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Chemical Composition of *Combretum erythrophyllum* Leaf and Stem Bark Extracts

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Abstract: Combretaceae is a large Angiosperm family that is highly sought after because of its pronounced medicinal value. *Combretum* is recognized as the largest genus, prevalent in southern Africa due to its extensive use in traditional medicine. This study aimed to provide a comparative analysis of the phytochemical constituents of the leaf and stembark extracts of *Combretum erythrophyllum (*Burch.) Sond. Leaf and stembark crude extracts were generated using hexane, chloroform, and methanol as the solvents of choice. Qualitative phytochemical tests indicated the presence of phytocompounds, including carbohydrates, alkaloids, sterols, phenols, fixed oils, and fats. Flavonoids were found within the leaf extracts only, while saponins, mucilage, and gums were specifically identified within the stembark extracts. The first reported gas chromatography–mass spectrometry (GC–MS) screening of *C. erythrophyllum* leaf and stembark extracts was conducted, yielding the identification of 266 phytocompounds. Major phytocompounds such as sitosterol and lupeol, which may have possible anti-cancer and anti-inflammatory properties, were identified. Furthermore, a pharmacogenetic evaluation was conducted. As a result, both the leaf and stem bark material were seen to fluoresce a wide array of colors (brown, red, green, and blue colorations), indicating the presence of beneficial phytometabolites and their use in medicinal applications. Given the wide array of proposed medicinal benefits associated with the presence of phytocompounds identified within *C. erythrophyllum*, this species should be considered for its medicinal importance. The isolation and extraction of these beneficial compounds open further avenues for their use in the pharmaceutical industry.

Keywords: bioactivity; *Combretum* species; phytochemistry; phytometabolites; traditional medicine

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1. Introduction

Among several cultures and ethnic groups around the world, medicinal plant species are known to act as an ancient therapeutic tool [1,2]. Medicinal flora is known to contain metabolites that may effectively improve an individual's state of health [3,4]. Due to extensive research into the benefits of medicinal plants, first-world countries are now integrating naturally derived medicines into mainstream health care systems. Growing interest in the study of medicinal plants has led to the exuberant boost of its global economic value [5]. The global medicinal plant industry was valued at an estimated USD 60 billion in 2003, whereas in 2012, a single avenue of the industry, traditional Chinese medicine, was valued at USD 83 billion, thus proving its exponential growth among communities [6,7]. The World Health Organization mentions that 80% of the global population relies on the utilization of medicinal plants for the treatment of numerous ailments [8–10]. Thus,

it has become of the utmost importance to promote extensive studies integrating the safe use and conservation of these medicinal plants [7,11,12]. The pharmacological use of medicinal plant species has numerous benefits with minimal side effects [9,13,14]. Known benefits associated with this naturally derived form of medication include cost-efficiency, accessibility, and a role in physiological effectiveness [15].

Medicinal properties are attributed to the secondary metabolites found in plants [16]. Synthesized primary metabolites aid in cell functioning, plant growth, and development [15]. While secondary metabolites are plant-specific and are produced as part of the plant defense system against pests and pathogens [16,17]. Secondary metabolites are classified into three main classes, namely, alkaloids (nitrogen-containing compounds), terpenes, and phenolic compounds [18]. They are exuded by specialized secretory tissues such as resin ducts, mucilage ducts, laticifers, salt glands, hydathodes, and trichomes [19–21]. Microbial pathogens are continuously evolving; hence, advanced remedial methods need to be introduced [22]. Scientists are now turning towards medicinal plant research to curb microbial pathogen growth. Continued research and analysis of current pathogens are crucial in order to develop efficient, naturally derived, plant drug-delivery methods [23].

The medicinal use of plant species from the family Combretaceae is of high interest on the African continent [24]. Literature suggests the hardy nature and predominant presence of medicinally important phytometabolites within these plants [25–27]. Traditionally, *Combretum erythrophyllum* is known to assert high medicinal value [23]. This tree is commonly known as the river bushwillow and is a deciduous indigenous tree that thrives in areas with good groundwater levels. Traditional uses include the treatment of venereal diseases, abdominal pain, and sores, among many others [24]. Pharmacological studies have shown that species of *Combretum* have antibacterial, anti-inflammatory, antifungal, genitourinary, cytotoxic, and mutagenic properties [23,28]. Epidemiological studies highlight the occurrence of antioxidant compounds within the species [23]. A recent study conducted by Mtunzi et al. [23] indicated the prominent antibacterial effects of *C. erythrophyllum* leaf extract against multiple bacterial strains.

Phytochemical analysis can be conducted to detect the presence of phytometabolites of therapeutic importance. A detailed analysis of a plant's biologically active metabolite profile can prove to be beneficial in new drug discoveries [29]. The measure of the medicinal value of a plant is directly linked to its phytochemical constituents, such as alkaloids, phenols, and essential oils [30]. Further studies demonstrating the true medicinal worth of traditionally utilized species will be highly beneficial, not only for further use in the modern drug development industry but also to communities. There have been studies released on the phytochemical constituents and possible biological activity of the leaves of *Combretum erythrophyllum*; however, there have been limited or no studies released on the phytochemical constituents and biological activity of the stembark. Hence, this study aims to provide a comparative analysis of the phytochemical constituents of the leaf and stembark extracts of *C. erythrophyllum* by conducting a phytochemical analysis, thin-layer chromatography (TLC), energy-dispersive X-ray (EDX), gas chromatography–mass spectrometry (GC–MS), and powder microscopy. Information regarding both beneficial and potentially harmful compounds present within *C. erythrophyllum* was elucidated through the emanating study.

2. Materials and Methods

2.1. Phytochemistry

2.1.1. Plant Collection

The leaves and stem bark of *Combretum erythrophyllum* were obtained from the University of Kwa-Zulu Natal (Westville), Durban, South Africa (29°49′ S; 30°59′ E). A voucher specimen was previously submitted to the Herbarium (13476/2), University of KwaZulu-Natal, Westville Campus. The collected fresh material was left to dry at ambient temperature, ~25 °C for six weeks. The dried leaves were then crushed using a blender

(Philipps HR7762, China) to obtain a fine powder-like material. Because of the hardy nature of stembark, samples were subsequently freeze-dried using liquid nitrogen and crushed. The material was stored away until further use.

2.1.2. Extraction

Approximately 25 g of crushed leaf and stem bark material were added to 125 mL of hexane (organic solvent) in separate round-bottom flasks. These were attached to the reflux apparatus, placed within an Electrothermal EMV0250/CE MK5 heating mantle and distillation commenced via the electrical application of heat. After approximately three hours of heating at 35 °C, the extract was filtered using a funnel and filter paper. This process was repeated three times to allow maximum extraction of compounds. Thereafter, this process was repeated using, chloroform followed by methanol as the organic solvent of choice (non-polar to polar solvents). The crude extract was then utilized for phytochemical analysis and thin-layer chromatography.

2.1.3. Phytochemical Analysis

Various chemicals were added to portions of crude extracts to detect the presence of bioactive phytocompounds (adapted from Jaradat et al. [31]).

Fixed Oils and Fats

Spot test: Crude extract (0.1 mL) was spotted on a sheet of filter paper and left to dry. The presence of an oil ring indicated a positive detection of fixed oils.

Saponins

Foam test: Crude extract (0.5 mL) was added to 10 mL of distilled water and shaken rapidly, by hand or 15 min. The presence of saponin was indicated by a 1–2 cm layer of foam on the surface of the solution.

Froth test: Crude extract (2 mL) was diluted using 20 mL of distilled water and shaken rapidly, by hand for 15 min. A froth layer of 1 cm was an indicator of the presence of saponin.

Phenols

Ferric chloride test: Two drops of $FeCl_3$ were mixed with 1 mL of crude extract. The presence of phenol was indicated by a green/black color change.

Lead acetate test: A 5% lead acetate solution (1 mL) and 5 mL of crude extract were combined. The generation of a white precipitate indicated the presence of phenolic compounds.

Carbohydrates

Molisch test: A 0.2 mL solution of alcoholic α-naphthol was added to 1 mL of crude extract and swirled. Concentrated H_2SO_4 (0.5 mL) was gently added to the solution and the visualization of a purple/red ring between solvent layers indicated the presence of carbohydrates.

Benedict's test: Crude extract (2 mL) was combined with 1 mL of Benedict's reagent. The solution was then boiled in a water bath at 60 °C for 2 min. Reducing sugars were indicated by the formation of orange-red precipitation.

Fehling's test: Crude extract (1 mL) and Fehling's A and B (1 mL) solutions were added to a test tube. The resulting solution was boiled in a water bath. Reducing sugars were indicated by the formation of red precipitation.

Glycosides

Salkowski's test: Two drops of Salkowski reagent were added to 2 mL of crude extract. Glycosides were observed by the formation of a red ring.

Sterols

Chloroform (3 mL) and sulfuric acid (two drops) were added to 2 mL of crude extract. The visualization of a red and green ring between the layers indicated the presence of sterol and cholesterol, respectively.

Alkaloids

Mayer's test: Mayer's reagent (1 mL) was added to 2 mL of crude extract. A yellow precipitate was an indication of the presence of alkaloids.

Wagner's test: Wagner's reagent (1 mL) was added to 2 mL of crude extract. A brown/red precipitate was indicative of the presence of alkaloids.

Dragendorff's test: Dragendorff's reagent (1 mL) and 2 mL of crude extract were combined. A red precipitate indicated the presence of alkaloids.

Flavonoids

Shinoda test: Ribbons of Magnesium(2) were added to 3 mL of crude extract. Once dissolved, 2 drops of concentrated Hydrochloric acid were added. A red color change indicated the presence of flavonoids.

Mucilage and Gums

The crude extract (1 mL) was infused with two drops of 0.5% Ruthenium Red. Mucilage and/or polysaccharides were positively identified by a red color change.

2.1.4. Thin-Layer Chromatography (TLC)

The prepared extracts were evaluated using thin-layer chromatography. This generalized analysis was conducted to identify phytometabolic compound classes found in the extracts. Using a pencil, a baseline was drawn onto the edge of a silica gel 60 F254 plates. The plate was spotted with a droplet of each crude extract and placed into a beaker containing a mobile solution of 9 parts toluidine: 1-part ethyl acetate: 0.5-part formic acid: 0.5-part glacial acetic acid. The beaker was covered with foil and the solution was left to move up the plate. By active capillary action of the solution moving up the silica plate, a number of compound classes were visualized. A non-polar compound is known to travel faster in comparison to a polar compound [32]. The plate was viewed under ultraviolet light at wavelengths 254 and 360 nm. The plate was then exposed to a solution comprising: 0.5 mL anisaldehyde solution, 10 mL glacial acetic acid, 85 mL methanol, and 5 mL sulfuric acid. After exposure to this solution, the plate was left in an oven at 105 °C for 5–10 min.

Retention values (Rf) were calculated using the following formula:

$$\text{Rf} = \frac{\text{Distance travelled by compound}}{\text{Distance of the solvent front}}$$

2.1.5. Gas Chromatography–Mass Spectrometry (GC–MS)

The generated extracts were analyzed using GC–MS (QP-2010 SE Shimadzu, Japan), with a Rx_5Sil MS (Restek, Rxi-GC columns) capillary column. Helium was used as the carrier gas and all relevant flow rates were noted. The total flow rate was 4.9 mL min^{-1} while the column and purge flow rates were recorded as 0.96 mL min^{-1} and 3.0 mL min^{-1}, respectively. Temperatures of the injection port, detector, and oven were maintained at 220 °C, 320 °C, and 150 °C, respectively. After a holding period of 2 min, the oven temperature was increased and held at 295 °C. The applied injection volume was 1 μL and the scan speed was recorded to be 3333 m. The sensitivity and accuracy of the equipment were

tested using relative standards and calibration methods. The extracts were run in the splitless mode for 37 min. As a result, molecular structures and mass were key features analyzed in compound identification. The National Institute of Standards and Technology (NIST) database was used for compound identification [33,34].

2.1.6. Energy-Dispersive X-ray (EDX)

An energy dispersion X-ray analysis was performed to determine the elemental composition of leaf and stem bark material. Measures of 0.2 mgs of powdered leaf and stem bark material were placed on the surface of individual sterile aluminum stubs. These stubs were then sputter-coated with gold using the Quarum Q150 RES gold coater. The samples were subsequently viewed and analyzed using the Field Emission Gun Scanning Electron Microscope Zeiss FEGSEM Ultra Plus (Carl Zeiss, Germany), and AzTec software (Oxford Instruments, UK).

2.2. Fluorescence Microscopy

Powdered leaf and stem bark material were utilized for this analysis. A portion of dried leaf/stem bark material was sprinkled onto a clean glass slide and submerged within 1–2 drops of the prepared reagent of choice. The prepared slide was then gently swirled and left to incubate in a cool, dry, dark environment for 2–3 mins (until all the reagent was absorbed). The slides were viewed using the Nikon compound and fluorescence microscope, Nikon eclipse, 80 i, and Nikon DS-Fi1 microscopy (NIS-Elements D) using both brightfield light and ultraviolet 2a (330/380 nm). The following reagents were utilized for this study: water, sulfuric acid, acetic acid, aqueous sodium hydroxide, hydrochloric acid, ethanol, ethyl acetate, hexane, chloroform, methanol, petroleum ether, diethyl ether, and acetone.

3. Results and Discussion

3.1. Phytochemical Analysis

Preliminary phytochemical tests indicated the presence of multiple phytocompounds (Table 1). The polarity of compounds differs; hence, a variety of organic solvents were utilized to extract a maximum number of compounds present [32]. The following compounds were detected within the crude leaf extracts, namely: carbohydrates, alkaloids, flavonoids, sterols, phenols, as well as fixed oils and fats (Table 1). Phytochemical analysis of the stembark extracts indicated the presence of the following compounds: carbohydrates, alkaloids, sterols, phenols, saponins, mucilage, and gum, as well as fixed oils and fats (Table 1). Comparative analysis between the dried leaf and stem bark samples showed a higher compound variety within the stembark. The leaf (hexane) extract and stembark (methanol) extract showed the highest number of phytometabolites present within this species.

Table 1. Phytochemical analysis of *Combretum erythrophyllum* leaves and stembark.

Test	Leaves			Stembark		
	Hexane	Chloro Form	Methanol	Hexane	Chloro Form	Methanol
Carbohydrates						
Molisch test	++	++	++	++	++	++
Benedicts test	-	++	++	-	-	++
Fehlings test	++	++	-	+	++	++
Alkaloids						
Mayers test	++	-	++	++	+	+
Wagners test	++	++	-	++	+	-
Dragensdorffs tes	++	-	++	++	+	+
Flavonoids						
Lead acetate test	++	+	+	-	-	-
Saponins						
Froth test	-	-	-	-	+	-
Foam test	-	-	-	+	-	+
Glycosides						
Sulfuric acid test	+	++	+	-	-	++
Sterols						
Sterols test	++	-	+	++	+	+
Chloroform test	-	+	-	+	-	++
Phenols						
Ferric trichloride test	+	+	++	+	+	++
Mucilage and Gums						
Ruthenium red test	-	-	-	-	-	++
Fixed Oils and fats						
Filter paper test	++	-	+	-	-	++

Intensity of reaction: (-) negative reaction, (+) slight colour change and (++) Intense colour change.

Eloff et al. [26] highlighted the presence of alkaloids, tannins, phenols, and saponins within numerous *Combretum* species. In lieu, the presence of the above-mentioned phytometabolites was noted within C*. erythrophyllum* (Table 1). Alkaloids were indicated by the formation of a reddish/brown ring produced upon the addition of Dragendorff's reagent. The color change indicates the formation of ion pairs, which occurs between metal atoms (of the reagent) and the nitrogen molecules (of alkaloids) present [35]. The detection of alkaloids in both leaf and stembark extracts of *C. erythrophyllum* (Table 1) denotes its prominent use in traditional medicine [26]. Alkaloids are a diverse group of nitrogen-containing phytometabolites that have been key components in traditional medicine for centuries [30,36]. Research suggests that the therapeutic use of alkaloids can be dated back to the 19th century, when substances were isolated mainly for their narcotic and analgesic components [37]. Continued research has broadened the utilization of alkaloids in the pharmaceutical industry [38]. Pharmacological activities of alkaloids include their uses as muscle relaxants, analgesics, antitussive agents, local anesthetics, as well as anti-cancer, anti-hypertensive, and antiseptic agents [36]. Thus, the detection of alkaloids within extracts of *C. erythrophyllum* (Table 1) emphasizes the importance of their reported traditional use in treating sores and wounds, among many other applications in medicine [26]. *Combretum* species such as *Combretum sokodense* Engl. and *Combretum racemosum* P.Beauv also tested positive for the presence of alkaloids within generated crude extracts [39,40]. Although beneficial, incorrect alkaloid dosages could be lethal [26]. Most commonly isolated alkaloids include quinine, morphine, caffeine, strychnine, and cinchonine [41].

While medically important in plants, these compounds aid in chemical defense and successful pollination [42].

Using the spot test, the presence of fixed oils and fats (essential oils) was identified by the production of an oil ring on filter paper. Table 1 indicates the presence of essential oils in tested crude extracts, which highlights their possible medicinal value, like other members within this genus [23]. Essential oils are hydrophobic, volatile compounds that are found in an estimated 10% of the world's known flora [43].The biological activity of essential oils is based on their chemical constituents [44]. They consist mainly of esters, hydrocarbons, oxides, ketones, and aldehydes. Esters, oxides, and aldehydes are known to exhibit antimicrobial, antiseptic, and spasmolytic properties, while hydrocarbons and ketones are known for their cell-regenerating, sedative, neurotoxic, antitumor, and antibacterial capabilities [13,45]. The positive result for oils within the leaf and stembark extracts of *C. erythrophyllum* (Table 1) emphasizes the link between their reported traditional use in treating bacterial diseases and the identified metabolite [23]. In addition, these oils are commonly known for their enchanting scents. In plants, the scent is used to ward off preying herbivores and to protect against pathogens [44].

The stem bark extracts tested positive for saponins, mucilage, and gums, which were not detected in the leaf extracts (Table 1). In addition, the presence of a foam layer above the diluted solution indicated the presence of saponins within the stembark extract. Saponins are amphipathic compounds that are classified by their structural differences [29]. These compounds are characteristically identified by the formation of foam upon agitation within an aqueous solution [46]. Desai et al. [47] elaborated on the plethora of medicinal properties associated with saponins. These include antibacterial, antifungal, and anti-inflammatory properties, in addition to lowering the cholesterol levels and improving host immune systems. With reference to the above, pharmacological studies have found *C. erythrophyllum* to have antibacterial, anti-inflammatory, antifungal, genitourinary, cytotoxic, and mutagenic properties [24]. *Combretum* species such as *Combretum sokodense* and *Combretum racemosum* also tested positive for the presence of saponins within the stem extract [39,40]. In addition, saponins are known to protect leaf surfaces by acting as deterrents to pathogens and preventing the growth of mold as surface agents [46].

Glycosides are naturally occurring compounds found in flora and were present within both the leaf and stembark extracts (Table 1). These are non-volatile, amorphous compounds that are highly prized in the medicinal industry, because of their numerous benefits [29]. These molecules are characteristically attached to a cyclic form of sugars and are divided into various types such as; phenolic glycosides, cardiac glycosides, cyanogenic glycosides, anthraquinone glycosides, saponin glycosides etc [48]. In addition, glycosides protect plants from diseases and pests [49]. Glycosides isolated from members within the *Combretum* genus are known to exhibit antifungal, anti-inflammatory, and molluscicidal effects [29]. Promising results obtained from previous research of C*ombretum* species such as *Combretum racemosum* show that glycosides are beneficial phytometabolites [39,50,51].

Phenolic compounds are structurally diverse secondary plant metabolites comprising numerous groups such as tannins, flavonoids, combretastatin, and quinones, amongst serval others [29,52]. The presence of tannins and phenols was indicated within the generated crude extract by a black/green color change upon the addition of $FeCl_3$. Phenolic compounds were positively identified across all tested extracts (Table 1). These compounds are known to express the following major effects: anti-mutagenic, anti-inflammatory, apoptosis-inducing, anti-carcinogenic, and antioxidant activity [53]. In addition, Masoko et al. [54] associate the presence of tannins and saponins within *Combretum loefl*, with its anti-fungal activity. Combretastatin is a phenol that may be present within members of the *Combretum* genus. Pettit et al. [55] first isolated combretastatin from a common Bushwillow tree known as *Combretum caffrum* and analyzed the benefits of this molecule in reducing cancer effects. Preliminary phytochemical analysis conducted on *Combretum albidum* G. Don. *Combretum racemosum,* and *Combretum sokodense* extracts also indicated

the presence of phenolic compounds [39,40,56]. Additionally, flavonoids were identified by the generation of a white precipitate upon the addition of lead acetate to the crude leaf extracts. As shown in Table 1, leaf extracts showed the presence of flavonoids, which were absent from the stembark extracts. Flavonoids are the largest phytometabolite group within the phenolic compound class [57]. Thus far, over 9000 flavonoids have been identified and isolated [58]. Common flavonoids include kaempferol, quercetin, and naringin [58]. Flavonoids are known for their anti-cancer, antiviral, anti-allergic, anti-inflammatory, cholesterol-reducing, and most importantly, antibacterial properties [59,60]. Current research suggests the use of flavonoids in reducing the symptoms of menopause as well as lowering the risk of osteoporosis [61,62]. In a study by Berkoff [63], it was observed that flavonoids reduced the release of inflammatory mediators within the cell membrane, thus exhibiting anti-inflammatory effects. With *C. erythrophyllum*, Martini et al. [64] attributed the plant's anti-bacterial effects to the presence of seven different flavonoids. Another study highlighted the presence of flavonoids within plant cell tissues, which serve as chemical barriers against microorganisms, possibly due to the permeability of flavonoids [65]. This supports the traditional use of *C. erythrophyllum* leaves to treat inflammation and disease [24,66]. In flora, these compounds are known to serve a role in flavor profile development, fruit coloration, and defense [67]. Studies show that exposure to high levels of ultraviolet (UV) radiation results in an overall increase in the presence of flavonoids and phenols [53]. As seen in Table 1, the intense presence of phenols in both leaf and stembark extracts correlates with findings from Harborne and Williams [67] suggesting that increased polyphenol levels were a direct defense mechanism against UV damage.

3.2. Thin Layer Chromatography (TLC)

TLC analysis resulted in the visualization of approximately 36 different compound classes (Figure 1) when viewed under UV light (254 nm and 360 nm) and after exposure to anisaldehyde solution. Different compound classes were noted by the appearance of bands that resulted from the upward movement of the mobile solution on the plate. Compounds found closer to the baseline are more polar than those compounds which have traveled further up [32]. A leaf hexane compound band had the highest Rf value of 0.89 while the stem bark chloroform compound class had the lowest Rf value of 0.06, thus indicating their possible polarity and distance traveled within the plate. As compounds move up the plate, colorless bands were formed; thus, the TLC plates were viewed under UV light of different wavelengths to aid visualization. Under normal light (Figure 1), hexane, chloroform, and methanol leaf extracts were shown to have eight, one, and three bands, respectively, whereas hexane, chloroform, and methanol stem bark extracts revealed eleven, two, and five bands, respectively. When viewed under UV light (Figure 1b,c), six additional bands were indicated in the chloroform and methanol leaf extracts, as well as in the methanol stembark extract.

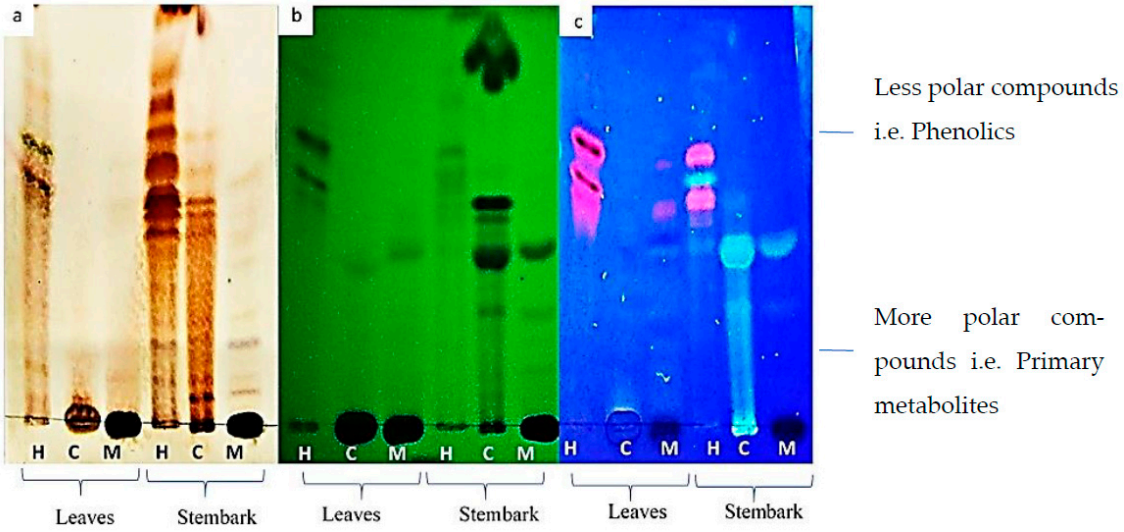

Figure 1. Thin layer chromatographs showing the separation of the hexane, chloroform and methanolic extract of the leaves and stembark of C. erythrophyllum using solvent system 9 toluidine: 1 Ethyl acetate: 0.5 Formic acid: 0.5 Glacial acetic acid (**a**) after spraying with anisaldehyde solution, (**b**) under 254 nm and (**c**) under 360 nm.

3.3. Energy-Dispersive X-ray (EDX)

The elemental composition of powdered (dried) leaf and stem bark material was characterized using the EDX analysis. Carbon (C), oxygen (O), chlorine (Cl), potassium (K), and calcium (Ca) were found in both the extracts (Table 2). Fundamentally, the presence of these elements is justified as they are essential for plant growth and development [15]. The presence of C, O, and Cl indicates an active metabolizing system [6]. The dense presence of calcium within the stembark (26.08%) may be denoted by the presence of crystals found within [68]. In addition, the dense presence of chlorine may indicate the presence of a gelatinous covering across the leaf and stem bark surfaces [69]. Overall, the leaf extract was shown to encompass a wider variety of elements as opposed to the stem bark (Figure 2). Furthermore, the powdered leaf material was characterized by the presence of Magnesium (Mg), Aluminum (Ag), and Silicon (Si). Notably, the presence of Mg, Al, and K (essential nutrients) is well established in medicinal plants [70]. The distinct presence of these compounds in the leaf material suggests that the leaf may be more medicinally inclined in comparison to the stembark. The presence of silicon within the leaf material is highly beneficial to the plant. Ecologically, this element may decrease the transpiration rates (which in turn, prevents water loss) and protects the plant against a range of biotic and abiotic stresses [70].

Table 2. EDX elemental composition of leaf and stembark.

Element	Composition (%)	
	Leaf	Stembark
C	74.67	52.015
O	21.01	17.32
Cl	0.43	1.22
K	2.05	6.37
Ca	0.92	23.08
Al	0.14	-
Mg	0.56	-
Si	0.17	-

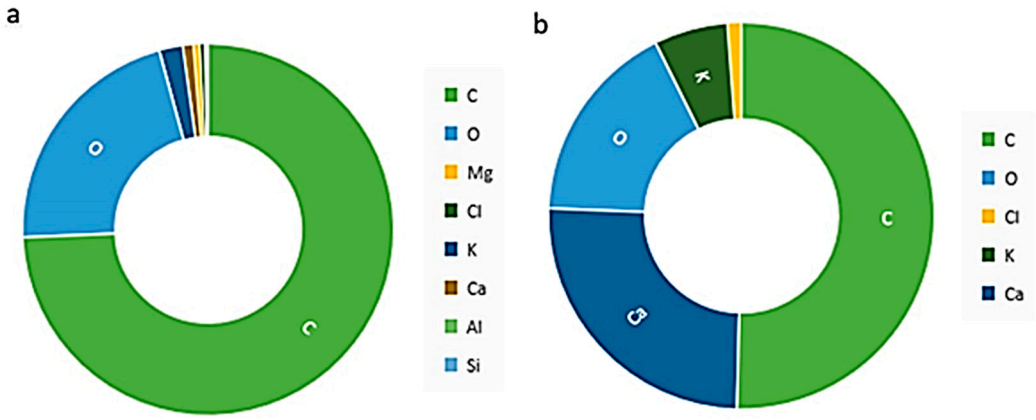

Figure 2. (**a**) Elemental composition of leaf; (**b**) elemental composition of stembark.

3.4. Gas Chromatography–Mass Spectrometry (GC–MS)

Gas chromatography is a compound identification technique that allows for the qualitative and quantitative evaluation of samples [71]. In comparison, mass spectrometry allows for an extremely accurate molecular analysis; hence, the collaboration of both techniques allows for the best form of identification and separation of molecules [56]. Compounds identified through this study are known to have numerous pharmacological properties that are prevalent in plant medicinal research. The chemical constituents of *C. erythrophyllum* leaf and stembark extracts (hexane, chloroform, and methanol) were evaluated using GC–MS. This was the first report of GC–MS analysis of *C. erythrophyllum*. GC–MS screening of the leaf and stembark extracts resulted in the identification of 45, 25, and 196 phytocompounds within the hexane, chloroform, and methanolic extracts, respectively (Tables 3 and 4; Figures S1 and S2 of the supplementary materials).

Table 3. GC–MS phytochemical analysis of *C. erythrophyllum* leaf extract.

No	Phytochemical Compound	CAS NO.	Solvent	RT	Peak (%)	Pharmacological Activity and References
1	Phenol, 2,4-bis(1,1-dimethylethyl)-	96-76-4	Chloroform Hexane	12.715 12.719	2.17 2.77	Antibacterial activity [31]
2	*n*-Pentadecanol	629-76-5	Chloroform	16.901	1.23	Antioxidant and antidiabetic [72]
3	Phytol, acetate	0-00-0	Methanol	17.052	3.09	Unknown
4	*n*-Heptadecanol-1	1454-85-9	Methanol	17.522	6.13	Anti-oxidant [5,73]
5	Pentadecanoic acid	1002-84-2	Chloroform Hexane Methanol	17.770 17.773 18.327	2.82 2.93 3.80	Possible cancer prevention [58,73]
6	Phytol	150-86-7	Hexane	19.124	1.05	Anti-microbial, anti-inflammatory and possible cancer prevention [74]
7	9-Octadecen-1-ol, (Z)-	143-28-2	Methanol	19.287	14.44	Emollien and delivery of medication [73]
8	cis,cis,cis-7,10,13-Hexadecatrienal	56797-43-4	Hexane	19.447	2.68	Antioxidant, antifungal and antibacterial [75]
9	*n*-Nonadecanol-1	1454-84-8	Methanol	19.509	6.33	Antioxidant, anti-inflammatory, and possible cancer prevention [70,76]

10	Eicosanoic acid	506-30-9	Hexane	19.670	1.51	Anti-inflammatory, anti-diabetic, anti-bacterial and anti-oxidant [30]
11	Octadecanoic acid	57-11-4	Hexane	19.670	4.82	Lowers cholesterol, antimicrobial, and anticancer activity [58]
			Chloroform	19.678	5.39	
12	Phytol	150-86-7	Methanol	19.741	4.10	Antimicrobial, anti-inflammatory, and anticancer [74]
13	Thiophene, 2-butyl-5-hexyl-	4806-12-6	Methanol	24.534	2.74	Unknown
14	13-Docosenamide, (Z)-	112-84-5	Hexane	24.606	1.31	anti-oxidant and anti-microbial [77]
			Chloroform	24.634	2.36	
15	Tetratetracontane	7098-22-8	Hexane	26.745	6.74	Cytoprotective and antioxidant [71]
16	1-Heptacosanol	2004-39-9	Methanol	27.334	2.98	Membrane stabilizer [5,73]
17	beta-Sitosterol	83-46-5	Hexane	28.384	2.21	Chronic wound treatment, anti-inflammatory and anti-proliferation [73]
			Methanol	29.247	9.06	
18	9,19-Cyclolanost-25-en-3-ol, 24-methyl-, (3.b,24 S)	511-61-5	Methanol	30.022	2.78	Unknown

Table 4. GC–MS phytochemical analysis of *C. erythrophyllum* stembark extract.

No	Phytochemical Compound	CAS NO.	Solvent	RT	Peak (%)	Pharmacological Activity and References
1	Phenol, 2,4-bis(1,1-dimethylethyl)	96-76-4	Hexane	12.716	1.95	Antibacterial activity [37]
2	*n*-Pentadecanol	629-76-5	Chloroform	16.906	1.38	Antioxidant and antidiabetic [14]
3	*n*-Heptadecanol-1	1454-85-9	Methanol	17.523	3.57	Antioxidant [5,78]
4	Pentadecanoic acid	1002-84-2	Hexane	17.738	3.56	Possible cancer prevention [30,78]
5	9-Octadecen-1-ol, (Z)-	143-28-2	Methanol	19.285	9.31	Anti-neoplastic, Antioxidant, Natural moisturizer [78]
6	*n*-Nonadecanol-1	1454-84-8	Methanol	19.509	4.28	Anti-acne, anti-inflammatory, and possible cancer prevention [58,65]
7	Octadecanoic acid	57-11-4	Hexane	19.675	2.15	Lowers cholesterol, antimicrobial, and anticancer activity [30,33]
			Chloroform	19.644	0.68	
8	Decanedioic acid, dibutyl ester	109-43-3	Hexane	19.667	1.23	Antimicrobial, Antifouling activity [5]
9	2-methyloctacosane	0-00-0	Hexane	19.936	1.39	Unknown
10	Eicosane	7098-22-8	Hexane	23.236	5.10	Antioxidant [79]
11	Phthalic acid, di(4-methylhept-3-yl) ester	117-81-7	Chloroform	24.525	31.17	Antioxidant, Antimicrobial Allelopathy [80]
12	Terephthalic acid, dodecyl 2-ethylhexyl ester	6422-86-2	Chloroform	24.578	32.93	Antioxidant, Hypocholesterolemic activity, Antimicrobial [81]
13	13-Docosenamide, (Z)-	112-84-5	Hexane	24.593	2.70	Anti-oxidant and anti-microbial [29]
			Chloroform	24.687	0.996	
			Methanol	25.169	2.62	

14	Tetratetracontane	7098-22-8	Hexane	26.080	3.38	Cytoprotective and antioxidant [66]
15	1-Heptacosanol	2004-39-9	Methanol	26.783	1.02	Treats Diabetes, Potential anticancer activity, Antioxidant, Antimicrobial [5]
16	Silane	0-00-0	Hexane	28.901	1.39	Unknown
17	beta-Sitosterol	83-46-5	Methanol	29.247	4.20	Chronic wound treatment, anti-inflammatory and anti-proliferation [78]
18	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one	0-00-0	Methanol	29.748	2.59	Unknown
19	Androst-5-en-17-ol, 4,4-dimethyl	0-00-0	Methanol	30.088	2.08	Unknown
20	Lupeol	545-47-1	Methanol	30.221	11.32	Treats Arthritis, Treats Diabetes, Potential anticancer activity [30]
21	Lup-20(29)-en-3-ol, acetate, (3.beta.)	1617-68-1	Methanol	32.432	15.51	TreatsDiabetes, Potential anticancer activity [30]

The highest numbers of phytocompounds were detected within the methanolic extracts, thus making it of prime interest. Within the leaf methanolic extract, 107 phytocompounds were identified, whereas 95 phytocompounds were noted within the stembark extract. Through the conducted analysis, novel compounds were noted. Within the leaf extract, the compound which exhibited the highest peak area (14.44%) was 9-octadecen-1-ol, (Z). Within stem bark extract, Lup-20(29)-en-3-ol acetate (3. beta) had the highest peak area (15.51%). Overall, the abundance of phytocompounds detected across all tested extracts in the literature has highlighted the medicinal importance of tetratetracontane, eicosane, octadecanoic acid, terephthalic acid, lupeol, and phytol [80,82]. Tetratetracontane had the highest peak within the hexane leaf extract (retention time: 26.745, peak area: 6.74%); this compound is highly prized for its antibacterial and antioxidant properties [83,84]. Eicosane had the highest peak area within the hexane stembark extract (retention time: 23.236, peak area: 5.10%). This is an alkane compound known for its prominent use in the cosmetic, lubricant, and petroleum industries. In addition, this compound is utilized in traditional medicine as an antioxidant, anti-bacterial, and anti-fungal agent [85]. Octadecanoic acid had the highest peak area within the chloroform leaf extract (retention time: 19.678, peak area: 5.39%); this compound is known for its use as a cosmetic, lubricant, and fragrant applications. In addition, octadecanoic acid is known for its traditional medicinal use as an antioxidant and antimicrobial agent and is also known to lower cholesterol levels [86]. Terephthalic acid had the highest peak area within the chloroform stem bark extract (retention time: 24.578, peak area: 32.93%); this compound is known for its prominent use in traditional medicine as an antioxidant agent with hypocholesterolemic activity [87]. Lupeol is known for its anti-inflammatory properties and is an effective therapeutic tool in aiding cancer treatment [88]. Other known pharmacological activities include the prevention or treatment of arthritis and diabetes [80]. Phytol is known to exhibit antioxidant, anticancer, hypocholesterolemic, anticoronary, antimicrobial, antiarthritic, anti-inflammatory, antidiabetic, and immunostimulatory properties [82,84]. Such findings indicate possible pharmacological activities associated with this species and the phytocompounds found within.

3.5. Fluorescence Microscopy

Powdered leaf and stem bark material were utilized for this analysis. The fluorescence classification of crude powdered samples was evaluated under bright and UV2a (330/380 nm) light. The emanating assay revealed various and distinct color changes for the varied samples (Table 5). Upon the addition of different reagents, noticeable color changes were observed at various wavelengths. The color change occurs when molecules emit light as they return from an excited to non-excited state [89]. Excited molecules scatter the absorbed light while fluorescing [89]. Research indicates that various phytocompounds fluoresce at different wavelengths, many can do so under normal bright light whilst others, such as alkaloids, require the aid of UV intervention [90]. The noticeable color changes of the crude-powered samples were distinct and highly beneficial in identifying the possible phytochemical compounds of the sample [78]. Furthermore, the emanating analysis depicts the presence of two trichome types (peltate scale and non-glandular), various cellular components and the distinguishable color changes noted when viewed using the UV2a (330/380 nm) adapter (Figure 3 and Table 5). Overall, this analysis is a cost-effective means of classification and identification of proposed medicinal plants. In order to establish the true ethnobotanical value of a plant, its botanical characterization needs to be fully explored [89]. Hence, the information retrieved via this analysis is prudent in establishing the true ethnobotanical value of *C. erythrophyllum*.

Table 5. Powder microscopical analysis of the leaves and stembark of *Combretum erythrophyllum.*

	Leaves		**Stembark**	
	Bright Light	**UV (ex330/380)**	**Bright Light**	**UV (ex330/380)**
Powder only	Dark Green	Blue, Red	Brown	Green, Blue
Powder + water	Greenish brown	Orange, Blue	Brown	Green, Blue, Red
Powder + 50% H_2SO_4	Brown	Orange, Blue	Brown, Red	Green, Blue
Powder + acetic acid	Brown	Red, Blue	Brown	Green, Blue
Powder only + aqueous NaOH	Brown	Blue	Brown	Green, Blue, Red
Powder + HCl	Greenish brown	Orange, Blue	Brown, Red	Green, Blue, Red
Powder + ethanol	Greenish brown	Orange, Blue	Brown, Red	Green, Blue, Red
Powder + ethyl acetate	Greenish brown	Orange, Blue	Brown, Red	Green, Blue, Red
Powder + hexane	Brown	Green, Blue	Brown	Green, Blue, Red
Powder + chloroform	Greenish brown	Green, Blue, Red	Green, Brown, Red	Green, Blue, Red
Powder + methanol	Greenish brown	Orange, Blue	Brown, Red	Green, Blue, Red
Powder + petroleum ether	Greenish brown	Orange, Blue	Brown, Red	Green, Blue, Red
Powder + diethyl ether	Greenish brown	Orange, Blue	Brown, Red	Green, Blue, Red
Powder + acetone	Greenish brown	Orange, Blue	Brown, Red	Green, Blue, Red

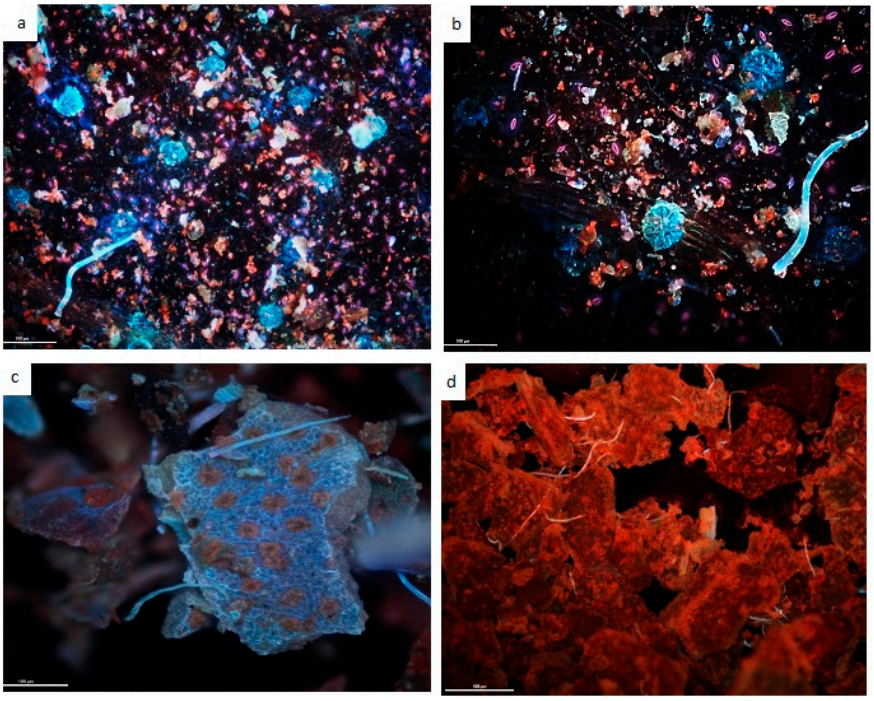

Figure 3. Fluorescence micrographs of powder leaf and stem bark material viewed under UV2a (330–380 nm); (**a**) powdered stem bark material emersed in water, (**b**) powdered leaf material emersed in water, (**c**) powdered leaf material emersed in acetic acid, and (**d**) powdered leaf material immersed in ethanol.

4. Conclusions

This study aimed to investigate the presence and composition of phytometabolites from the leaf and stembark extracts of *C. erythrophyllum*. Through the analyses, it can be concluded that *C. erythrophyllum* is indeed a plant worthy of being considered for its medicinal properties. The presence of phenols, sterols, flavonoids, saponins, and alkaloids possibly indicates antiviral, antibacterial, and antioxidant properties within the plant. However, further studies aimed at identifying individual compounds, isolation, phytometabolite quantification, and the feasibility of utilizing these extracts as beneficial medicinal extracts should be explored. In addition, anticancer, antioxidant, and anti-microbial assays should be conducted to evaluate the biological activity of this species. It is possible that this plant could be the future panacea in treating numerous ailments.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae8080755/s1, Figure S1: Total ion chromatograph of the (a) hexanolic, (b) chloroform and (c) methanolic leaf extracts of Combretum erythrophyllum; Figure S2: Total ion chromatograph of the (a) hexanolic, (b) chloroform and (c) methanolic stem bark extracts of Combretum erythrophyllum.

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