1}}\right)^{0.34} \quad (1)$$

Where Tr,1 and Tr,2 are the reduced temperatures at states 1 and 2, and δ_1 and δ_2 are the solubility parameters at these states. State 1 can be regarded as the reference state.

$$T_r = \frac{T}{T_c} \quad (2)$$

Where T is the temperature of interest and Tc is the critical temperature.

Based on the experimental setup of CXLE in Fig. 11, the solubility parameters of the CO_2-cosolvent system are affected by thermodynamic parameters such as temperature, pressure and CO_2 mole fraction. Cosolvents such as methanol, ethanol, ethyl lactate, acetone, ethyl acetate and n-hexane can be expanded by CO_2. Therefore, understanding the phase behaviour of the CO_2-cosolvent system is essential for identifying optimal extraction conditions. The liquid phase is the most effective during extraction. In the liquid phase, CO_2 is fully dissolved in the cosolvent, but in the two-phase (liquid - vapour), CO_2 is partly dissolved, resulting in the active phase (liquid phase) having a low concentration, and therefore poor selectivity, compared to the same CO_2 mole fraction in the liquid phase. Following the experimental setup of CXLE shown in Fig. 11, CO_2 is preconditioned to 0 °C before mixing with the cosolvent [139], and the pressure can be varied (i.e. from 4 to 8 MPa), depending on the experimental setup and the phase behaviour of the expanded solvent. At these states, CO_2 exists as a compressed/subcooled liquid with molar volume ranging from 49.097 to 47.123 cm^3/mol, respectively [140].

To account for the effects of both temperature and pressure, the solubility parameters for dispersion, polar, and hydrogen bonding energy of CO_2 at 0 °C and varying pressures can be calculated using Equation (3)-(5) [133]. It should be noted that molar volume is an intensive thermodynamic property which is a function of both temperature and pressure.

$$\delta_{\mathrm{d}} = \delta_{\mathrm{dref}} \left(\frac{V}{V_{ref}}\right)^{-1.25} \quad (3)$$

$$\delta_{\mathrm{P}} = \delta_{\mathrm{Pref}} \left(\frac{V}{V_{ref}}\right)^{-0.5} \quad (4)$$

$$\delta_{\mathrm{h}} = \delta_{\mathrm{href}} \times e^{\left[1.32\times10^{-3}\left(T_{ref}-T\right)+\ln\left(\frac{V_{ref}}{V}\right)^{0.5}\right]} \quad (5)$$

Where δ_{dref}, δ_{pref} and δ_{href} are the reference solubility parameters for dispersion, polar and hydrogen bonding, respectively, at the reference temperature and pressure, V_{ref} and T_{ref} are the reference molar volume and temperature, respectively.

Effect of the CO_2 Mole Fraction in CXLE

To counter the effect of CO_2 mole fraction, the solubilities of the mixture (CO_2 and cosolvent) are calculated using Equation (6). The molar volumes of both CO_2 and cosolvent before mixing can be obtained from the NIST database [140]. After mixing CO_2 and cosolvent the resulting molar volume (v_{mix}) and temperature (T_{mix}) of the mixture can be obtained from the NIST standard reference database accessed through “Reference fluid properties” (REFPROP) software or by using equations of states such as perturbed chain statistical associating fluid theory (PC-SAFT) [141] for polar solvents or the Peng Robinson (PR) [142] for non-polar compounds when REFPROP does not converge. The mixture of CO_2-cosolvent is again preconditioned in a water bath or any working fluid to the extraction temperature, thus equations (3), (4) and (5) are again used to estimate the new solubilities at extraction conditions.

$$\delta_{mix} = \left(\delta_c - \delta_s\right)\frac{x_c v_c}{\left(v_c - v_s\right)x_c + v_s} + \delta_s \qquad (6)$$

Where δ_{mix} are the solubility parameters of a mixture (δ_d, δ_p or δ_h), δ_c and δ_s are the solubility parameters (δ_d, δ_p or δ_h) of CO_2 and the cosolvent, respectively, v_c is the molar volume of CO_2, vs is the molar volume of the solvent, and xc is the mole fraction of CO_2.

The solubilities of the mixture are calculated by varying the CO_2 mole fractions within a specified range from 0.1 to 0.9, at different pressures and temperatures.

Interaction of CO_2-Cosolvent With Bioactive Compounds

After identifying the solubilities of target compounds listed in Table 4 from selected Tanzanian medicinal plants and the solubilities of CO_2-cosolvent, the energy interaction difference can be calculated using Equation (7) [133].

$$\Delta\delta_t = \sqrt{\left(\delta_{d2} - \delta_{d1}\right)^2 + \left(\delta_{p2} - \delta_{p1}\right)^2 + \left(\delta_{h2} - \delta_{h1}\right)^2} \qquad (7)$$

Where $\Delta\delta_t$ is the total energy density interaction difference, δ_d, δ_p, and δ_h are Hansen solubility parameters for dispersion, polar and hydrogen bonding forces, respectively. The subscripts “1” and “2” denote solvent and solute, respectively.

The minimum energy interaction difference determines which solvent is suitable for a particular compound under certain conditions. At this stage, some estimations may have slightly deviated from the stated extraction conditions, or may estimate lower mole fractions and higher temperatures as the best interactions. However, under these conditions, selectivity decreases. Therefore, it is essential to conduct an optimisation experiment around the proposed region to identify the actual optimal conditions for the targeted bioactive compounds. In optimisation experiments, factors can be grouped into solubility factors, which relate to thermodynamic control, such as temperature, pressure and CO_2 mole fraction and mass transfer kinetics factors, which determine how quickly the solute moves to the solvent (flow rate and time). Typically, the solubility factors (thermodynamic controls) are optimised first, and the kinetic factors are evaluated at the optimal conditions [49, 143] to ensure that operational factors do not inherently limit the optimisation of solubility.

Scalability, Economic Feasibility and Environmental Impact of Using CXLE at the Industrial Scale in Tanzania

Transition of CO_2-expanded liquid extraction from laboratory to industrial scale requires a critical assessment of scalability, economic feasibility, and environmental implications. A pilot plant CXLE with a 50 L capacity and with an extraction vessel of a design pressure of 7.0 MPa, as shown in Fig. 10, has already been installed at the University of Dar es Salaam, Tanzania, purposely for extracting oils from agricultural residues like rice bran for rural electrification [144]. This provides a unique opportunity for process validation under near-industrial conditions. The CXLE pilot plant demonstrates its technical feasibility in the Tanzanian context and could serve as a basis for scale-up studies, process optimisation, and operator training. However, there is limited information on its techno-economic assessment, operating costs, and return on investment, creating a knowledge gap regarding its economic sustainability for industrial development.

Tanzania is abundant in medicinal plants; therefore, access to feedstock is not a limiting factor for CXLE. As this technology is newly adopted in Tanzania, major challenges may rely on spare supplies for maintaining high-pressure pumps, seals, connectors and valves, which may sometimes lead to extended down times. The main cost drivers in CXLE are: sourcing CO_2 and solvents, as well as the energy needed for compression by the main compressor and chillers. However, CO_2 is available from local producers and solvents are imported at competitive prices. Both CO_2 and solvents are recycled in CXLE, supporting its economic feasibility. Skilled labour, electricity, and capital amortization for high-pressure equipment must be carefully considered when assessing the potential for scaling up to full industrial production.

In addition to that, CXLE minimises the use of large volumes of organic solvents, reducing hazardous waste generation and aligning with principles of green chemistry and the circular economy. After leaving the separator tanks, the CO_2 gas is temporarily stored in atmospheric tanks and later pumped back to the extraction line by the compressor. This reuse of CO_2

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reduces its greenhouse gas footprint in the environment. The mild operating conditions (<40 °C and <8 MPa) generally lower energy requirements and preserve heat-sensitive bioactive compounds.

Fig. 10: Extraction vessel of pilot scale CXLE at the University of Dar es Salaam, Tanzania

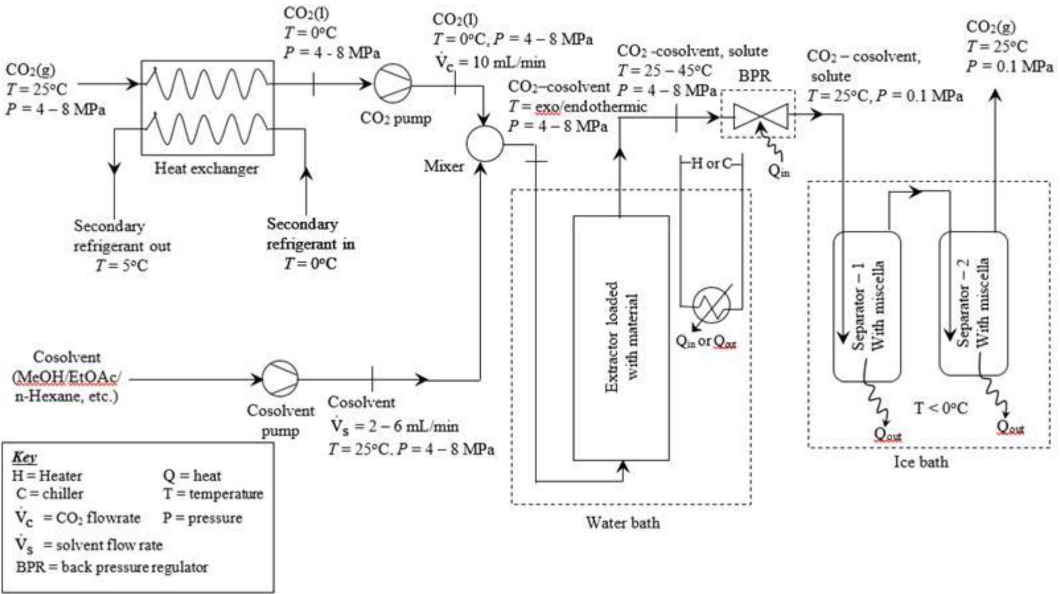

Fig. 11: A detailed block flow diagram of the CO_2-expanded liquid experimental setup, with various extraction and operation conditions at different stages

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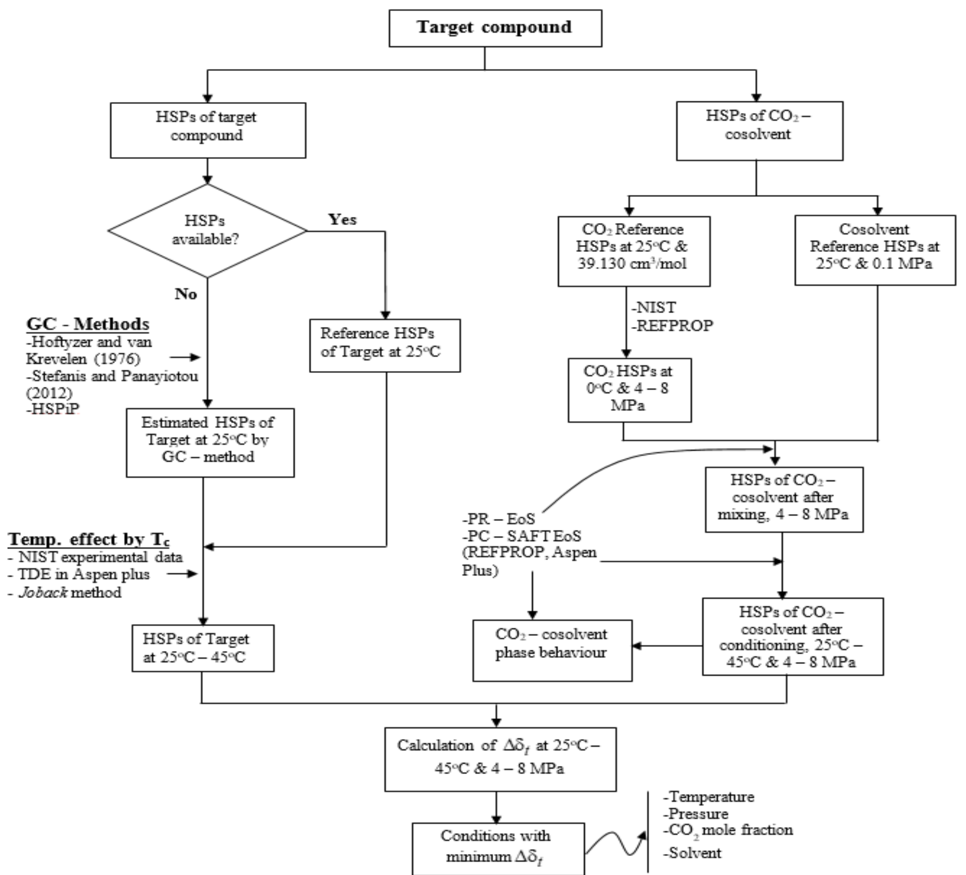

Fig. 12: Cosolvent screening steps to establish minimum energy interaction difference. HSP is the Hansen solubility parameter, $\Delta\delta_t$ is a minimum energy interaction difference. TDE is the thermodata engine, NIST is the National Institute of Statistics, GC is the group contribution method, PC-SAFT is the perturbed chain statistical associating fluid theory equation of state, PR-EoS is the Peng-Robinson equation of state, REFPROP is Reference fluid properties

Discussion on the Efficacy of CO_2-Expanded Liquid in Extracting Bioactive Compounds

The analysis of CXLE studies summarised in Table 2 demonstrates the efficacy of extracting compounds from different plant matrices such as fruit peels, fruit pulps, seeds, resin, and grain husks from various plant species. These studies investigated CXLE's ability to recover various classes of bioactive compounds, such as polar phenolics and flavonoids, intermediate polar steroids, and nonpolar compounds like fatty acids and monoterpenes, which are the same compound classes covered in Table 4 of selected medicinal plants from Tanzania. Across the studies, CXLE demonstrated better performance than conventional methods. CXLE demonstrated selective extraction of monoterpenes, yielding 300% more than hydro-distillation and 13% - 60% more than maceration. However, the relationship between maceration and CXLE varied depending on the cosolvent used. The same expanded solvent was tested in its pure form with the maceration technique. When hexane was used as a cosolvent in CXLE and maceration for extracting most nonpolar monoterpenes without an OH group, it resulted in a lower deviation with the CXLE extract. Conversely, it caused a higher deviation when extracting OH-containing compounds, such as monoterpenoids and phenolics. The same trend was observed with expanded ethanol. CXLE extracted a terpenoid with an OH group, such as cis-verbenol (5), 28% more than maceration with ethanol, and surpassed maceration with ethanol by 60% when extracting the nonpolar monoterpene, α-pinene (4). This highlights the ability of polarity tuning in CXLE regardless of the cosolvent used. The superior performance of CXLE over maceration is also influenced by improved mass transfer properties of CXL within the plant matrix.

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Furthermore, CXLE demonstrates superior polarity tuning compared to SC-CO_2. From Table 2, CXLE shows 7% to 17% higher extraction efficiency than SC-CO_2 when extracting monoterpenes such as α-pinene (4) and cis-verbenol (5). The minor difference of 7% between CXLE and SC-CO_2 was observed in the extraction of the nonpolar monoterpene α-pinene (4). In contrast, a deviation of 17% was observed in the extraction of the slightly polar terpenoid cis-verbenol (5) [49]. When extracting polar compounds, CXLE achieved 82% higher efficiency compared to SC-CO_2 for compounds like the polyphenolic flavonoid, silymarin (6) [48]. The preferences of SC-CO_2, which tend to favour nonpolar compounds, highlight limitations in extracting intermediate and polar compounds. This insight encourages further investigation of CXLE as a promising method for extracting compounds with varying polarities. Studies summarised in Table 2 indicate that the optimal extraction conditions differ depending on the target compounds and the type of cosolvent used. Heavier and more polar molecules, like silymarin (6), are best obtained at lower CO_2 mole fractions, while the moderately polar and lighter molecules, like taxifolin (3), are extracted at slightly higher CO_2 mole fractions when using a mixture of CO_2, ethanol, and water. This understanding provides a basis for optimising CXLE for target compounds of diverse polarities.

Selectivity has a direct influence on the bioactivity of the resulting extracts. Depending on the polarity of unwanted compounds and the concentration of the target compound in the plant matrix, the optimal extraction conditions for optimal yield (w/w) sometimes do not always align with the best conditions for selectivity of target compounds [35]. The highest yield is mainly achieved at lower CO_2 mole fractions due to poor selectivity of the extractant, while the selectivity for the target compound tends to increase at higher CO_2 mole fractions. Optimising overall yield may not fully reflect the extract's functional potential. Therefore, prioritising the selectivity of bioactive compounds over yield is a reliable strategy when the primary purpose of the extract is bioactivity.

Although CXLE has been extensively studied for its ability to extract bioactive compounds from different plant matrices, most studies have mainly concentrated on anti-inflammatory, antioxidant activities, and the reduction of micellar cholesterol (RMC) of the extracts [35, 47, 48]. Based on this review, no evident work was observed to directly test CXLE extracts in antimicrobial assays, creating a knowledge gap in assessing their therapeutic potential. Furthermore, due to the selective nature of CXLE, it can suppress contaminants that might otherwise interfere with extract penetration and diffusion through bacterial biofilms and the cell wall. Therefore, future research should go beyond antioxidant and anti-inflammatory assessment and explore the antimicrobial properties of CXLE extracts, helping to establish their bioactivity profile.

In addition to that, CXLE has also been studied in non-pharmaceutical domains, particularly for recovering oils from agricultural products for biodiesel production [31, 32, 45]. In these studies, CXLE effectively suppressed contaminants such as phospholipids and waxes, which are known to reduce fuel performance. This achievement highlights the capacity of CXLE to improve product quality by reducing interfering compounds in the extract. Based on these principles, CXLE can be extended to the extraction of bioactive compounds with antibacterial potential by minimising impurities that interfere with diffusion into bacterial cells.

Summarised studies in Table 2 have examined CXLE in various plant matrices, each with distinct compositions and targeted bioactive compounds. As a result, variations in reported yields and selectivity make it challenging to compare performance across studies. The optimal extraction conditions have been reported over a broad range of pressures from 5 to 25 MPa, temperatures between 25 to 75 ºC and CO_2 mole fractions of 0.15 to 0.82, spanning different plant materials, compound classes and cosolvents. These differences complicate direct comparison of extraction efficiency across studies. Both mild conditions (16.6 - 33.4 ºC, 3.3 - 7.2 MPa) and more intense conditions (40 - 80 ºC, 8 - 30 MPa) have yielded reasonable results, indicating that extraction efficiency may be less dependent on pressure or temperature and more strongly enhanced by solvent composition in its liquid phase. Previous studies suggest that pressure has a limited impact when the CO_2 - cosolvent mixture remains in the liquid phase [48, 145]. Therefore, elevating the temperature while increasing the pressure to keep the phase liquid appears sufficient for optimal recovery and potentially reduces energy consumption. However, extracting at higher temperatures risks thermo-degrading thermolabile compounds, as reported by Cunico et al. [146], who observed thermo-degradation of quercetin (2) at 50 ºC in CXLE.

1 D-limonene **2** Quercetin

3 taxifolin **4** ∝ - pinene **5** cis-verbenol

6 Silymarin

7 β-sitosterol

Fig. 13: Chemical structures of compounds (1 - 7)

Future Research Direction

Research on CXLE should emphasise a deeper understanding of the solubility properties and the phase behaviour of CO_2-solvent systems, which helps in accurately determining and locating extraction conditions [31, 139]. The chosen extraction conditions define the state properties, diffusivity, and transport behaviour of the CO_2-cosolvent mixture in interaction with the target bioactive compound. In addition to that, future research can explore the extraction of polar and intermediate polar bioactive compounds using CXLE under milder conditions (< 8 MPa and < 40 ºC), as achieved with essential oils and fatty acids [31, 47, 139]. This approach will help preserve thermolabile compounds, thereby enhancing the recovery of bioactive compounds with antibacterial potential. After process optimisation, it is essential to validate the antimicrobial efficacy of CXLE extract. When compound separation is necessary, combining CXLE with chromatographic techniques will enable simultaneous extraction and separation of bioactive compounds in a single step [147]. By utilising CXLE pressure and microfilters, adding another column packed with silica gel (Si-OH for nonpolar or reversed Si-OR for polar) in series can reduce downstream processing steps and improve the purity of separated compounds.

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Conclusion

In conclusion, the review underlines the potential of using CO_2-expanded liquid extraction for producing bioactive compounds from Tanzanian medicinal plants. This method appears to be a promising alternative to conventional extraction techniques, offering a greener approach that yields high-quality extracts. The bioactive compounds extracted from these plants are known to have notable antibacterial properties against a range of pathogenic bacteria, indicating their potential for developing new antibacterial agents. The review also emphasises the importance of optimising extraction parameters to maximise the yield of bioactive compounds and validating their antibacterial efficacy, to integrate the chromatographic technique for further separation of compounds in a single step with high purity. It emphasises the need for further research to explore a wider range of medicinal plants in Tanzania and to confirm the clinical applications of the bioactive compounds. Combining traditional medicine knowledge with innovative extraction technologies could pave the way for novel treatments and contribute to the global fight against antibiotic resistance. This study serves as a baseline for future explorations into Tanzania's rich botanical heritage and its previously unexplored therapeutic potential.

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Author's Contributions

Wajihu Ahmada: Collected information on ethnomedicinal knowledge of selected medicinal plants currently used to treat various ailments, including taking photographs of these plants from Bukoba, Tanzania and prepared the draft manuscript.

Emrod Elisante: Contributed to the knowledge of extraction techniques, their cost-effectiveness, and the potential of CO_2-expanded liquid extraction for high-value products.

Neema Msuya: Contributed to the knowledge of bioactive compounds and their antimicrobial properties.

Kando Janga: Contributed to the knowledge of optimisation of CO_2-expanded liquid extraction parameters and proofreading the article.

Ambrose Itika: Contributed to the knowledge of conventional extraction techniques in relation to CO_2-expanded liquid extraction.

Ethics

This review article is original and presents newly analysed information from the cited literature. The authors declare that they have no conflict of interest.

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