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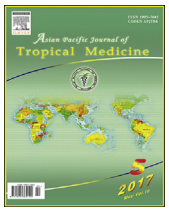

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# Traditional usage, phytochemistry and pharmacology of *Croton sylvaticus* Hochst. ex C. Krauss

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ABSTRACT

*Croton sylvaticus* (*C. sylvaticus*) is widely used as herbal medicine by the indigenous people of tropical Africa. The potential of *C. sylvaticus* as herbal medicine, the phytochemistry and pharmacological properties of its parts used as herbal medicines are reviewed. The extensive literature survey revealed that *C. sylvaticus* is traditionally used to treat or manage at least 24 human and animal diseases and ailments. The species is used as herbal medicine for diseases and ailments such as abdominal pains, boils, fever, inflammation, malaria, rheumatism, swellings and tuberculosis and as ethnoveterinary medicine. Multiple classes of phytochemicals such as alkaloids, anthraquinones, essential oils, flavonoids, lignan, phenolics, sterols, tannins and terpenoids have been isolated from the species. Scientific studies on *C. sylvaticus* indicate that it has a wide range of pharmacological activities which include antibacterial, antifungal, anti-inflammatory, antioxidant, larvicidal and effects on the central nervous system. Although studies have confirmed that *C. sylvaticus* has a wide range of bioactives, further research on the exact bioactive molecules and mechanisms of action are required.

## 1. Introduction

*Croton sylvaticus* Hochst. ex C. Krauss. (*C. sylvaticus*) is a species of the genus *Croton* L., Euphorbiaceae family. There has been a tremendous interest in the medicinal uses and pharmacological properties of *C. sylvaticus* in recent years, see Schmelzer [1] and references there in. *C. sylvaticus* is an important medicinal plant in tropical Africa with potential of providing important pharmaceutical products for sale in local, regional and international markets [1]. Previous research by Netshiluvhi [2] revealed that *C. sylvaticus* is popular and widely used as herbal medicine in South Africa and also scarce or potentially vulnerable in some communities in the KwaZulu Natal province in South Africa. Bark and roots of *C. sylvaticus* are sold as herbal medicines in informal herbal (*muthi*) markets in KwaZulu Natal province [2,3], Cape Peninsula, Western Cape province [4] and Gauteng province in South Africa [5,6]. It is important to assess if there is correlation between the ethnomedicinal uses of *C. sylvaticus* and the recent documented phytochemical and pharmacological properties of the species. Therefore, the present review complies the fragmented information on traditional uses, phytochemistry, pharmacology and toxicology of the species. It is hoped that this information will highlight the importance of *C. sylvaticus* and will provide a new direction for researchers in the future.

## 2. Botanical profile and taxonomy of *C. sylvaticus*

The genus name "*Croton*" was derived from a Greek word "kroton", meaning thick, in reference to thick smooth seeds, a common feature of most *Croton* species which belong to the Crotonoideae subfamily of the Euphorbiaceae family [7]. The specific name "*sylvaticus*" means sylvan, forest loving or the woodland croton [8] as the species is common in forests or associated with dense woodland or sometimes a pioneer species in woodlands or forests. Historical names or synonyms of *C. sylvaticus* are: *Croton sylvaticus* Pax, *Croton bukobensis* Pax, *Croton elskensi* De Wild., *Croton oxypetalus* Müll. Arg., *Croton sphaerocarpum* Kuntze, *Croton sphaerocarpus* Kuntze, *Croton stuhlmannii* Pax and *Croton verdickii* De Wild. [9,10]. *C. sylvaticus* is a semi-deciduous shrub or small to large tree that has been recorded in Angola, Cameroon, Central African Republic, Congo, Democratic Republic of Congo (DRC), Ethiopia, Gabon, Guinea, Ivory Coast, Kenya, Liberia, Uganda, Malawi, Mozambique, Nigeria, South Africa,

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South Sudan, Sudan, Swaziland, Tanzania, Zambia and Zimbabwe [1]. Research by Schmelzer [1] revealed that *C. sylvaticus* is relatively common in many parts of tropical Africa except in West Africa where it is quite rare. It occurs in semi-deciduous savannah, secondary forest and mixed evergreen forest, often on rocky slopes, in river gully forest and on rocky outcrops, from sea-level up to 1700 m altitude [1].

## 3. Ethnomedicinal uses of *C. sylvaticus*

A leaf decoction of *C. sylvaticus* is used as a body wash to treat oedema caused by kwashiorkor and tuberculosis in Kenya and Tanzania [11–13]. Leaf, root and stem bark decoction is taken orally as remedy for tuberculosis in the Democratic Republic of Congo (DRC), Kenya and Tanzania [11–14], while wood scrapings are rubbed onto the feet of a person with elephantiasis in the DRC [15]. In Kenya and Tanzania, a leaf or root infusion is taken as a purgative [11–13], while in Gabon, the seed and seed oil are taken as strong purgatives [15]. In Kenya, a leaf or root extract is taken as remedy for inflammation and malaria [11,13], while in Uganda, leaf decoction is herbal medicine for hypermenorrhea or heavy menstrual bleeding [16]. In Tanzania, leaf extract is used as herbal medicine for cancer and malaria [12,17,18], leaf juice is used as drops for ear infections [19] and roots are used as poultices for boils [12]. In South Africa, bark, leaf or root decoction is used as herbal medicine for abdominal disorders, dropsical swellings, febrile, fever, indigestion, internal inflammation, intestinal disorders, pleurisy, rheumatism and uterine disorders [8,20–23]. Research in South Africa by Stafford *et al.* [24] revealed that a patient suffering from insomnia is made to inhale fumes from ground leaves of *C. sylvaticus* mixed with leaves of other *Croton* species burnt on hot coals. The bark and seeds of *C. sylvaticus* are used by small-scale farmers as ethnoveterinary medicine in east and southern Africa. The powdered bark is used as a remedy for gall sickness in cattle in Swaziland [25], while the seed is used to repel and control ticks in Kenya [26]. In Mozambique, the bark of *C. sylvaticus* is used as a fish poison [8] (Table 1).

## 4. Phytochemistry

Considerable pharmacological potential of *C. sylvaticus* has been documented through detection, isolation and purification of its natural products via advances in spectrometric techniques such as Fourier transform infrared spectroscopy (FTIR), mass spectrometry (MS), gas chromatography-mass spectrometry (GC–MS) and nuclear magnetic resonance (1D, 2D, $^{1}$H and $^{13}$C NMR) for structural elucidation of new and complex compounds. Advanced research through FTIR, MS, GC–MS, 1D, 2D, $^{1}$H and $^{13}$C NMR spectroscopy enabled researchers to have a better understanding of the correlations between molecular conformation and biological activities of the natural compounds of *C. sylvaticus* and its importance as herbal medicine. The bark of *C. sylvaticus* is one of the most commonly stocked products on the informal herbal (muthi) markets in South Africa [2–6] and Grace *et al.* [28] tried to authenticate dried bark of the species using thin layer chromatography (TLC). This study showed that dried bark of *C. sylvaticus* is often confused with dried bark of *Acacia sieberiana* DC., *Acacia xanthophloea* Benth. and *Albizia adianthifolia* (Schum.) W. Wight, other three plant species sold as herbal medicines. Grace *et al.* [28] argued that the notable similarities in the phytochemical fingerprints of *Acacia sieberiana*, *Acacia xanthophloea*, *Albizia adianthifolia* and *C. sylvaticus* may be an indicator of close usage relationships as similarities shown by TLC chromatograms may sometimes explain the phytochemical properties common to bark products that are purposefully substituted for one another, particularly in cases where taxonomically unrelated species are used.

**Table 1**
Ethnomedicinal uses of *C. sylvaticus* in tropical Africa.

| Use | Plant parts used | Country practiced | References |
|---|---|---|---|
| Abdominal disorders | Bark decoction taken orally | South Africa | [21] |
| Bleeding gums | Charred, powdered bark taken orally | South Africa | [25] |
| Body swelling due to kwashiorkor or tuberculosis | Body washed by leaf or root decoction | Kenya, Tanzania | [11–13] |
| Boils | Roots used as poultices | Tanzania | [12] |
| Cancer | Leaf decoction taken orally | Tanzania | [17,18] |
| Chest complaints | Bark decoction taken orally | South Africa | [25,27] |
| Dropsical swellings | Bark decoction taken orally | South Africa | [21] |
| Ear infections | Leaf juice used as drops | Tanzania | [19] |
| Elephantiasis | Wood shavings rubbed onto affected feet | DRC | [15] |
| Excessive menstrual bleeding (hypermenorrhea) | Leaf decoction taken orally | Uganda | [16] |
| Febrile | Bark decoction taken orally | South Africa | [22] |
| Fever | Root decoction taken orally | South Africa | [23] |
| Indigestion | Root decoction taken orally | South Africa | [23] |
| Inflammation | Leaf or root decoction taken orally | Kenya, South Africa | [11,13,21] |
| Insomnia | Fumes from ground leaves mixed with other *Croton* species on hot coals inhaled | South Africa | [24] |
| Intestinal disorders | Bark decoction | South Africa | [20] |
| Malaria | Bark, leaf or root decoction | Kenya, Tanzania | [11–13] |
| Pleurisy | Leaves, roots made into poultices | South Africa | [8,20,23] |
| Purgative | Leaf, root infusion, seeds or seed oil | Gabon, Kenya, Tanzania | [11–13,15] |
| Rheumatism | Bark decoction taken orally | South Africa | [8,20] |
| Tuberculosis | Leaf, root or stem bark decoction taken orally | DRC, Kenya, Tanzania | [11–14] |
| Uterine disorders | Bark decoction | South Africa | [21] |
| **Ethnoveterinary medicine** | | | |
| Gall sickness in cattle | Powdered bark | Swaziland | [25] |
| Tick prevention and control | Seed | Kenya | [26] |

Various reports on the phytochemical screening of *C. sylvaticus* leaves, roots and stem bark [17,18,29–34] confirm the presence of alkaloids, anthraquinones, essential oils, flavonoids, lignan, phenolics, sterols, tannins and terpenoids (Table 2). Phytochemical screening of the aqueous and methanol root and stem bark extracts of *C. sylvaticus* by Ndunda [33] showed predominance of sterols and terpenoids. Alkaloids, anthraquinones, flavanoids, phenolics and tannins were found in trace amounts [33]. The methanol extract of the stem bark of *C. sylvaticus* were found to have low total phenolic content [TPC: (1.89 + 0.02)%–(1.14 + 0.01)% *w/w* equivalent of gallic acid] [33].

Mwangi *et al.* [29] analysed the essential oil isolated by hydrodistillation from *C. sylvaticus* leaves revealing the presence of β-caryophyllene oxide **1** (35.1%) and α-humulene-1,2-epoxide **2** (12%) as the major constituents. The petroleum ether extract of the non-volatile constituents isolated from the stem bark yielded hardwickiic acid **3**, β-sitosterol **4** and stigmasterol **5** [29]. Kapingu *et al.* [17,18] isolated three compounds, namely 2-[N-(2-methylbutanoyl)]-N-phenyl-ethylglutarimide **6**, lup-20(29)-en-3β-ol **7** and ent-(12R)-methyl-15.16-epoxy-9.10-friedolabda-5(1O),13(16),14-trien-19-oate20.12 lactone **8** from the leaves of *C. sylvaticus*. The phytochemical investigation of the leaf extracts of *C. sylvaticus* carried out by Langat *et al.* [30] yielded a novel lactonized clerodanefurano diterpenoid, along with clerodane diterpenoids namely sylvaticinol **9** and 3-hydroxy-3-((*Z*)-4-hydroxy-but-1-enyl)-2,2,4-trimethyl-cyclohexanone **10**. Further phytochemicals analysis of the stem bark and leaves of *C. sylvaticus* by Langat [40] yielded *trans*-phytol **11**, 15,16-dihydroxy-*trans-ent*-cleroda-3,13-diene **12**, 15-acetoxy-2-oxo-*trans-ent*-cleroda-3,13-diene **13**, t*rans*-annonene **14**, 15-acetoxy-*trans*-cleroda-3,13-diene **15**, 15-hydroxy-*trans*-cleroda-3,13-dien-15-ol **16**, lupenone **17**, 3β-acetoxylup-20(29)-ene

**Table 2**

Chemical compounds isolated and characterized from *C. sylvaticus*.

| No | Compound | Plant part | Method of characterization | Reference(s) |
|---|---|---|---|---|
| | **Essential oils** | | | |
| 1 | β-Caryophyllene oxide | Leaves | GC, GC–MS | [29] |
| 2 | α-Humulene-1,2-epoxide | Leaves | GC, GC–MS | [29] |
| | **Ent-clerodane diterpenoids** | | | |
| 3 | Hardwickiic acid | Stem bark | GC, GC–MS | [29] |
| 26 | 15-Acetoxy-ent-3,13E-clerodadiene | Roots | 1D, 2D, $^{1}$H, $^{13}$C NMR, GC–MS | [34] |
| 27 | 15-Formate-ent-3,13E-clerodadiene | Roots | 1D, 2D, $^{1}$H, $^{13}$C NMR, GC–MS | [34] |
| 28 | Ent-3,13E-clerodadien-15-ol | Roots | 1D, 2D, $^{1}$H, $^{13}$C NMR, GC–MS | [34] |
| | **Phytosterol** | | | |
| 4 | β-sitosterol | Stem bark | GC, GC–MS | [29] |
| 5 | Stigmasterol | Stem bark | GC, GC–MS | [29] |
| | **Glutarimide alkaloid** | | | |
| 6 | 2-[N-(2-methylbutanoyl)]-N-phenyl-ethylglutarimide | Leaves | GC–MS | [17,18] |
| 29 | Crotohalimaneic acid | Roots | 1D, 2D, $^{1}$H, $^{13}$C NMR, GC–MS | [34] |
| | **Triterpenoids** | | | |
| 7 | Lup-20(29)-en-3β-ol | Leaves | GC–MS | [17,18] |
| 17 | Lupenone | Leaves, stem bark | GC–MS | [31] |
| 18 | 3β-Acetoxylup-20(29)-ene | Leaves, stem bark | GC–MS | [31] |
| 19 | β-Amyrin | Leaves, stem bark | GC–MS | [31] |
| | **Halimane diterpenoid** | | | |
| 8 | Ent-(12R)-methyl-15.16-epoxy-9.10-friedolabda-5(1O), 13(16),14-trien-19-oate20.12 lactone 8 | Leaves | GC–MS | [17,18] |
| | **19-Norclerodane diterpenoid** | | | |
| 11 | Sylvaticinol | Leaves | NMR, FTIR, MS | [30] |
| | **Nor-cyclofarnesene sesquiterpenoid** | | | |
| 12 | 3-Hydroxy-3-((*Z*)-4-hydroxy-but-1-enyl)-2, 2, 4-trimethyl-cyclohexanone | Leaves | NMR, FTIR, MS | [30] |
| | **Acyclic diterpenoid** | | | |
| 9 | Trans-phytol | Leaves, stem bark | GC–MS | [31] |
| | **Trans-ent-clerodane diterpenoids** | | | |
| 12 | 5,16-Dihydroxy-trans-ent-cleroda-3,13-diene | Leaves, stem bark | GC–MS | [31] |
| 13 | 15-Acetoxy-2-oxo-trans-ent-cleroda-3,13-diene | Leaves, stem bark | GC–MS | [31] |
| 14 | Trans-annonene | Leaves, stem bark | GC–MS | [31] |
| | **Trans-clerodane diterpenoids** | | | |
| 15 | 15-Acetoxy-trans-cleroda-3,13-diene | Leaves, stem bark | GC–MS | [31] |
| 16 | 15-Hydroxy-trans-cleroda-3,13-dien-15-ol | Leaves, stem bark | GC–MS | [31] |
| | **Nor-cyclo-farnesene sesquiterpenoid** | | | |
| 20 | (+)-[5R, 6S, 9R]-4,5-dihydroblumenol A | Leaves, stem bark | GC–MS | [31] |
| | **Ferulate derivatives** | | | |
| 21 | Lignoceryl trans-ferulate | Leaves, stem bark | GC–MS | [31] |
| | **Lignans** | | | |
| 22 | (+)-Syringaresino | Leaves, stem bark | GC–MS | [31] |
| 23 | 2′-(3″,4″-dihydroxyphenyl)-ethyl-4-hydroxybenzoate | Leaves | MS, 1D, 2D NMR | [32] |
| | **Flavonols** | | | |
| 24 | 3,3′,4′,5,7-Pentahydroxyflavone | Leaves | MS, 1D, 2D NMR | [32] |
| 25 | 3,4′,5,7-Tetrahydroxyflavone | Leaves | MS, 1D, 2D NMR | [32] |
| | **Labdane diterpenoid** | | | |
| 30 | Labda-13E-ene-8α,15-diol | Roots | 1D, 2D, $^{1}$H, $^{13}$C NMR, GC–MS | [34] |

**18**, β-amyrin **19**, (+)-[5*R*, 6*S*, 9R]-4,5-dihydroblumenol A **20**, lignoceryl *trans*-ferulate **21** and (+)-syringaresino **22**. Adero-gba *et al.* [32] isolated 2′-(3″,4″-dihydroxyphenyl)-ethyl-4-hydroxybenzoate **23**, 3,3′,4′,5,7-pentahydroxyflavone **24** and 3,4′,5,7-tetrahydroxyflavone **25** from methanol leaf extracts of *C. sylvaticus*. Ndunda *et al.* [34] isolated some compounds from root methanol and dichloromethane (1:1) extract of *C. sylvaticus* namely hardwickiic acid **3**, stigmasterol **5**, 15-acetoxy-*ent*-3,13*E*-clerodadiene **26**, 15-formate-*ent*-3,13*E*-cler-odadiene **27**, *ent*-3,13*E*-clerodadien-15-ol **28**, crotohalimaneic acid **29** and labda-13*E*-ene-8α,15-diol **30**.

## 5. Pharmacological activities

A number of pharmacological activities of *C. sylvaticus* have been reported in literature justifying some of its ethnomedicinal uses. Some of the listed pharmacological activities may not relate directly to the ethnomedicinal uses of *C. sylvaticus*, but may provide some insight into its potential therapeutic value and pharmacological properties. A wide range of biological activities have been reported including antibacterial [33,35], antifungal [33,36], anti-inflammatory [37,38], antioxidant [33,38] effects on the central nervous system (CNS) [24,32], larvicidal [39] and mutagenic activities [32,40,41].

### 5.1. Antibacterial

Selowa *et al.* [35] evaluated antibacterial activities of *n*-hexane, dichloromethane, ethyl acetate, acetone and methanol leaf extracts of *C. sylvaticus* against *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* using bioautography and the serial microdilution methods. Results from Selowa *et al.* [35] showed that *C. sylvaticus* inhibited weakly *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa and Staphylococcus aureus* at the constant concentration of 1.25 mg/mL. Similarly, Ndunda [33] evaluated antibacterial activities of aqueous and methanol root and stem bark extracts of *C. sylvaticus* against *Bacillus subtillis*, *Escherichia coli* and *Staphylococcus aureus* using disc diffusion method with gentamycin and dimethyl sulfoxide (DMSO) as positive and negative controls respectively. The extracts were tested at high concentrations of 100 mg/mL, 50 mg/mL, 25 mg/mL and 10 mg/mL. Methanol extract of the stem bark of *C. sylvaticus* was the only crude extract that inhibited the growth of a bacteria strain, *Bacillus subtillis* at 10 mg/mL. The compound, 2-[N-(2-methylbutanoyl)]-N-phenyl-ethylglutarimide **6** that was isolated from the roots of *C. sylvaticus* showed some antibacterial activities towards *Ba-cillus subtillis* with minimum inhibitory concentration (MIC) value of <12.5 μg/mL [33]. These findings somehow confirm the species' antibacterial potential and its usefulness in the treatment and management of boils in Tanzania [12] and tuberculosis in DRC [14], Kenya [11,13] and Tanzania [12].

### 5.2. Antifungal

Mokoka *et al.* [36] evaluated antifungal activities of hexane, dichloromethane, acetone and methanol leaf extract of *C. sylvaticus* against *Cryptococcus neoformans* using bioautography and microdilution assays. *C. sylvaticus showed* promising antifungal activity against *C. neoformans*, with average minimum inhibitory concentration (MIC) of 0.07 mg/mL. Similarly, Ndunda [33] evaluated antifungal activities of aqueous and methanol root and stem bark extracts of *C. sylvaticus* against *Aspergillus niger*, *Cryptococcus neoformans* and *Candida albicans* using disc diffusion method with nystatin and dimethyl sulfoxide (DMSO) as positive and negative controls respectively. The extracts were tested at high concentrations of 100 mg/mL, 50 mg/mL and 25 mg/mL. The root and stem bark aqueous extracts were active towards *Candida albicans* at the lowest concentration tested of 25 mg/mL. The compound, hardwickiic acid **3** that was isolated from the roots of *C. sylvaticus* was found to inhibit the growth of *Candida albicans* with MIC value of <12.5 μg/mL [33].

### 5.3. Anti-inflammatory

Jäger *et al.* [37] evaluated aqueous and ethanolic bark extracts of *C. sylvaticus* in an *in vitro* assay for cyclooxygenase inhibitors with indomethacin as the control. The ethanolic extract of *C. sylvaticus* showed an inhibition of 59% which was lower than 66.5% inhibition exhibited by the indomethacin control. Based on these results, there might be a rationale for the ethnopharmacological claim that *C. sylvaticus* possess anti-inflammatory properties. Frum and Viljoen [38] evaluated anti-inflammatory activities of aqueous and meth-anol root extracts of *C. sylvaticus* through assessment of the 5-lipoxygenase inhibitory activity by using a threefold stepwise dilution method with dimethyl sulfoxide (DMSO) and Tween®20 as negative controls and nordihydroguaiaretic acid as positive control. The aqueous and methanol root extracts dis-played 5-lipoxygenase inhibitory activity with an $IC_{50}$ value <66 ppm [37]. These findings by Jäger *et al.* [37] and Frum and Viljoen [38] corroborate traditional uses of *C. sylvaticus* in the treatment and management of inflammatory conditions such as skin infections [11–13] and oxidative stress related diseases such as insomnia [24], abdominal disorders [21] and internal inflammations [11,13,20,21].

### 5.4. Antioxidant

Frum and Viljoen [38] the antioxidant activities of aqueous and methanol root extracts of *C. sylvaticus* using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity. The aqueous and methanol root extracts of *C. sylvaticus* dis-played DPPH antioxidant activity with an $IC_{50}$ of 11.28 ± 0.23 ppm and 11.28 ± 0.23 ppm respectively [38]. Similarly, Ndunda [33] evaluated the antioxidant activities of methanol stem bark extract of *C. sylvaticus* using the DPPH radical scavenging method using ascorbic acid as the control. The methanol extract of the stem bark of *C. sylvaticus* was also found to have low antioxidant potential ($IC_{50}$ > 1000 μg/mL compared to ascorbic acid, $IC_{50}$ = 9.51 + 0.22 μg/mL) [42]. The documented antioxidant activities of root [38] and stem bark [33] extracts are probably due to the presence of flavonoids and phenolics [42].

### 5.5. Effects on the central nervous system (CNS)

Stafford *et al.* [24] evaluated the $GABA_A$-benzodiazepine receptor-binding activities of ethanol bark extract of *C. sylvaticus* by assessing the binding of $^3$H-Ro 15-1788 (flu-mazenil) to the benzodiazepine site. *C. sylvaticus* extract showed some dose-dependent activity. Aderogba *et al.* [32] evaluated

acetylcholinesterace (AChE) inhibiting properties of 20% aqueous methanol, dichloromethane, ethyl acetate, n-butanol and n-hexane extracts and compounds isolated from *C. sylvaticus* using the microplate technique. The $IC_{50}$ values of the compounds, 2′-(3″,4″-dihydroxyphenyl)-ethyl-4-hydroxybenzoate **23**, 3,3′,4′,5,7-pentahydroxyflavone **24** and 3,4′,5,7-tetrahydroxyflavone **25** ranged from 60.7 to 415.0 μg/mL while the $IC_{50}$ values of the plant crude extract and solvent fractions ranged from 235.0 to 4695.0 μg/mL [32]. Ranking the fractions according to their $IC_{50}$ values resulted in this order of potency: ethyl acetate > *n*-hexane > *n*-butanol > dichloromethane [41]. The activity demonstrated by the compounds, 2′-(3″,4″-dihydroxyphenyl)-ethyl-4-hydroxybenzoate **23**, 3,3′,4′,5,7-pentahydroxyflavone **24** and 3,4′,5,7-tetrahydroxyflavone **25** suggests that they could be effective in the management of neurodegenerative disorders. Compounds with AChE inhibitory effects are known to be helpful in the treatment of a number of neurodegenerative disorders such as Alzheimer's' disease, Parkinsonism, myasthenia gravis and senile dementia [43]. These findings by Stafford *et al.* [24] and Aderogba *et al.* [32] corroborate the traditional use of *C. sylvaticus* as herbal medicine for insomnia in South Africa [24].

### 5.6. Larvicidal

Kihampa *et al.* [39] evaluated the larvicidal activities of chloroform, methanol and pet ether of root and stem bark of *C. sylvaticus* against the malaria vector, *Anopheles gambiae* s.s. Giles larvae bio-assayed following WHO susceptibility protocols. *C. sylvaticus* crude extracts showed some activity with root bark extracts exhibiting $LC_{50}$ values between 110 and 163 ppm and stem bark exhibiting $LC_{50}$ values between 232 and 246 ppm [39]. The results suggest that the investigated plant extracts are promising as larvicides against *An. gambiae* s.s. Giles mosquitoes and could be useful leads in the search for new and biodegradable plant derived larvicide products.

### 5.7. Toxicity and mutagenic activity

Elgorashi *et al.* [40] investigated genotoxicity potential of bark, leaf and twig dichloromethane and 90% methanol extracts of *C. sylvaticus* using the Ames test, micronucleus test, comet assay and VITOTOX® test. All dichloromethane extracts showed mutagenicity or DNA damage in micronucleus test and comet assay tests, while bark and twig 90% methanol extract showed DNA damage in micronucleus test only [30]. Similarly, Taylor *et al.* [41] evaluated genotoxic activity of *C. sylvaticus* in human peripheral blood lymphocytes using the micronucleus test, with further testing of select extracts using the alkaline comet assay. Dichloromethane and methanol-water extracts of bark, leaf or twig of *C. sylvaticus* gave positive results for genotoxicity in both the micronucleus and comet assay tests. Several samples tested positive in the micronucleus test and the comet assay, indicating that they damage the DNA of human white blood cells *in vitro*. Those extracts that tested positive in both the micronucleus and comet assays should be treated with particular care as compounds in the extracts cause direct and extensive damage to the DNA. Aderogba *et al.* [32] evaluated the mutagenic activity of three compounds, 2′-(3″,4″-dihydroxyphenyl)-ethyl-4-hydroxybenzoate **23**, 3,3′,4′,5,7-pentahydroxyflavone **24** and 3,4′,5,7-tetrahydroxyflavone **25** isolated from *C. sylvaticus* using the Ames test using the Salmonella microsome assay based on the plate-incorporation procedure with *Salmonella typhimurium* tester strains TA98 and TA100. The three compounds, 2′-(3″,4″-dihydroxyphenyl)-ethyl-4-hydroxybenzoate **23**, 3,3′,4′,5,7-pentahydroxyflavone **24** and 3,4′,5,7-tetrahydroxyflavone **25** showed no mutagenic effects against *Salmonella typhimurium* tester strains TA98 and TA100. Recently, Omosa *et al.* [44] evaluated the cytotoxicity of dichloromethane and methanol (1:1) extract of *C. sylvaticus* stem bark using the resazurin reduction assay against CCRF-CEM leukemia cell line. The dichloromethane and methanol extract of *C. sylvaticus* stem bark displayed cytotoxicity towards leukemia CCRF-CEM cells with $IC_{50}$ value of 23.5 μg/mL [42].

Research done by Mwangi *et al.* [29] on mice showed that an aqueous extract of the stem bark of *C. sylvaticus* prolonged ether anaesthesia. reduced exploratory activity exhibited muscle relaxant activity and analgesic activity [29]. Moshi *et al.* [45] evaluated toxicity of aqueous ethanol whole stem of *C. sylvaticus* using the brine shrimp lethality test. The concentration killing 50% ($LC_{50}$) of the shrimps was 29 μg/mL. Kapingu *et al.* [17,18] evaluated cytotoxic properties of pure compounds 2-[N-(2-methylbutanoyl)]-N-phenyl-ethylglutarimide **6**, [lup-20 (29)-en-3β-ol] **7** and [(ent-(12R)-methyl-15.16-epoxy-9.10-friedolabda-5(1O),13(16),14-trien-19-oate20.12lactone] **8** isolated from the leaves of *C. sylvaticus* against brine shrimp (*Artemia salina*) larvae. Only 2-[N-(2-methylbutanoyl)]-N-phenyl-ethylglutarimide **6** showed high cytotoxic activity with a $LC_{50}$ (95% CI) value of 0.074 μg/mL when tested *in vitro* while [lup-20 (29)-en-3β-ol] **7** and [(ent-(12R)-methyl-15.16-epoxy-9.10-friedolabda-5(1O),13(16),14-trien-19-oate20.12lactone] **8** were inactive, their $LC_{50}$ values were 308 and 312 μg/mL respectively. These results obtained from mutagenic and cytotoxic evaluations indicate the possibility that *C. sylvaticus* may be toxic or contain some cytotoxic compounds.

## 6. Conclusion

*C. sylvaticus* has been used in tropical Africa as herbal medicine for many centuries. However, chemical profiling and phytochemical research carried out so far on the species are limited. More research is required and future research should focus on more comprehensive chemical characterization of both crude and pure extracts. From literature, it is documented that some members of the genus *Croton* are reputedly toxic, and therefore, medicinal use of *C. sylvaticus* is potentially dangerous [44,45]. The use of *C. sylvaticus* bark as fish poison in Mozambique [8] suggests that the bark has toxic properties. The cytotoxicity and mutagenic evaluations done so far on *C. sylvaticus* extracts [17,18,29,32,40,41,43] indicate the possibility that *C. sylvaticus* may be toxic or contain some cytotoxic compounds, and therefore, these results raise concern about the safety of the long-term use of *C. sylvaticus* as herbal medicine. It is therefore, recommended that detailed phytochemical studies of *C. sylvaticus* and its phytochemical properties, especially the mechanisms of action of its bioactive constituents should be done, aimed at assessing the correlation between ethnomedicinal uses and pharmacological activities of the species. There is need for extensive *in vivo* experiments to validate the existing pharmacological activities. However, because *C. sylvaticus* contains potentially toxic compounds, its toxicological properties need to be properly established to ensure that potentially toxic components are kept below tolerance levels.

## Conflict of interest statement

The author declares that he has no conflict of interest.

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