REVIEW
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Clausena anisata (Willd.) Hook.f. ex Benth. (Rutaceae): ethnomedicinal uses, phytochemistry, pharmacological activities, toxicity, and clinical application

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Author contributions

Timothy Omara was responsible for the original draft writing; Ambrose K. Kiprop, Viola J. Kosgei and Sarah Kagoya were responsible for reviewing, editing, and supervision; Timothy Omara was responsible for methodology.

Competing interests

The authors declare no conflicts of interest.

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Abbreviations

C. anisata, *Clausena anisata* (Willd.) Hook.f. ex Benth.; *V. cholerae*, *Vibrio cholerae*; *P. acnes*, *Propionibacterium acnes*; *C. maculatus*, *Callosobruchus maculatus*; *A. obtectus*, *Acanthoscelides obtectus*; *C. quinquefasciatus*, *Culex quinquefasciatus*; *A. aegypti*, *Aedes aegypti*; *A. stephensi*, *Anopheles stephensi*; *A. variegatum*, *Amblyomma variegatum*; BW, body weight; QE, querctein equivalent; GAE, gallic acid equivalent; TPC, total phenolic content; TFC, total flavonoid content; Eos, essential oils; DPPH, 2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing power; ABTS, 3-ethylbenzthiazoline-6-sulfonic acid; EC_{50}, half-effective concentration; BHT, butylated hydroxytoluene; IC_{50}, inhibitory concentration; SC_{50}, scavenging activity; LC_{50}, lethal concentration; DCM, dichloromethane; $sPLA_2$, secretory phospholipase A2; 15-LOX, lipoxygenase; HL-60, human leukaemic; ACE, angiotensin-converting enzyme; ZOI, zone of inhibition; COX, cyclooxygenase; SNPs, silver nanoparticles; LD_{50}, lethal doses; AChE, acetylcholinesterase; IL, interleukin.

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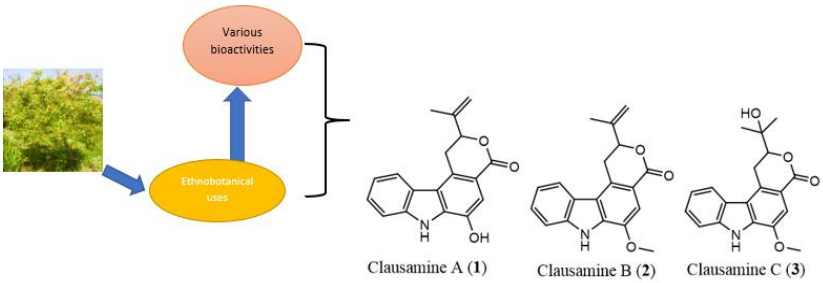
Abstract

Clausena anisata (Willd.) Hook.f. ex Benth. is a plant extensively used in traditional medicine. Here, a synopsis of the research on various aspects of *Clausena anisata* (Willd.) Hook.f. ex Benth. is presented. An electronic literature review revealed that formulations containing *Clausena anisata* (Willd.) Hook.f. ex Benth. are used to manage and treat diabetes, eye problems, malaria, snake envenomation, malignancies, as well as venereal, gastrointestinal, reproductive, respiratory, dermatological, and odonatological ailments. To date, 90 compounds have been isolated and characterized from extracts of *Clausena anisata* (Willd.) Hook.f. ex Benth. Crude extracts and isolated compounds from *Clausena anisata* (Willd.) Hook.f. ex Benth. possess anti-inflammatory, antimicrobial, antioxidant, antiplasmodial, antiproliferative, anti-human immunodeficiency virus-1, antimycobacterial, antihypertensive, and antidiabetic activities. These bioactivities can be attributed to alkaloids, coumarins, limonoids, and phenylpropanoids present in different parts of the plant. Although some studies have indicated moderate toxicity of the extracts, some of the dominant compounds in this species, such as estragole and carbazole alkaloids, are mutagenic or cytotoxic. A clinical trial utilizing a Ghanaian herbal formulation containing *Clausena anisata* (Willd.) Hook.f. ex Benth. was found to be effective in reducing pain associated with osteoarthritis. Research progress to date supports the traditional use of this species in herbal medicine. However, these studies do not explain the relationships between traditional uses, pharmacological activities, and mechanisms of action. Thus, further studies should be designed to understand the biochemical properties and physiological effects of *Clausena anisata* (Willd.) Hook.f. ex Benth. extracts, facilitating the development and utilization of this medicinal resource.

Keywords: carbazole alkaloid; coumarin; limonoid; phenylpropanoid; essential oil



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Highlights

This report presents a holistic overview of the ethnobotany, phytochemistry, and ethnopharmacology of *Clausena anisata* (Willd.) Hook.f. ex Benth., the only Rutaceous member of the *Clausena* genus extensively used in African traditional medicine.

Medical history of objective

Clausena anisata is a plant grown for its medicinal use in Asia. This plant is also widely used in traditional medicine in Africa; its leaves are usually placed under the bed or carried along during war by the Maasai because of its odoriferous properties. Plant twigs are used to steam patients to chase/dispel or warn off vengeful spirits.

Background

Clausena anisata (Willd.) Hook.f. ex Benth. (*C. anisata*) is a member of the Rutaceae (*Citrus*) family [1]. The *Clausena* genus was named after the Norwegian clergyman, Peder Claussøn Friis; the genus comprises at least twenty-five known botanical species in Southern Asia, Australia, Africa, and the Pacific islands; these species were described in 1768 by the Dutch botanist Nicolaas Laurens Burman [2–4]. The major botanical synonyms of *C. anisata* total 43, including *C. dunniana* and *C. willdenovii* Wt. & Arn., *C. abyssinica* (Engl.) Engl., *C. inaequalis* (D.C.) Benth, *C. dentata* (Willd.) M. Roem., and *Amyris anisata* Willd. [5–7].

C. anisata is a deciduous shrub, small tree, or small tree with usually imparipinnate but sometimes paripinnate compound leaves densely dotted with glands and have a strong aniseed-like scent when crushed (Figure 1) [8] (https://commons.wikimedia.org/wiki/File: Clausena_anisata_1.jpg, This file is licensed under the Creative Commons Attribution-Share Alike 4.0 International license). Leaves are usually clustered at twig ends. The flowers are small and white, with orange-yellow stamens. The inflorescence forms a branched axillary spray [9]. The fruit is a berry, approximately 1.3 cm wide, while the seeds are oblong in shape [7]. The plant is popularly known as “horsewood” or “maggot killer”, chiefly for its fetid leaves, which, when bruised, give rise to “Perdepis” (horse urine in the Afrikaans dialect) [10, 11].

C. anisata is the only member of *Clausena* genus in tropical Africa [12, 13]. Its country-specific geographical range includes forests, riverine thickets, and bushvelds from Guinea to Sierra Leone eastwards to Ethiopia, Sudan, and southward to the Cape in South Africa, only avoiding the driest regions [14–19]. The species has also been recorded in tropical and Southeast Asia, growing in India, Nepal, and Sri Lanka, extending as far as Queensland in northeastern Australia and some Pacific islands (Figure 2) [3, 11, 17, 20–27]. It is grown for its medicinal flavored oil in Malaysia, Indonesia, Vietnam, Thailand, and the Philippines (in the Philippine local brandy Anisdos) [1, 3, 7, 20, 28]. In Kenya, plants are usually placed under the bed, used for sleeping, or carried in wars among the Maasai because of its odoriferous properties [29]. Some of the local names of *C. anisata* used across the indigenous communities are summarized in Table 1 [30–85]. Its native ranges are tropical (South Africa, India, and South Central China) [21].

As a revered medicinal plant, *C. anisata* has been widely researched on. Two review articles have been published on this species, focusing on its bioactivity and phytochemistry [14, 86]. Given the exponential development of research approaches, analytical instruments, and strides in research concepts, many new features of the species have been determined recently. Nevertheless, there has been no up-to-date review of all the relevant research aspects of *C. anisata*. Therefore, this review is a holistic synopsis of the ethnomedicinal uses, phytochemistry, and ethnopharmacology of *C. anisata*, highlighting recent developments regarding the species and directions for future research.

Ethnomedicinal uses of C. anisata

C. anisata whole plants, stems, roots, leaves, flowers, twigs, stems, and root bark, singly or in combination, are utilized in African folk medicine for various human and animal ailments (Table 2) [87–123]. Targeted ailments include malaria, fever, colds, bacterial and fungal infections, venereal diseases (gonorrhea and syphilis), cough (tuberculosis), malignancies (breast and prostate cancer), and metabolic disorders, such as diabetes mellitus and coronary and renal diseases. The ethnoveterinary uses of this species include the management of maggots (myiasis) in cattle, back leg (Abakorpa), anthrax (Abasenga), helminthiasis, and other endoparasites. Multiple therapeutic remedies involving *C. anisata* have also been reported. For example, decoctions of *C. anisata* leaves mixed with *Lippia javanica* and *Eucalyptus grandis* leaves are used for respiratory ailments in South Africa [35]. In Ethiopia, the leaves of *C. anisata*, *Solanecio gigas*, and *Justicia schimperiana* are pounded together and applied dermally to treat skin irritation [64]. In another area in Ethiopia, inhaling *C. anisata* root powder with the roots of *Croton macrostachyus* and *Capparis tomentosa* is used as a treatment for devil attacks [65]. In South West Nigeria, a mixture of *C. anisata*, *Afraegle paniculata*, and *Azadirachtha indica* is administered to treat gut disturbance, and a concoction of the same called “Agbo” is used among the Yoruba as an antimalarial medicine [75]. In Nigeria, the Yoruba use a decoction of *C. anisata*, *Ageratum conyzoides*, *Momordica charantia*, and *Uvaria chamae* leaves (one glass cup thrice daily until the symptoms subside) for cancer management [77]. A similar decoction involving the roots of *C. anisata*, *Garcinia kola*, *Astonia boonei*, *Culcasia scandens*, and *Alafia barteri* is used to treat breast pain in Nigeria [19]. In Rwanda, *C. anisata* leaves, aerial parts of *Polydora serratuloides*, and leaves of *Kalanchoe* species are used to prepare decoctions for asthma treatment [83]. In Tanzania, a decoction of the roots of this species, along with those of *Byrsocarpus boiuinianus*, *Albizia anthlmintica*, and *Carpolobia goetzei*, are used to treat constipation in children [124].

The leaves (branches/aerial parts) and stem bark are the most used parts for preparing herbal remedies. Phytochemical, pharmacological, and toxicity studies have primarily investigated these aspects. This is

Figure 1 Clausena anisata tree. Source: Wikimedia Commons.

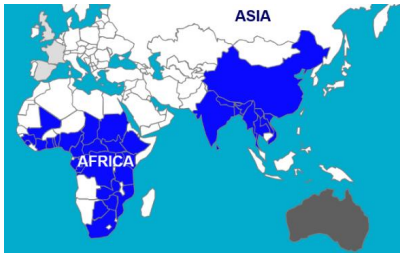

Figure 2 Geographical distribution of *C. anisata* (in blue) based on Table 1 and other reports. *C. anisata*, *Clausena anisata* (Willd.) Hook.f. ex Benth.

Table 1 Local names of *C. anisata* used across indigenous communities

Folk name (local language)	Country	References
Umsanga (isiZulu), Iperepesi, Umtuto (Xhosa), Perdepis (Afrikaans), Isifudu (Zulu), Isifuthu, Umnukambhiba, Umnukelambiba, Umsanka, Umwashampunzi	South Africa	[30–38]
Mtondombare, Mnyapala, Munyapala (Digo), Kumunyabubi, Kisimbari, Shihunya bukundu (Luhya), Mukibia, Chesamishiet, Chebunoiwo (Marakweta), Matathi, Mutathi (Kikuyu), Mjavikali (Swahili), Chepkolol (Tugen), Siska (Boran), Kathimi kapala (Giriama), Kithiw'a, Muthungwa (Kamba), Siunya (Luo), Olmatasia (Maasai), Mukithia (Meru), Arawithargi (Sanya), Kalagapala-uvumba (Duruma), Olmatasia	Kenya	[39–53]
Nawawayo (NgaKaramojong), Munawaidudu/Mufunya idudu (Lusoga), Omutanu	Uganda	[54–56]
Ayida (Asante-Twi), Saman ndobir, Samandua	Ghana	[9, 57]
Gbossouazohouin	Benin	[58, 59]
Olmaa'ii, Ulumaa'i, Hulimay (Afan oromo), Ulumaa/Limich, Limche (Afan Oromo/Amaric), Etunisha, Zumi	Ethiopia	[60–71]
Mkoma vikali (Kwere, Nyangalio), Mjavikali, Msongambwa (Luguru), Mtanwa (Zinza), Mjafari	Tanzania	[10, 44, 72, 73]
Mbiet ekpene (Ibibio), Atabari Obuko, Agbasa (Yoruba)	Nigeria	[6, 19, 74–79]
Eyra	Togo	[80, 81]
Muvengahonye (Shona)	Zimbabwe	[82]
Umuno (Kinyarwanda)	Rwanda	[83]
Kattukariveppila/Kariveppila, Suganthaveppu, Potti/Pothi (Tamil, Malayalam), Ana	India	[7, 84]
Nampi (Tagalog)	Philippines	[85]

C. anisata, *Clausena anisata* (Willd.) Hook.f. ex Benth.

Table 2 Ethnobotanical uses of *C. anisata* reported by ethnobotanical studies

No.	Ailment (s) treated/ethnoveterinary uses	Part (s) used	Method of preparation and administration	Country/region	Authors
1	Diabetes, analgesic	R, L, St	Cooked in food as a culinary recipe	South Africa, Tanzania	[10, 36, 87]
2	Malaria, fever, colds, coughs, stroke, *Portabarak*	L, R, Bk, RB, St	Decoction taken, roots chewed for colds & coughs, leaf infusion taken, or steam inhaled.	Kenya, Nigeria, South Africa, Togo, Benin, Ethiopia	[15, 36, 39–42, 44, 50, 58, 81, 88–93]
3	Heart, liver and kidney diseases, jaundice, constipation	R, L, B, St	Decoction taken	Kenya, Tanzania, Togo	[40, 48, 80, 81, 85, 94]
4	Convulsions, gonorrhea, epilepsy, syphilis, fungal infections	L, SB, R	Decoction taken. For epilepsy, the steam of leaves or roots are inhaled.	Tanzania, Uganda, Kenya, Nigeria, Benin	[44, 54, 59, 72, 73, 95–101]
5	Measles, toothache (toothbrush), ear infections, fever, influenza, eye problems, evacuant, headache, rheumatism, respiratory ailments (ulceration of the lung, tuberculosis, bronchial problems, asthma), sore throat	SB, RZ, L, R, St, Tw	Decoction taken, used as a chewing stick or chewed, placed and held on the aching tooth. For ear problems, juice of leaves is used as ear drop. Twigs used as a tooth brush. Leaf decoction taken for asthma.	Nigeria, Uganda, South Africa, Kenya, Benin, West Africa, Ethiopia, Rwanda	[22, 34, 35, 44, 45, 49, 62, 63, 74, 78, 83, 102–111]
6	Hypotension, hypertension, as an emetic, blood cleanser, ascariasis	B, L, St	Decoction of bark or leaves and stems taken.	Kenya, Ethiopia, South Africa	[45, 68, 91, 112]

Table 2 Ethnobotanical uses of *C. anisata* reported by ethnobotanical studies (*continued*)

No.	Ailment (s) treated/ethnoveterinary uses	Part (s) used	Method of preparation and administration	Country/region	Authors
7	Snakebites, colic pain, worms (children), sweating, analgesic (breast pains), as a tonic (*umuthi omhlophe*)	L, R	Boil with rock salt and drink 250 mL or smear powder on bite.	Uganda, Kenya, South Africa, Nigeria	[19, 36, 44, 54, 113, 114]
8	Breast and prostate cancer, cancer (unspecified), skin irritation, evil eyes, spirits, mental illness/madness	L, R, SB, Tw, Fr	Leaf powder in honey eaten. For irritation, leaves mixed with other leaves applied after pounding. Root powder inhaled for evil eyes. For spirits, patient is steamed using twigs steam. Fruit or leaves chewed for madness.	Ethiopia, Kenya, Uganda, Nigeria	[47, 56, 61, 64–67, 69, 77]
9	Menstrual disorders, ease child birth, after-birth problems (post-partum haemorrhage, delayed/protracted labour), and increase lactation	L, R	Pounded roots used in soup; woman drinks it before bedtime or roots are chewed.	Nigeria, Kenya	[19, 44, 115, 116]
10	Calf diarrhoea (*Aremor*), worm infection in cattle, repellent	R	Infusion given	Uganda, Kenya, Cameroon	[55, 117, 118]
11	Maggots (myiasis), back leg (*Abakorpa*), anthrax (*Abasenga*) and helminthiasis	L	Crushed & packed onto wounds to expel maggots, decoction administered nasally for back leg/orally for anthrax/helminthiasis.	Zimbabwe, South Africa, Ethiopia, Kenya	[70, 119–121]
12	Mosquito, house fly, bedbug and cockroach repellent	L, St	By hanging or smoking	South Africa, Ethiopia	[30, 122]
13	Insecticide against stored-grain pests	SB, L, F, WP	Decoction taken orally, powdered and licked; sliced bark chewed; cold infusion taken orally.	Benin, Cameroon, Kenya	[115, 123]

F, flowers; Fr, fruit; L, leaves; R, roots; RB, root bark; St, stem; SB, stem bark; Tw, twigs; WP, whole plant; RZ, rhizome; *C. anisata*, *Clausena anisata* (Willd.) Hook.f. ex Benth.

explained by the high yield associated with them because of their high potential to accumulate, concentrate, and store therapeutic phytochemicals responsible for the treatment of various ailments [125]. The main methods of herbal preparation and administration are decoctions, cold infusions, pounding dried samples into powder and then licking, preparing poultices that are applied topically, squeezing fresh samples, and mixing with bathing water or direct chewing of the different parts (Table 2). In some instances, as is the case in the Xhosa of South Africa, the plant organ is cooked in food as a condiment [36]. Herbal remedies are rarely prepared by steam inhalation. For example, *C. anisata* twigs are used to steam patients to chase away revengeful spirits [17, 56].

Phytochemical profile of C. anisata

Phytochemical screening of extracts of *C. anisata* leaves, stem bark, root bark, stem, and roots indicated the presence of therapeutic secondary metabolites, including flavonoids, saponins, coumarins, phenols, alkaloids, phytosterols, tannins, phenols, triterpenes, steroids, cardiac glycosides, proanthocyanins, terpenoids, and saponosides [6, 9, 31, 60, 75, 82, 126–132].

Some studies have quantified the secondary metabolites in *C. anisata*. A multiflora quantitative study in Ghana indicated that methanolic leaf extracts of this species had 0.87 ± 0.43 g/L of gallic acid, 0.41 ± 0.20 g/L of vanillic acid, 0.99 ± 0.47 g/L of syringic acid, 0.74 ± 0 g/L of 2, 5-dihydroxy benzoic acid, 0.61 ± 0.30 g/L of caffeic acid, 0.55 ± 0.27 g/L of rosmarinic acid, and 0.60 ± 0.34 g/L of *p-coumaric* acid as the major phenolic compounds [133]. Working with *C. anisata* acetonic leaf extracts, the total phenolic content (TPC) and total flavonoid content (TFC) were reported to be 109.63 ± 7.62 mg gallic acid equivalent (GAE)/g and 159.01 ± 1.88 mg querctein equivalent (QE)/g, respectively [134]. In Zimbabwe, a TPC of 0.11 ± 0.004 mg tannic acid equivalent per 100 mg of leaves was reported [82]. Another team reported that the TPC, TFC, and proanthocyanidin content of *C. anisata* leaves were 29.50 ± 1.26 mg GAE/g, 0.28 ± 0.03 mg CTE/g, and 1.96 ± 0.23 mg CTE/g, respectively [135].

A report from South Africa indicated TPC values of 31.30 ± 0.05 and 28.10 ± 0.99 mg GAE/g, TFC values of 11.70 ± 0.17 and 7.60 ± 0.20 mg CE/g, and total iridoids values of 3,019.60 ± 63.35 and 3,264.70 ± 96.40 μg harpagoside equivalents per gram for stored and fresh leaves and twigs of *C. anisata* extracted with 50% methanol, respectively [136]. In the same study, the corresponding values of condensed tannins were 1.39 ± 0.03 and 1.33 ± 0.02% in dry matter, respectively. The extracts had no free gallic acid, while only the stored samples had gallotannins quantified to be 68.45 ± 5.14 GAE/g [136]. Kadiri et al. indicated that *C. anisata* roots had 0.32 ± 0.03, 0.72 ± 0.03, 3.79 ± 0.26, 1.86 ± 0.03, and 0.05 ± 0.01 mg/g of tannins, saponins, alkaloids, flavonoids, and phenols, respectively [19]. Another investigation recorded 6.69 ± 0.54 mg GAE/g and 7.45 ± 0.64 mg GAE/g as the TPC values of ethanolic extracts of *C. anisata* leaves and stems, respectively; the corresponding TFC values were 2.46 ± 0.08 mg QE/g and 3.93 ± 0.122 mg QE/g [128]. The polyphenol content of hydroalcoholic leaf and stem extracts of *C. anisata* was 62.8% and 36.8%, respectively, according to Agbor et al. [126].

To date, 90 compounds have been isolated and characterized from extracts of *C. anisata*. Alkaloids, coumarins (sometimes substituted), limonoids, terpenoids, and phenylpropanoids are the major groups of compounds that have been characterized using chromatographic and spectroscopic techniques such as gas chromatography-mass

spectrometry, ultraviolet-visible, fourier-transform infrared, mass spectrometry, nuclear magnetic resonance spectroscopy, and complementary techniques such as heteronuclear multiple bond correlation spectroscopy and distortionless enhancement by polarization transfer spectroscopy.

Alkaloids

The characteristic class of phytochemicals in rutaceous plants (of which *C. anisata* is a member) is carbazole alkaloids with complex structural diversity. They frequently occur in tandem with coumarins and phenylpropanoids (clausamines) and are classified as carbazole, lactone carbazole, and pyranocarbazole alkaloids. These alkaloids are responsible for the poor odor and nasal membrane irritation of this species [137]. In total, 35 alkaloids (1–35) have been reported in *C. anisata* (Table 3) [138–147]. Three novel and naturally isolated lactonic carbazole alkaloids (clausamines: A, B, and C) were identified in acetonic extracts of *C. anisata* branches.

Coumarins

Coumarins are a characteristic class of heterocyclic compounds in which the benzene ring is fused to a pyrone ring. In *C. anisata*, furanocoumarins, geranyl coumarins, and furanocoumarin lactones have been isolated (Table 4) [148–154]. A total of 38 coumarins (36–73) have been identified in this species.

Tetranortriterpenes (limonoids)

Limonoids are chemically characterized by variations in the furanolactone core structure, a prototypical structure made of four six-membered rings with an adjoining furan ring. Six tetranortriterpenoids (74–79) were isolated from *C. anisata* stem bark and roots; these included limonin (74), zapoterin (75), clausenolide (76), clausenolide-l-ethyl ether (77), clausenarin (11β-hydroxydeacetylnomilin) (78), and 1-*O*-methylclausenolide (79) [142, 155].

Other compounds

Other compounds not categorized into the preceding chemical groups have been isolated and characterized from *C. anisata* (Table 5) [156, 157]. Some of these compounds include chlorophyll derivatives (80, 81), peptide derivatives (82–84), and sterols (85, 86).

Table 3 Alkaloids isolated from *C. anisata*

Compound	Part (s) used	References
Clausamine A (1)	Branches	[138, 139]
Clausamine B (2)	Branches, stem	[138, 140]
Clausamine C (3)	Branches, stem	[138–140]
Ekeberginine (4)	Branches, roots, stem bark	[138, 139, 141–143]
Methyl carbazole-3-carboxylate (5)	Branches	[138, 139]
Clausine E (6)	Stem, branches	[139]
Clausine F (7)	Stem, branches	[139, 140]
O-demethylmurrayanine (8)	Branches, stem bark, roots	[138, 139, 143]
Clausamine D (9)	Stem, branches	[139, 140]
Clausamine E (10)		
Clausamine F (11)	Branches	[139]
Clausamine G (12)		
Clausenol (13)		
Clausenine (14)	Stem bark	[144]
1-Methyl-3, 4-dimethoxy-2-quinolone (15)		
3-Formyl-1-hydroxycarbazole (16)	Stem bark, roots	[143]
Heptaphylline (17)		[143, 145]
Girinimbine (18)	Roots, stem bark	[141–143]
3-Methylcarbazole (19)	Stem bark, roots	[140, 143]
Mukonal (20)		
Glycosinine (21)		
Mukonidine (22)	Stem	[140]
Furanoclausamine A (23)		
Furanoclausamine B (24)		
Clausamine H (25)	Stem bark	[142]
Murrayamine-A (26)	Roots, stem bark	[141, 142]
Oxypeucedanin (27)	Not specified	[146]
N-methylswietenidine-B (28)		
Clausenamide (29)	Stem bark, roots	[143]
Mupamine (30)	Stem bark, roots	[143, 147]
Clausanitin (31)		
Atanisatin (32)	Stem bark, roots	[143]
Clausine B (33)	Leaves	[127]
Clausenocoumarin (34)	Leaves	
Heptaphyline (35)	Roots	[60]

C. anisata, *Clausena anisata* (Willd.) Hook.f. ex Benth.

Table 4 Coumarins isolated from *C. anisata*

Compound	Part (s) used	References
Chalepin (36)	Roots, root bark, leaves	[60, 142, 146, 148, 149]
Osthol (37)	Root bark, roots	[25, 145, 148]
3-(1, 1-dimethyl allyl) xanthyletin (38)	Stem bark, root bark	[142, 148]
Xanthoxyletin (39)	Root bark, stem bark, whole plant	[142, 148, 150]
Imperatorin (40)	Aerial parts, roots, stem bark, leaves, whole plant	[60, 142, 145, 150–152]
Xanthotoxol (41)	Aerial parts, roots	[145, 151]
Lansamide-I (42)	Aerial parts	[151]
Indicolactone (43)		
Anisolactone (44)		
2',3'-Epoxyanisolactone (45)		
Heliettin (46)	Stem bark, roots, whole plant	[150, 153]
Gravelliferone methyl ether (47)	Stem bark, whole plant	[142, 150]
Swietenocoumarin I (48)	Stem bark, roots, whole plant	[153]
Triphasiol (49)	Leaves, whole plant	[150, 152]
Capnolactone (50)		
Anisocoumarin A (51)	Roots, stem bark, leaves, whole plant	[145, 150, 152, 153]
Anisocoumarin B (52)		
Anisocoumarin C (53)		
Anisocoumarin D (54)		
Anisocoumarin E (55)		
Anisocoumarin F (56)	Leaves, whole plant	[150, 152]
Anisocoumarin G (57)		
Anisocoumarin H (58)		
Anisocoumarin I (59)		
Umbelliferone (60)	Leaves	[152]
Isoponcimarin (61)	Leaves	
Anisocoumarin J (62)	Leaves	
Unidentified compound with chromene skeleton (63)	Roots	[60]
Gravelliferone (64)	Stem bark, whole plant	[142]
5,7-Dimethoxy-8-(3-*O*-methylbut-2-*O*-enyl) coumarin (65)	Stem bark	[142, 150]
Excavatin D (66)	Leaves	[142]
7-Methoxy-6 (2'-oxo-3'-methyl butyl) coumarin (67)		
(R)-(+)-6-(20-hydroxy-30-methyl-30-butenyl)-7-methoxycoumarin (68)		
7-((E)-7-hydroxy-3,7-dimethylocta-2,5-dienyloxyl)-coumarin (69)		
Phellopterin (70)	Stem bark	
Bergapten (71)	Leaves	
Isooxypeucedanin (72)	Leaves	
Seselin or 2',2'-dimethylpyranocoumarin (73)	Leaves	[154]

C. anisata, *Clausena anisata* (Willd.) Hook.f. ex Benth.

Table 5 Other compounds isolated from *C. anisata* extracts

Compound	Part (s) used	References
13^2(R)-hydroxypheophyton a (80) 13^2(S)-pheophyton a (81) N-benzoyl-L-phenyl alaninyl-N-benzoyl-L-phenyl alaninate (82) Aurantiamide acetate (83)	Leaves	[142]
N-benzoyl-2-hydroxy-2-(4-methoxyphenyl) ethylamine (84) Sitosterol (85) Stigmasterol (86) Quercetin 3,4-dimethyl ether (87)	Roots, stem bark	[141, 143]
(*S/R*)-3-(1,1,3-trimethyl-2-butenyl)-5-(2,3-dihydroxy-3-methylbutane)-7-methyl-2*H*-1-benzopyran-2-one (88)	Leaves	[127]
1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester (89)	Leaves	[156]
Trans-4-hydroxy-N-methylproline (90)	Leaves, stem	[157]

C. anisata, *Clausena anisata* (Willd.) Hook.f. ex Benth.

Compounds from essential oils (Eos) and volatile fractions of *C. anisata*

In addition to the isolated compounds, more than 150 phytoconstituents (91–340) have been identified in Eos and volatile fractions of *C. anisata* leaves, fruits (fruit pericarp), and seed in Supplementary Table S1. The dominant components of the Eos include (*E*)-anethole, limonene, γ-terpinene, sabinene, estragole or methyl chavicol, sabinene, germacrene D, and Z (*β*)-ocimene [158–162]. An overview of the composition of Eos from *C. anisata* growing in different parts of the world indicates that they have at least six chemovarieties: (i) estragole (methyl chavicol), (ii) ocimenone, (iii) (*E*)-anethole, (iv) (*E*)-foeniculin, (v) sabinene, and (vi) β-pinene [118]. These disparities in the constitution of Eos are explained by differences in climatic conditions, seasons, soil composition, and the intraspecific chemical polymorphism and extraction technique(s) used in Eos extraction [157, 163–166]. Overall, the Eos of *C. anisata* are rich in phenylpropanoids.

Pharmacological activities of C. anisata Eos, extracts, and isolated compounds

The pharmacological potential of Eos, crude extracts, and therapeutically active compounds (Figure 3) from *C. anisata* was evaluated. The reported biological activities include antioxidant, antimicrobial (antiviral, anti-human immunodeficiency virus-1, antibacterial, antifungal, and antidiarrheal), anti-inflammatory, anticancer (antitumor/antiproliferative), antidiabetic, antimycobacterial, immunomodulatory, analgesic, antipyretic, antiplasmodial, anticonvulsant, and anthelminthic activities. In addition, the antifeedant, repellent, and pesticidal activities of this species have been explored extensively.

Antioxidant/antiradical activity

The total in vitro antioxidant activity of Eos and extracts of *C. anisata* was assessed using multiple radical generating systems such as 2,2diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, nitric oxide, ferric reducing power (FRAP), and 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid (ABTS) assays. These methods have been used in tandem in most studies to provide elaborate insights into the antioxidant potential of the species. In Zimbabwe, methanolic *C. anisata* leaf extracts afforded 80.90% ± 0.78% DPPH inhibition [82].

Another study reported that the Eos of *C. anisata* had a half-effective concentration (EC_{50}) of 6.53 mg/L against 524 µg/L for butylated hydroxytoluene (BHT) in a β-carotene-linoleate system [167]. The authors reported an EC_{50} (10 days) of 1.77 mg/L against 156 µg/L for BHT and 2.66 mg/L and 150 µg/L for FRAP and DPPH assays, respectively [167]. The Eos of another collection of *C. anisata* had half inhibitory concentration (IC_{50}) of 3.40 g/L found to be close and comparable to that of BHT (IC_{50} = 42 µg/L), thymol (IC_{50} = 333 µg/L), and carvacrol (IC_{50} = 666 µg/L) [26]. A multiflora screening study in Ghana recorded a radical scavenging activity of 14.50 ± 0.06% for *C. anisata* methanolic leaf extracts in a DPPH assay [131].

The scavenging activities of the three solvent extracts of *C. anisata* were evaluated using DPPH, nitric oxide, FRAP, and ABTS assays in Supplementary Table S2 [31]. Acetonic extracts have been reported to exhibit strong antioxidant activities in vitro, which could explain the widespread medicinal use of *C. anisata* leaves and bark in the management of oxidative stress-induced diseases. Investigations of the antiradical activities of *E*-ocimenone rich-Eos of *C. anisata* leaves have indicated that it had lower radical scavenging activity (SC_{50} = 5.10 g/L), which was less than that of the standard compound (BHT, SC_{50} = 0.01 g/L) [168]. Investigating the effect of storage on the antioxidant potential of fresh and stored *C. anisata* leaves and twigs, Amoo et al. recorded EC_{50} of 26.80 ± 2.06 and 33.20 ± 3.89 µg/mL, respectively [136].

Similarly, Arsia, et al. reported maximum DPPH scavenging activities of 74.1%, 74%, 70.8%, 67.1%, and 55.1% for ethyl acetate/chloroform, acetonic, aqueous, hexane, and alcoholic leaf extracts of *C. anisata*, respectively [129]. Silver nanoparticles synthesized from ethanolic extracts of *C. anisata* leaf extracts had DPPH scavenging activity of 71.6% at 500 µg/mL, comparable to 77.38% inhibition of the standard ascorbic acid at the same concentration [169]. The IC_{50} value (200 µg/mL) of the Silver nanoparticles was equal to that of ascorbic acid. Recently, Pavela et al., using DPPH and ABTS assays, reported that the antioxidant activity of *C. anisata* Eos as measured by IC_{50} values were 1.81 ± 0.01 mg/mL and 1.30 ± 0.02 mg/mL [118]. The Trolox equivalent antioxidant concentration, as assessed using FRAP, was 151 ± 10.20 µmol TE/g. The high antioxidant potential obtained in the DPPH and ABTS assays was attributed to the abundance of anethole in the Eos, as previously reported by Senatore et al. [170]. The low reducing power in the FRAP assay was speculated to be elicited by the synergistic action of its other constituents, such as *p*-cymene, terpinene, and (*E*)-methyl isoeugenol [118]. Recently, the ethanolic extract of *C. anisata* leaves and stems was shown to possess strong antioxidant activity, with an IC_{50} of 34.46 µg/mL that the species requires a comprehensive evaluation of its antioxidant potential [157].

Antimicrobial activity

Eos. Employing the hole-plate diffusion method, volatile oils (1:l, 1:2, 1:5, and 1:10 dilutions) of *C. anisata* leaves exhibited dose-dependent inhibitory activity against *Aeromonas hydrophila* (zone of inhibition (ZOI) = 7.80–8.90 mm), *Alcaligenes faecalis* (ZOI = 10.20–12.70 mm), *Bacillus subtilis* (ZOI = 6.20–13.90 mm), *Beneckea natriegens* (ZOI = 14.30–19.70 mm), *Brochothrix thermosphacta* (ZOI = 5.40–6.90 mm), *Citrobacter freundii* (ZOI = 7.60–8.40 mm), *Clostridium sporogenes* (ZOI = 7.0–9.60 mm), *Enterobacter aerogenes* (ZOI = 7.90–9.70 mm), *Enterococcus faecalis* (ZOI = 12.20–15.80 mm), *Erwinia carotovora* (ZOI = 7.30–8.70 mm),

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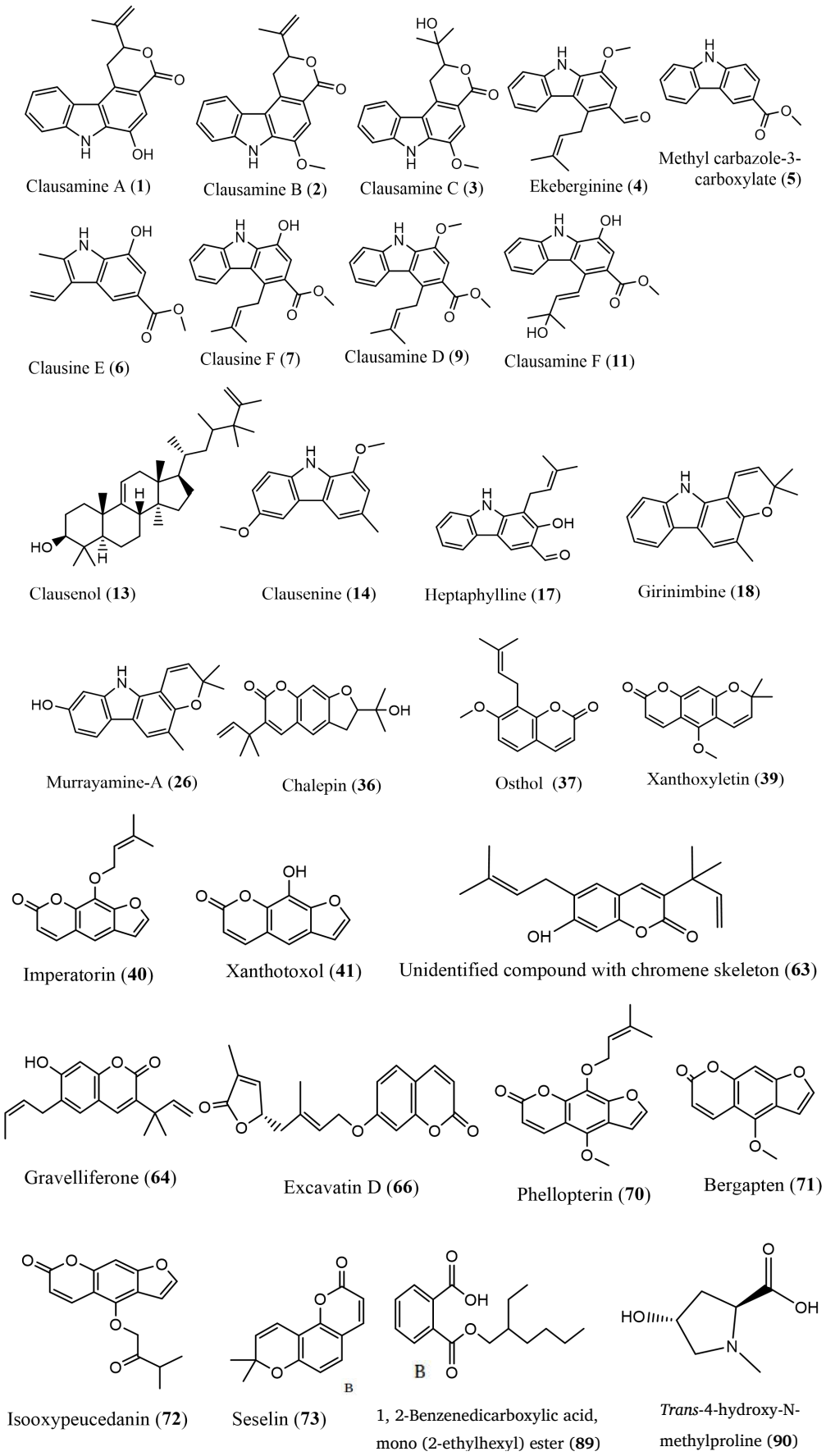

Figure 3 Chemical structures of some compounds isolated from *C. anisata* that exhibited biological activities. *C. anisata*, *Clausena anisata* (Willd.) Hook.f. ex Benth.

Flavobacterium suavoeolens (ZOI = 8.90–16.30 mm), *Lactobacillus plantarum* (8.30–8.90 mm), *Leuconostoc cremoris* (ZOI = 9.40–10.10 mm), *Klebsiella pneumoniae* (ZOI = 6.30–7.70 mm), *Micrococcus luteus* (ZOI = 7.0–7.70 mm), *Proteus vulgaris* (ZOI = 5.90–6.60 mm), *Pseudomonas aeruginosa* (ZOI = 5.70–6.50 mm), *Salmonella pullorum* (ZOI = 6.60–7.50 mm), *Staphylococcus aureus* (ZOI = 8.50–9.90 mm), *Serratia marcescens* (ZOI = 10.0–12.70 mm), and *Yersinia enterocolitica* (ZOI = 6.0–7.80 mm) [162]. The oils had no activity against *Escherichia coli*, *Acinetobacter calcoacetica*, *Brevibacterium linens*, or *Moraxella* species. Fungal species *Geotrichum candidum* (85%–98%), *Aspergillus parasiticus* (44%–84%), *Candida albicans* (26%–72%), *Penicillium citrinum* (57%–71%), and *Alternaria alternata* (42%–69%) similarly showed significant sensitivity to the volatile oils, with *Aspergillus flavus* (28%–47%), *Aspergillus niger* (19%–56%), *Aspergillus ochraceus* (25%–57%), *Chaetomium globosom* (28%–48%), and *Fusarium culmorum* (15%–67%) inhibition at 1, 2, 5, and 10 µL/mL of the Eos [162]. Yaouba et al. determined the antifungal activities of *C. anisata* leaf Eos against *Aspergillus flavus*, *A. niger*, *Aspergillus parasiticus*, and *Fusarium moniliforme* [168]. The growth of the fungi was completely inhibited when assessed at 4, 5, 5, and 5 mg/mL of the oils.

The Eos of *C. anisata* leaves were found to have Minimum inhibitory concentration (MIC) values of 62.50 µg/mL and 125 µg/mL each, respectively, against *Salmonella typhimurium* (*S. typhi*) and *Micrococcus luteus*, *Proteus mirabilis*, *Staphyloccoccus aureus* (*S. aureus*), and *Psudomonas aeruginosa* (*P. aeruginosa*) [84]. *Bacillus subtilis*, *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*), and *Proteus vulgaris* had MIC values of 250 µg/mL each, while beta-hemolytic *Streptococcus pyogenes* had an MIC of 500 µg/mL. A comparative assessment of the antimicrobial potential of the leaf Eos of three chemo-varieties of *C. anisata* was undertaken. Estragole-rich Eos of *C. anisata* had the highest antimicrobial activity against *Shigella* species and *Escherichia coli*, with a ZOI of 17 mm and 16.mm, respectively [171]. The oils produced lower bioactivities against *Salmon ella typhi* (ZOI = 13 mm), *Staphyloccoccus aureus*, and *Proteus species* (ZOI = 11 mm in each case). In contrast, *Psudomonas aeruginosa* and all fungi had a ZOI less than or equal to 9 mm. Another chemo-variety (*trans*-anethole-rich) of oil showed the highest antimicrobial activity against *Shigella* species (ZOI = 12 mm), *Salmonella typhimurium* and *Escherichia coli* (ZOI = 11 mm in each case). The other microorganisms evaluated had a ZOI of less than or equal to 10 mm. The third chemo-type (feniculin-rich) Eos were inactive against all tested pathogenic microorganisms with ZOI less than 10 mm [171]. A similar small ZOI (6 ± 0 mm at 10 µL) was later recorded for *C. anisata* leaf Eos against *Escherichia coli* by Sessou et al. [172]. The Eos constituent's linalool, ∝ -terpinene, γ-terpinene, *cis*-sabinene hydrate, and sabinene from *C. anisata* have been previously shown to be responsible for the antibacterial and antifungal activities of Eos in this species [162].

Extracts. Ndukwe et al. reported that extracts of *C. anisata* twigs and stems (used as chewing sticks in Nigeria) were inactive against *Staphyloccoccus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Psudomonas aeruginosa* [78]. A similar observation was made by Tafadzwa in which methanolic extracts of *C. anisata* tuber extracts had a ZOI of 1.25 ± 0.50 mm against *Psudomonas aeruginosa* while *Staphyloccoccus aureus*, *Streptococcus* Group A, and *Escherichia coli* were not inhibited [82]. This corroborated the findings of Kemoli et al., who found that aqueous extracts of *C. anisata* stem and bark had no antibacterial activity against oral pathogens, such as *Streptococcus mutans*, *Streptococcus sobrinus* (clinical strains), *Porphyrimonas gingivalis*, *Prevotella melaninogenicus*, and *Bacteroides oralis* [53]. While verifying the same traditional claim in the Ethiopian context, *C. anisata* dichloromethane/methanol and aqueous extracts were reported to possess only moderate bioactivity against *Bacillus cereus*, *Staphyloccoccus aureus*, *Enteroccocus faecalis*, *Streptococcus mutans*, *Klebsiella pneumoniae*, *Escherichia coli*, *Candida albicans*, *Candidaneoformans*, and *Lactobacillus acidophilus* with MIC values between 3 to 8 mg/mL [71]. A bioautographic investigation of selected Southern African plant species used to traditionally manage helminth infections indicated that *C. anisata* exhibited excellent antifungal activities with MIC of 0.02 mg/mL and therapeutic index of 2.65 against *Aspergillus fumigatus* [173]. It also had the lowest cytotoxicity, with a median lethal concentration (LC_{50}) of 0.17 mg/mL.

The antimicrobial activity of ethanol leaf extract of *C. anisata* was assessed, using agar well diffusion and micro-dilution methods, against clinical isolates of *Candida albicans*, gram-positive bacteria (*Bacillus subtilis*, *Staphyloccoccus aureus*, *Enterococcus faecalis*, and *Bacillus thuringiensis*), and gram-negative bacteria *Psudomonas aeruginosa* and *Proteus vulgaris*) [9]. The extracts had MIC values in the range of 0.50 to 7 mg/mL against gram-positive bacteria, 2.50 to 1 mg/mL against gram-negative bacteria, and 5.50 mg/mL against *C. albicans*. Upon fractionation, the methanolic fraction gave the highest bioactivity, with MICs ranging from 0.60 to 5 mg/mL and 1 to 3 mg/mL for gram-positive and gram-negative bacteria, respectively. The chloroform fraction had higher MIC values spanning from 3 to 7.50 mg/mL and 2 to 6.50 mg/mL for gram-positive and gram-negative bacteria, respectively. The last fraction, obtained from petroleum ether, had MICs spanning from 4.50 to 8 mg/mL for gram-positive and gram-negative bacteria. However, Arsia et al. indicated that ethyl acetate/chloroform extract had higher bacteriostatic potential against *Corynebacterium* and *Proteus* species with a ZOI of 43 mm and 32 mm for *Staphylococcus epidermis* [129].

Another bioassay investigating chloroform, ethyl acetate, and methanol extracts of *C. anisata* fruits, leaves, stem bark, twigs, and roots reported MIC values of 0.78 to 6.25 mg/mL (for *Psudomonas aeruginosa*), 1.56 to 6.25 mg/mL (for *Salmonella typhimurium*, *Salmonella kisarawe*, and *Klebsiella oxytoca*), 3.13 to 6.25 mg/mL (for *Proteus mirabilis*), 3.13 to 12.50 mg/mL (for *Escherichia coli* and *Klebsiella pneumoniae*), and 3.13 to 50 mg/mL for *C. albicans* [174]. Qualitative antibacterial activity analysis (bioautography) was performed to assess the bioactivity of the constituents of the acetonic leaf extracts of *C. anisata* against *Streptococcus pyogenes*, *Escherichia coli*, and *S. aureus*. Three fractions of the extract had remarkable activity against the tested pathogens (with ZOI ranging from 6 ± 0.50 mm to 9.10 ± 0.10 mm), comparable to that of standard drugs (amoxicillin and ciprofloxacin with ZOI = 12.33 ± 1.50 mm and 12.33 ± 0.57 mm against *Streptococcus pyogenes*) [127].

Mutuku indicated that methanol extracts of *C. anisata* (stem bark) had significant antibacterial potential against methicillin-resistant *Staphyloccoccus aureus* with MIC and minimum bactericial concentration values of 0.06 mg/mL and 0.78 mg/mL, respectively [175]. Agbor et al. reported that hydroalcoholic extracts of *C. anisata* leaves and stems were active against some isolated periodontal microorganisms (*Streptococcus mutans*, *Lactobacillus* species, *Aggregatibacterium actinomycetemcomitans*, *Fusobacterium nucleatum*, and *Prevotella intermedia*), with MICs between 50 and 100 mg/mL [126]. The lowest MIC was 50 mg/mL for *Aggregatibacterium actinomycetemcomitans* and *Fusobacterium nucleatum* isolates for the leaf extract and *Aggregatibacterium actinomycetemcomitans* for the stem bark extract. A recent investigation by Machumi et al. indicated that *C. anisata* leaf extract and petroleum ether, dichloromethane, ethyl acetate, and alkaloidal fractions exhibited marked antifungal activity against *Cryptococcus neoformans* and *C. albicans* with MIC ranging from 0.39 to 6.25 mg/mL [164]. The study further indicated that the traditional claim of curative potential of this species varies with the harvest season, with the highest activity in the rainy season than in the dry season.

Isolated compounds. The carbazole alkaloids clausenol and clausenine, isolated from the ethanolic stem bark of *C. anisata* were evaluated for their antimicrobial activity [144]. Clausenol was reported to be active against gram-positive and gram-negative bacteria and fungi, with MIC values of 7, 14, 12, 14, 13, 5, and 2 µg/mL against *Escherichia coli*, *B. subtilis*, *Salmonella typhimurium*, *Psudomonas aeruginosa*, *Staphyloccoccus aureus*, *Candida albicans*, and *Trychophyton rubrum*, respectively. The corresponding MIC for clausenine were 40, > 100, > 100, 16, > 100, 16, and 5 µg/mL, respectively. However, these MICs were higher than those of the commercial antibiotics (penicillin, streptomycin, griseofulvin, and

nystatin) used in this study [144]. These observations agree with a later work of Amoo et al., wherein fresh and stored leaves and twigs of *C. anisata* elicited antimicrobial activity with MIC and minimum microbicidal concentration values of 1.56–6.25 mg/mL and 6.25 mg/mL or greater against *Candida albicans*, *Psudomonas aeruginosa*, and *Staphyloccoccus aureus* [176].

Tatsimo et al. assessed the antibacterial activities of crude methanolic and hexane extracts of *C. anisata* and 21 isolated compounds; they reported that residues of crude leaf and stem bark extracts had broad-spectrum antibacterial activity against *Vibrio cholerae* (*V. cholerae*) SG24, *V. cholerae* CO6, *V. cholerae* NB2, *V. cholerae* PC2, *Shigella flexneri* 2a, and *Staphyloccoccus aureus* ATCC 25,923 [142]. Among the isolated compounds, imperatorin had the highest activity, with an MIC of 34.66 lg/mL. This was followed by phellopterin (MIC = 61.33 lg/mL), murrayamine-A (MIC = 64 lg/mL), 7-methoxy-6 (2'-oxo-3'-methyl butyl) coumarin (MIC = 69.33 lg/mL), chalepin (MIC = 69.33 lg/mL), 7-((*E*)-7-hydroxy-3, 7-dimethylocta-2, 5-dienyloxyl)-coumarin (MIC = 82.66 lg/mL), 13^2 (*R*)-hydroxypheophyton *a* (MIC = 128 lg/mL), ekeberginine (MIC = 144 lg/mL), girinimbine (MIC = 144 lg/mL), isooxypeucedanin (MIC = 149.33 lg/mL), xanthoxyletin (MIC = 160 lg/mL), 1-*O*-methylclausenolide (MIC = 160 lg/mL), clausamine H (MIC = 235.33 lg/mL), bergapten (MIC = 256 lg/mL), gravelliferone (MIC = 277.33 lg/mL), and 3-(1,1-dimethyl allyl) xanthyletin (MIC = 352 lg/mL) [142]. Time-kill kinetic studies of imperatorin against *V. cholerae* CO6 and *V. cholerae* SG24 (as a function of incubation time) indicated that it caused an appreciable reduction (approximately 6-log growth reduction) of bacteria, comparable to that of ampicillin when their minimum bactericidal concentrations were used within 6–10 h [142].

Recently, heptazoline, chalepin, imperatorin, and an incompletely identified compound with a chromene skeleton (63) isolated from the dichloromethane (DCM)/methanol extract of *C. anisata* roots were screened using the agar diffusion method against strains of *Staphyloccoccus aureus*, *Escherichia coli*, *Psudomonas aeruginosa*, and *B. subtilis* [60]. Heptazoline and imperatorin had comparable antibacterial activity against *Staphyloccoccus aureus* and *B. subtilis* (with ZOI of 14 mm and 13 mm, respectively) to that of ciprofloxacin (ZOI = 15 mm) at 20 µg/mL. Interestingly, chalepin has a comparatively higher bacteriostatic potential against *B. subtilis* (ZOI = 16 mm zone of inhibition) than ciprofloxacin (ZOI = 15 mm) [60].

Anti-inflammatory activity

Crude ethanolic leaf extracts of *C. anisata* (39–117 mg/kg) induced a significant dose-dependent reduction in induced inflammation and fever. These activities were speculated to have been caused by various compounds reported for this species [6]. Another assay assessed stored and fresh methanolic extracts of *C. anisata* leaves and twigs for their cyclooxygenase (COX) (COX-1 and COX-2) inhibition potency [176]. The extracts caused 26.7% to 65.3% inhibition of the COX enzymes. Interestingly, the inhibition of COX-1 by stored *C. anisata* differed significantly from that of the fresh parts. The anti-inflammatory potential of the extracts on prostaglandin synthesis during the inflammation cascade through inhibition of COX enzymes (especially COX-2) was asserted to be through this pathway, as is the case with nonsteroidal anti-inflammatory allopathic drugs [176].

Another report found that crude acetonic extracts of *C. anisata* leaves exhibited the best inhibitory activity against nitric oxide production (96.9% inhibition) when tested at 6.25 µg/mL, comparable to that of quercetin (91.08% inhibition) [134]. Owing to the release of nitric oxide contributes to inflammation, the extract proved to be a potential scavenger or inhibitor of nitric oxide synthase activity and/or its expression. In vitro, 30 mg/kg of *C. anisata* extracts dwindled nitric oxide production and pro-inflammatory cytokines such as interleukin (IL)-6 and prostaglandin E2 in lipopolysaccharide-stimulated RAW 264.7 cells [20]. The extract further diminished the expression of pro-inflammatory mediators such as COX-2. While in vivo, *C. anisata* extract significantly reduced inflammatory cell numbers in bronchoalveolar lavage fluid and suppressed pro-inflammatory cytokine levels, including tumor necrosis factor-α, IL-6, and IL-1β, as well as reactive oxygen species production in bronchoalveolar lavage fluid. Taken together, these findings demonstrate that the extracts inhibited inflammatory responses in a mouse model of lipopolysaccharide-induced acute lung injury and stimulated RAW 264.7 cells. Thus, *C. anisata* extract is a potential candidate for development as an adjunctive treatment for inflammatory disorders such as acute lung injury [20].

In vivo anti-inflammatory and analgesic investigations of ethanolic extracts of *C. anisata* roots revealed that it had a significant anti-inflammatory effect at 1,000 mg/kg in a carrageenan-induced edema model [145]. In acetic acid-induced writhing and hot plate tests, it had a strong but dose-dependent analgesic effect, with maximum analgesic activity of 72.1% at 1,000 mg/kg [145]. The isolated compounds (osthol 37 and anisocoumarin B 52) displayed the highest anti-inflammatory activity at 9 mg/kg, which was better than that of indomethacin. Heptaphylline (17) was the most potent (48.7%), while others (except xanthotoxol 41) showed significant analgesic activity at 6 mg/kg (higher than diclofenac). The analgesic activity of 52 (50.4%) was the highest among the isolates tested and the standard, tramadol, in the hot plate test. The nonselective opioid receptor antagonist naloxone abolished the analgesic effect of the crude extract and the tested isolates (41 and 52) in the hot plate test, suggesting an effect via the central opioidergic system [145].

The potential of selected 21 species used against *Propionibacterium acnes* (*P. acnes*) in South African herbal medicine has been assessed [157]. Interestingly, the ethanolic extracts of *C. anisata* leaves and stems inhibited the growth of *P. acnes* with an MIC of 31.25 µg/mL, but the isolated compound (*trans*-4-hydroxy-1-methyl-L-proline 90) had an MIC value > 500 µg/mL. The anti-inflammatory activity of *C. anisata* was evaluated along with its probable mechanism of action using lipase and hyaluronidase [157]. Cells stimulated with *P. acnes* and treated with 50, 25, 12.50, and 6.25 µg/mL of *C. anisata* extracts had diminished Interleukin-8 production at concentrations of 322.48, 365.98, 383.62, and 409.52 pg/mL, respectively. The extracts at 500 µg/mL resulted in 21.93% inhibition of lipase activity, which could be translated into their demonstrated potential to ameliorate sebum production associated with *Acne vulgaris* progression. At the same concentration, the extract portrayed 49.02% hyaluronidase activity inhibition, interpreted as its potential to deter the proliferation of *P. acnes* cells to adjoining cells [157].

Another probe assay was performed to evaluate the inhibitory activities of acetonic and aqueous extracts of *C. anisata* on secretory phospholipase A_2 ($sPLA_2$), lipoxygenase (15-LOX), and COX enzymes, which are South African plants used for skincare and beauty [177]. The extract had a high inhibitory activity against the COX-2 enzyme (IC_{50} = 15.18 and 6.89 µg/mL, respectively). The IC_{50} values for $sPLA_2$, 15-LOX, and COX-1 were 24.82 and 24.61 µg/mL, 67.62 and 41.45 µg/mL, and 4.29 and 3.13 µg/mL, respectively. Usually, free arachidonic acid released by $sPLA_2$ activity is metabolized by 15-LOX to form hydroxyeicosatetraenoic acid, resulting in atherosclerotic plaque formation. Given the results that indicated low inhibition of $sPLA_2$ encountered, it was deduced that the extracts exerted their inhibitory activity via other mechanisms [177]. It is recommended that the extracts have the potential to serve as nonselective inhibitors of COX-1 and COX-2 enzymes, implying that incorporation of the extracts selective for COX-2 and 15-LOX into beauty product formulations could enhance skin beauty by reducing scarring, dark spots, and uneven skin usually encountered with chronic inflammation and progression [177].

Anticancer activity

Various extracts and compounds isolated from this species elicited marked bioactivity against cancer cells (Table 6) [178–181]. Although other malignancies such as breast and prostate cancer have been treated using *C. anisata* leaves (Table 2), no study has evaluated the potential of the plant extracts against cell lines of these cancers. The mechanisms by which the evaluated extracts (compounds) mediate their antitumor activity have been elucidated in other

Table 6 Anticancer activities of extracts and isolated compounds from *C. anisata*

Extract/compounds	Cancer cell lines tested	Anticancer activity	References
Ethanolic leaf extract	Human epidermal melanocyte cell line (PCS-200-013) and cellular tyrosinase	Nontoxic to melanocytes at 15 μg/mL and but inhibited cellular tyrosinase with IC_{50} of 1.56 μg/mL	[178]
Hydroethanolic root extract	Jurkat (human acute-cell leukemia) cells	IC_{50} = 293.14 μg/mL	[179]
Leaves and stem bark ethanol extracts	HL-60 monocyte lymphoma (U937) cells	Low cytotoxicity with IC_{50} = 74.46 μg/mL	[157]
Leaves ethanol extract	Human rhabdomyosarcoma cancer cell line	CC_{50} of 8.83 μg/mL	[79]
Methanolic and DCM aerial part extracts	HL-60 cells	IC_{50} = 118.50 μg/mL and 225.40 μg/mL	[180]
Methanolic stem bark extract	Hoechst 33342, alamar blue, calcein-AM, glutathione depletion and oxygen-consumption assays	Minimal toxic dosage of < 7.81 to 250 μg/mL	[181]
Murrayamine-A, 3-(1, 1-dimethyl allyl) xanthyletin (38), gravelliferone (64), excavatin D (66), 7-((*E*)-7-hydroxy-3, 7-dimethylocta-2, 5-dienyloxyl)-coumarin (69), phellopterin (70), 1-*O*-methylclausenolide (79)	HeLa cells	For 70, LC_{50} was > 10 lg/mL. For the other compounds, LC_{50} = 1.14 to 3.26 lg/mL and selective to normal Vero cells (LC_{50} = 69.15–434.78 lg/mL)	[142]
Roots, leaves and bark extracts	Leukemia CCRF-CEM cells	Inhibited less than 50% of cancer cells at 40 mg/mL	[130]
Methanolic and hexanic leaves and stem bark extracts	HeLa cells	–	[142]
Clausamines B−E (2, 3, 9, 10), Clausine F (7), mukonal (20) and glycosinine (21)	HL-60	Clausamine E had the highest cytotoxicity (cell viability 47.3%); clausamines B and C had lower cytotoxicity (viability ~80%)	[140]
Clausamines A−E (1−3, 9, 10), ekeberginine (4), methyl carbazole-3-carboxylate (5), Clausine F (7) and *O*-demethylmurrayanine (8)	Epstein-barr virus early antigen activation induced by 12-*O*-tetradecanoylphorbol-13-acetate in Raji cells	All the compounds inhibited Epstein-Barr virus activation at 1 × 10^2 mol ratio	[139]

C. anisata, *Clausena anisata* (Willd.) Hook.f. ex Benth.; DCM, dichloromethane; IC_{50}, inhibitory concentration; CC_{50}, 50% cytotoxic concentration; LC_{50}, lethal concentration; HeLa, human cervical cancer; HL-60, human leukaemic; –, not mentioned.

Clausena species. For example, girinimbine (18) and other alkaloids isolated from *Clausena dunniana* and clausine E (from *Clausena vestita*) exhibit growth inhibitory activity by inducing apoptosis and cell cycle arrest in the S and G2/M phases [182, 183].

Antiparasitic (antiplasmodial/antimalarial and anthelmintic) activities

Extracts from *C. anisata* have been investigated using either nonradioactive antiplasmodial (in vitro) or four-day *Plasmodium berghei* ANKA suppressive (in vivo) assays. In an earlier study dichloromethane:methanol (1:1) extracts of *C. anisata* twigs and leaves were investigated using the parasite lactate dehydrogenase assay; the extracts had IC_{50} values of 18, 55, and > 100 μg/mL against the *Plasmodium falciparum* strain D10, and were subsequently not labeled as highly active antiplasmodial plants [184]. Another study using petroleum ether, dichloromethane, and methanolic extracts of *C. anisata* roots, stem bark, and leaves used a parasite lactate dehydrogenase assay and reported that the extracts had IC_{50} values between 5 and > 499 g/mL against the multidrug-resistant K1 strain of *Plasmodium falciparum* [185]. Similarly, chloroform, aqueous, and its fractions obtained from *C. anisata* leaves were assayed using Peter's 4-day test. Ethanolic and aqueous extracts and their fractions showed dose-dependent chemosuppressive and schizonticidal effects on (*P. berghei*) infection in mice (4-day test) with parasitemia [131]. Another in vivo assessment of the suppressive, curative, prophylactic, and acute toxicity properties of hexane and chloroform extracts of *C. anisata* stem bark against murine malaria has been performed [51]. The authors revealed that chloroform extract (500 mg/kg/day) had 66.1% and 73.4% parasite (*P. berghei*) reductions in the prophylactic and suppressive tests, respectively. The same dose of the hexanic extract had prophylactic effects (56.7% and 30.7%, respectively). The mean survival times of 12.3 days and 9.3 days, respectively, were recorded for the extracts in the curative test [51].

The crude ethanolic extract of *C. anisata* leaves exhibited anthelmintic activity, whereas the hexanic extract showed no bioactivity when tested at concentrations of 1–2 mg/mL in 2-hour and 7-day anthelmintic assays [186]. Similarly, organic (methanol and dichloromethane) extracts of *C. anisata* aerial parts displayed in vitro antitrypanosomal effects on the parasitic kinetoplastid (*Trypanosoma brucei brucei*) with IC_{50} values of 92.20 μg/mL and 49.80 μg/mL [180]. Christensen et al. assessed the antiparasitic potential of the ethanolic extract of *C. anisata* radix against the obligate anaerobic parasitic gut protist *Blastocystis* (subtype 4) and reported that the extract was among the best 24 plant extracts assayed, with an IC_{50} of 314 mg/mL after 24-hour incubation [187]. However, the authors noted that the antimicrobial activity of the extracts at 800 mg/mL could have influenced the anti-*Blastocystis* activity observed [187]. In another screening involving both African and Caribbean plants, ethanolic *C. anisata* root extract exhibited the highest anthelmintic activity against *Ascaris suum* with an EC_{50} value of 74 lg/mL [188].

A Ghanaian study screened the antiprotozoal efficacy and potential mechanisms of action of 112 crude extracts of indigenous plants against *Trypanosoma*, *Leishmania*, and *Plasmodium* parasites [179]. Hydroethanolic root extracts of *C. anisata* was found to possess cytotoxic effects to *Trypanosoma brucei brucei* (IC_{50} = 29.50 μg/mL), *L. donovani* (IC_{50} = 12.10 μg/mL), and *Plasmodium falciparum* (IC_{50} =

487.56 μg/mL). These results implied that the extracts elicited strong anti-Leishmania activity [179]. Another study in Cameroon reported that *C. anisata* leaf Eos had a very high IC_{50} (> 100 μg/mL) against *Trypanosoma brucei* [189].

Antidiabetic activities

The hypoglycemic effect of *C. anisata* root methanol extract was investigated in normal (normoglycemic) and streptozotocin-treated (90 mg/kg) diabetic Wistar rats [87]. Graded doses of *C. anisata* extract were administered to fasted normal and fasted diabetic rats. Relatively moderate to high doses of the extracts (100–800 mg/kg) induced a dose-dependent reduction in blood glucose concentrations in both normal and diabetic rats. At 800 mg/kg, the extract reduced the mean basal blood glucose concentrations of normal and diabetic rats by 57.52% and 51.3%, respectively. The mechanism of this activity was not elucidated, but it was expected to be elicited by the terpenoids and coumarins that are abundant in this species. The study findings agreed with the folkloric utilization of *C. anisata* roots in the treatment of adult-onset type-2 diabetes mellitus in South Africa [87].

The inhibitory effects of *C. anisata* extracts against human urinary α-amylase, α-glucosidase, and glucose 6-phosphatase in vitro, as well as rat α-amylase and α-glucosidase in vivo, were investigated [190]. These enzymes are associated with carbohydrate metabolism, and this study was conducted to establish the mechanism of hypoglycemic action of the leaf extracts. Aqueous and methanolic extracts reportedly had significant inhibition of α-amylase (> 80%) and hepatic glucose-6-phosphatase (60% and 58%). However, they are less potent inhibitors of α-amylase than acarbose [190]. Acetonic and hexanic extracts similarly showed significant inhibition (> 80%) of *Bacillus stearothermophillus* α-glucosidase. Thus, in vivo confirmation of the observed in vitro evaluation of phosphatase inhibition by the extracts and their hypoglycemic action is not mediated through the inhibition of intestinal carbohydrate hydrolyzing enzymes [190, 191].

In another study, aqueous and methanolic extracts of *C. anisata* leaves inhibited human urinary α-amylase with IC_{50} values of 1,947 and 2,436 μg/mL [33]. This inhibition has been suggested to be reversible and non-competitive. Acetonic and hexane extracts of *C. anisata* inhibited *Bacillus stearothermophilus* α-glucosidase, with IC_{50} values of 1,020 and 2,068 μg/mL. The aqueous and methanolic extracts of *C. anisata* leaves also inhibited hepatic glucose 6-phosphatase with IC_{50} values of 493.60 and 1,012 μg/mL, and the effect of the latter was far less than that of the reference inhibitor (sodium vanadate). Although both aqueous and hexane extracts of *C. anisata* leaves inhibited human urinary α-amylase and *B. stearothermophilus* α-glucosidase in vitro, the aqueous extract was more potent in in vivo inhibition of rat hepatic glucose 6-phosphatase than the known inhibitor of the catalytic subunit of this multi-component enzyme system [33].

Arsia et al. found that the percentage increase in glucose uptake into yeast cells was correlated with the glucose concentrations used when *C. anisata* leaf extracts were tested [129]. The aqueous and alcoholic leaf extracts of *C. anisata* produced maximum increase in 10 mM glucose concentration, i.e., 95% and 95.3% at 2,000 μg/mL. The alcoholic, aqueous, and acetone extracts showed appreciable (> 20%) enzyme inhibitory activity against human urinary alpha amylase, followed by hexane (3.4%) and ethyl acetate + chloroform extracts (8.1%).

The hypoglycemic effect of AgNPs synthesized using ethanolic leaf extract of *C. anisata* was assessed using glucose uptake by yeast cells, alpha-amylase inhibition assay, adsorption capacity, and glucose diffusion assay [169]. The silver nanoparticles (SNPs) had alpha-amylase inhibitory activity of 80.32% at 500 μg/mL, with IC_{50} of 100 μg/mL. Glucose uptake by yeast cells was found to be dose-dependent, with a maximum uptake found to be 68.29% at 10 mM, and the rate of glucose diffusion across the membrane ranged from 30 to 180 min [169]. The same team tested two doses (100 mg/kg body weight (BW) and 200 mg/kg BW) of crude leaf and root extracts and 5 mg/kg BW and 10 mg/kg BW of SNPs synthesized from the leaf and root extracts of *C. anisata* [192]. The extracts were administered to alloxan-induced diabetic rats for 30 days, and the results indicated that the activities (triglycerides, antioxidants, cholesterol, serum glucose, liver biomarkers, and gluconeogenic enzymes) were reduced. The extracts proved beneficial for the regeneration of pancreatic β-cells in alloxan-induced diabetic rats. SNPs from the root extracts at 10 mg/kg BW were the most potent [192].

Anticonvulsant activity

A lead study by Makanju highlighted that aqueous extracts of *C. anisata* root bark depresses the central nervous system with mild-to-moderate anticonvulsant activity at 250–500 mg/kg in mice [96]. A later report using the Gamma aminobutyrric acid-benzodiazepine receptor assay reiterated that the ethanolic extract of *C. anisata* bark and aerial parts had weak and dose-dependent anticonvulsant activity [193]. Later, Mbah and Kenechukwu observed that ethanolic extracts of the leaves and stem bark of this species had no anti-convulsive effect but sedative properties, with intraperitoneal administration of bark extract affording only partial protection at 800 mg/kg [194]. In extension of the foregoing study, the antiepileptic potential of *C. anisata* leaf, root and stem bark ethanolic extracts (labeled CALE, CARE, and CASE, respectively) against induced seizures was explored [132]. Their study found that CARE (800 mg/kg) delayed the onset of convulsions, providing 33.33% protection. The remaining extracts did not provide any appreciable protection. The authors reiterated that CARE-housed therapeutic compounds (primarily tannins and saponins) have potential benefits for petit mal epilepsy, giving a pharmacological reason for its claimed use in epilepsy phytotherapy [132]. Further research is required to confirm the efficacy of the compounds isolated from this species.

Antihypertensive activity

To validate the folkloric claim of using *C. anisata* leaves by hypertensive Africans, aqueous and ethanolic extracts were screened for the angiotensin-converting enzyme (ACE) inhibitors. The in vitro results indicated that extracts had 54% and 1% ACE inhibition [91]. Based on this observation, another study was undertaken to investigate the blood pressure-lowering potential of aqueous leaf extracts of this species in a spontaneously hypertensive rat model. The authors investigated whether the antihypertensive effects were mediated by diuresis, inhibition of the renin-angiotensin-aldosterone blood pressure control system, and/or possible negative inotropic or chronotropic cardiac effects. Extracts administered intra-arterially at 400 mg/kg BW reduced aortic blood pressure via the reduction in plasma angiotensin II levels, reaffirming the postulation by Duncan et al. [37, 91]. This bioactivity was predicted to be elicited by coumarins in the extracts because they have been previously shown to possess antihypertensive properties [195].

Antinociceptive and anti-arthritic properties

Hydroethanolic extract of *C. anisata* leaves at 400 mg/kg reduced the duration of paw licking/biting during the early (42.12%) and late (75.79%) phases of formalin-induced nociception [75]. Findings from the study illustrated that *C. anisata* elicits antinociceptive activity through interaction with opioidergic, noradrenergic, L-arginine-nitric oxide, and serotonergic pathways, as well as anti-arthritic properties conferred by its potential to inhibit the release of inflammatory mediators and oxidative stress [75].

Antifeedant, repellent, fumigant, and contact toxicity (pesticidal activities)

Eos. *C. anisata* extracts and Eos were bioassayed against houseflies (*Musca domestica* L.) and cockroaches (*Periplaneta americana* L.) for various insect control activities [196]. The Eos had knockdown effects on *Musca domestica* (KD_{50} = 0.74). The lethal doses (LD_{50}) of Eos were 2.21 and 0.86 g against flies and cockroach nymphs, respectively. The LD_{50} values of the solvent extractives were between 0.41 and 1,746.20 mg cm^2 for houseflies and 0.69 to 2.60 mg cm^2 for cockroaches. The

crude extracts elicited poor growth regulatory and anti-feedant effects [197].

In the western highlands of Cameroon, the incorporation of dry leaves of *C. anisata* into stored food products was investigated using leaf powder, Eos, and anethole (2) against *Callosobruchus maculatus* (*C. maculatus*) and *Callosobruchus chinensis* (Coleoptera, Bruchidae) in stored beans [198]. The dry leaves and Eos evoked significant reduction in the survival rate and number of progeny production, with up to 91% adult mortality and a reduction in adult emergence in treated grain at a dose of 5 g/50 g grain for powder or with 8 µL/40 g grain for Eos. The oils and anethole were markedly toxic to both pests, but anethole had a higher contact toxicity, particularly upon coating it on the grains (LD_{50} of Eos = 5, 97 and 4.30 µL/40 g grain and that of anethole = 0.37 and 0.65 µL/40 g grain). Both oils and anethole exhibit strong repellency against test insects [198].

Similarly, *C. anisata* leaf Eos were tested along with other 32 plant Eos and extracts against *C. maculatus* [199]. The number of eggs laid by the beetles on the treated bean seeds was lower than that of the untreated controls. The Eos caused repellence, with 19.8% percentage mortality. A previous study by the same group had indicated that distilled Eos of this species had no repellence activity against *C. maculatus* but prevented oviposition completely and reduced the longevity of adult beetles drastically at doses of 1.25 and 2.50 mL/kg [200]. The insecticidal efficacy of *C. anisata* Eos and an oil-aromatized clay powder against *Acanthoscelides obtectus* (*A. obtectus*) has been investigated [201]. Contact toxicity studies (evaluated through the coating of bean seeds) revealed significant dose-dependent mortality of *A. obtectus*. The aromatized clay powder exhibited greater toxicity (LD_{50} = 0.07 µL/g) than Eos (LD_{50} = 0.08 µL/g), indicating that the former could be used to stabilize the latter for greater insecticidal efficacy. The Eos and clay powder exhibited reduced toxicity after 24 and 36 h, respectively. The F1 progeny of *A. obtectus* production were reduced by clay and Eos. The highest recorded repellency of the Eos was 90% at 0.25 µL/cm^2, while it showed high fumigant toxicity (LC_{50} = 0.09 µL/cm^3) against the adult beetles [201].

Govindarajan found that leaf Eos of *C. anisata* (50, 100, 150, 200, and 250 ppm) elicited significant larvicidal activity with 24-hour LC_{50} values of 140.96, 130.19, and 119.59 ppm against *Culex quinquefasciatus* (*C. quinquefasciatus*), *Aedes aegypti* (*A. aegypti*), and *Anopheles stephensi* (*A. stephensi*) late III instar larvae [202]. Five isolated pure compounds from the Eos (β-pinene, sabinene, germacrene D, estragole, and linalool) tested at concentrations of 5–75 ppm had LC_{50} values of 23.17, 19.67, 16.95, 11.01, and 35.17 ppm against *A. stephensi*; 27.69, 21.20, 18.76, 12.70, and 38.64 ppm against *A. aegypti* and 32.23, 25.01, 21.28, 14.01, and 42.28 ppm against *C. quinquefasciatus* larvae [202]. An assay using Eos from *C. anisata* grown in Tanzania indicated that the oils had low repellency against *Anopheles gambiae* s.s. of the essential oil was low (1.1×10^{-5} g/cm^2), unlike those of Western Kenyan plant species, which had no mosquitocidal activity [108, 159]. Toxicity studies on estragole, a major component of most Eos from *C. anisata*, indicate that the compound is a naturally occurring genotoxic carcinogen unsafe for human use [203].

The variation in the constituents of *C. anisata* and *Plectranthus glandulosus* (*P. glandulosus*) Eos, and their levels in stored food products over time was investigated to establish their safety when used in the preservation of food products [204]. Corn grains and flour were treated with plant Eos and stored for 150 days, within which the composition of the Eos was extracted by hydrodistillation and analyzed by gas chromatography/flame ionization detector. Results indicated the concentration of the oils on food products reduced with storage time (half-life times recorded were 24.16 and 34.61 days for *C. anisata* and 25 and 38.75 days for *P. glandulosus*). At 150 days, only six constituents of *C. anisata* (linalool, estragole, methyl salicylate, *E*-ocimenone, thymol, and *E*-nerolidol) and three constituents of *P. glandulosus* (limonene, fenchone, and δ-3-carene) were found on the grains whereas ten (sabinene, linalool, estragole, methyl salicylate, *E*-ocimenone, *Z*-ocimenone, δ-elemene, *cis*-p-meth-2-en-1-ol, thymol, and *E*-nerolidol) and seven components (limonene, α-terpinolene, terpinene-4-ol, fenchone, isopulegone-4-methyl, piperitenone oxide, and germacrene D) were present in the flour. Thus, the persistent constituents were more than 62.5 times lower than the toxic concentration observed from the day of treatment, implying that they can safely be used to control insect pests on the corns [204]. With this efficacy, the authors recommended the use of Eos against storage pests, as it had comparable bioactivity to that of imidacloprid [205].

A knowledge-based Cameroonian investigation was undertaken to establish the larvicidal potential of Eos from *C. anisata* leaves and *Dysphania ambrosioides* aerial parts against *C. quinquefasciatus* (the lymphatic filariasis vector) and house flies (*Musca domestica*) [118]. A duplex mixture of the oils was also assessed for probable synergy with the insects, and the mechanism of insecticidal action (inhibitory action on acetylcholinesterase (AChE) was explored. It was found that *C. anisata* Eos were more toxic to third instar larvae of *C. quinquefasciatus* (LC_{50} = 29.30 µL/L) than *Musca domestica* (LC_{50} = 90.10 µg/adult), while the *Dysphania ambrosioides* Eos was more toxic to *Musca domestica* adults (LC_{50} = 51.70 µg/adult) than mosquito larvae (LC_{50} = 62.10 µL/L). The mixture of Eos had a synergistic effect against *C. quinquefasciatus* larvae with (LC_{50} of 19.30 µL/L), whereas this was not the case with *Musca domestica* adults (LD_{50} = 75.90 µg/adult). *C. anisata* Eos did not show any AChE inhibitory effect, though 77 ± 6.86% and 82.2 ± 3.74% AChE inhibition was previously reported at 1 mg/mL of its leaves and twigs methanol extract [136]. As *C. anisata* Eos are known to primarily house phenylpropanoids, their insecticidal activity could be mediated through the inhibition of the activities of cytochrome P450 detoxicative enzymes [206]. In another trial, estragole-rich Eos of *C. anisata* leaves completely inhibited the viability of larvae and the emergence of adult butterflies (*Sitotroga cerealella*) when tested at a dose of 0.50 µL/mL [207].

In another toxicological test, *C. anisata* volatile oils were investigated for their ovicidal, larvicidal, and adulticidal activities against eggs, larvae, and adult female *Anopheles coluzzii* at concentrations of 2%, 1.5%, 1%, and 0.5% [208]. The oils had significant inhibitory potential on *Anopheles coluzzii* eggs, with IC_{50} of 0.147%, while 100% mortality was recorded against the 3rd instar 48 h post-exposure to *C. anisata* Eos (LC_{50} = 0.44%). The knockdown effect of oil-impregnated nets was enhanced with increment in exposure time, with median knockdown times of 1.93, 18.58, 51.02, and 60.15 minutes, respectively for 2%, 1.5%, 1%, and 0.5% dilutions of the oils. The adulticidal effect of the oils 24 h post-exposure had LC_{50} value of 1.215% [208].

Mollong et al. assessed the acaricidal efficacy of *C. anisata* Eos on the larvae of *Amblyomma variegatum* (*A. variegatum*) (Acarina: Ixodidae), *Rhipicephalus* (*Boophilus*) (*Rh.* (*B.*)) *decoloratus*, and *Rh.* (*B.*) *microplus* ticks. A concentration of 0.06 µL/cm^2 resulted in 100% larval mortality in the tested tick species, with *A. variegatum* and *Rh.* (*B.*) *decoloratus* recording a similar LD_{50} of 0.02, whereas the LD_{99} of *Rh.* (*B.*) *decoloratus* was 0.08 [161]. Although the commercial acaricide (flumethrin) was more effective, with 100% mortality for *A. variegatum* at the lowest dilution of 0.01, these obtained results indicated that *C. anisata* Eos could be harnessed into a potential bio-acaricide [161]. The toxicity of the Eos was again tested on eggs and larvae of *A. variegatum* when diluted with palm kernel and *Jatropha curcas* oils as the vehicle [209]. At 0.12 µL/cm^2, the Eos inhibited the hatching of 95% of the eggs and provoked 60% mortality of the larvae after 24-h exposure. Despite its toxicity compared to the reference acaricide (bayticol), Eos can be harnessed for managing *A. variegatum* at the preimaginal stages [209]. A continuation of a previous study assessed the effects of *C. anisata* Eos and palm kernel oils on the biological parameters of engorged females of three major tick species in Togo. The Eos of *C. anisata* resulted in 100% mortality in all engorged females. A one-eighth dilution caused the destruction of 100% of eggs laid by *A. variegatum*. Palm kernel oils resulted in 100% mortality in female species of the genus *Rh.* (*B.*). The authors indicated that palm kernel oils had appreciable toxicity, indicating that the two oils could be harnessed as an alternative control for tick species.

Eos from *C. anisata* showed 82% fumigant toxicity (insect mortality) at 160 µL/L concentration after 24 hours against *Sitophilus oryzae* (L.) [210]. The oils had a median (LC_{50}) of 17.84 µL/L in fumigant toxicity, whereas maximum repellency (-0.25) was observed at 50 µL/L after 3 h. Eos (3, 6, and 12 µL/L) were reported to induce a significant reduction in the protein content and total esterase activity of *Sitophilus oryzae* [210].

Another group investigated Eos from *C. anisata*, *Crithmum maritimum*, *Heracleum sphondylium*, *Litchi chinensis* (*L. chinensis*), *Lippia alba*, *Pimpinella anisum*, and *Syzygium aromaticum* for their contact toxicity against the poultry red mite (*Dermanyssus gallinae*). The mechanism of action and efficacy of the oils were assessed using vapor-phase and residual toxicity tests [211]. It was found that Eos of *Syzygium aromaticum* had the highest contact toxicity (LC_{50} = 8.90 µg/mL), followed by *Crithmum maritimum* (LC_{50} = 23.70 µg/mL) and *L. chinensis* Eos (LC_{50} = 24.70 µg/mL). The Eos of *C. anisata* and *L. chinensis* showed the highest vapor toxicity among the Eos.

Extracts. The larvicidal potential of some selected traditional South African plant extracts, including that of *C. anisata* was assessed against *Anopheles arabiensis* to verify their use as mosquito repellents [38]. Dose-response bioassay results indicated that *C. anisata* leaf extracts had 100% and 88% larvicidal activity at 500 µg/mL and 250 µg/mL with LC_{50} of 112.70 µg/mL. Furthermore, the antifeedant activities of aqueous and organic (hexane, petroleum ether, chloroform, ethyl acetate, and methanolic extracts of *C. anisata* leaves and roots) extracts were evaluated against *Helicoverpa armigera* (H¨ubner) [23]. Root extracts had higher antifeedant activities, with petroleum ether and chloroform exhibiting the strongest activities, with concentrations eliciting 50% deterrence of 0.014% and 0.016%, respectively. Further fractionation of the root extracts resulted in one chloroform and one petroleum ether fraction that were active owing to the presence of osthol. The highest osthol concentration was observed in the chloroform root extract. The antifeedant activities of the root extracts, as measured by the 50% deterrence values, were highly correlated with their osthol contents. Approximately 99% of the variation in bioactivity of the root extracts could be accounted for by variation in osthol content; osthol, therefore, appeared to be an antifeedant component of *C. anisata* to *Helicoverpa armigera* [23].

The application of aqueous extracts of *C. anisata* leaves and stem bark along with other plant extracts to nematode-inoculated tomato plants was reported to significantly reduce (up to 86%) tomato root galling [135]. In another study, powdered *C. anisata* leaves admixed with maize grains were tested on Cameroonian and German strains of *Sitophilus zeamais* and *Prostephanus truncatus* to assess their insecticidal potential and their effects on progeny production, grain damage, and population increase [212]. Significant treatment, exposure time, and insect strain affected the adulticidal potential of the powder. The treatment also significantly affected the number of progeny produced, and the percentage reduction in adult emergence relative to the control, while 3-months monitoring results indicated that the powder significantly reduced the rate of increase in the population of the insects [212].

Experiments were performed to evaluate the fungitoxicity of methylene chloride/methanol leaf extracts of seven plants (including *C. anisata* leaves) on *Phytophthora infestans* development [213]. Plant extracts at 3% were assessed for their potential to inhibit/retard sporangial germination, latent and incubation periods, lesion size, and late blight severity in tomato plants. *C. anisata* along with *Entandrophragma angolense*, *Ageratum houstonianum*, and *Tephrosia vogelli* extracts provided excellent inhibition (3, 3.68, 0.18, and 2.18 cm^2, respectively) of late blight disease progression, with comparable fungitoxic effects to that of the commercial fungicides metalaxyl (0.40 cm^2) and maneb (1.33 cm^2), utilized as the positive controls in the study. The study concluded that the extracts could be incorporated into an integrated pathogen management program for tomato late blight [213]. Another report assessed the efficacy and toxicity of some acetonic extracts of plants used in ethnoveterinary medicine in South Africa on egg hatching and larval development of *Haemonchus contortus* [214]. *C. anisata* had EC_{50} values of 1.80 ± 0.09 and 2.07 ± 0.15 mg/mL in egg hatching and larval development assays, respectively. The extract was the least cytotoxic, with an LC_{50} of 0.17 mg/mL and a selectivity index of 0.10 and 0.08 for the two assays.

The larvicidal activity of 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester (339) isolated from the ethyl acetate extract of leaves of *C. anisata* was evaluated in the fourth instar larvae of *C. quinquefasciatus*, *A. aegypti*, and *A. stephensi*. The compound elicited 100% larval mortality against *A. aegypti* and *A. stephensi* at 40 ppm, with LC_{50} values of 8.94 and 9.23 ppm [156]. The compound showed 98% mortality, with an LC_{50} value of 12.07 ppm against *C. quinquefasciatus*.

The different bioactivities of *C. anisata* extracts and oils against insects have been attributed to the abundance of compounds such as coumarins, limonoids, and alkaloids [215]. For example, coumarins have been known to elicit insecticidal, feeding deterrence, and repellent activities through bioenergetic disruption of muscles or exerting neurotoxicity by ACE inhibition by blocking the octopamine receptor [216, 217]. Specifically, furanocoumarins may alter the detoxification capacity of organisms through the reversible and/or irreversible inhibition of cytochrome P450 detoxification enzymes [218]. On the other hand, alkaloids deter feeding by inhibiting impulse generation in sugar-sensitive cells in lepidopterans or by competitively blocking sucrose responses [14]. Their insecticidal potential varies for different target insects but is usually mediated by exerting effects on ACE and sodium channels [219, 220]. Limonoids, on the other end of the spectrum, have recognized insect antifeedant activities, usually mediated through suppression of the phagostimulant receptor and stimulation of deterrent cells located in the medial sensillum of insects [221]. The growth-regulating activities of this chemical group are achieved through the direct action on enzymes and other physiological regulatory processes necessary for normal insect growth or insect progenies [14].

Molecular docking studies

In silico methods have gained attention in modern drug design because they have the ability to understand drug-receptor interactions and quantum chemical properties. Eswaramoorthy et al. confirmed the mode of antibacterial binding and other properties of heptaphyline (35) and three coumarins (36, 40, and 63) isolated from *C. anisata* [60, 222]. Docking studies showed that compound 40 exhibited better docking scores against both DNA gyrase B and the LasR-binding domain compared to ciprofloxacin. In contrast, toxicological predictions revealed that these compounds are non-cytotoxic, non-hepatotoxic, non-carcinogenic, non-irritant, and immunogenic. Compound 40 had a large electronegativity, mutagenicity value, global softness, and global electrophilicity comparable to that of ciprofloxacin, suggesting that it could elicit better bioactivity and chemical reactivity with considerable intramolecular charge transfer between the electron-donor and electron-acceptor groups [222]. Molecular docking of *trans*-4-hydroxy-1-methyl-L-proline (90) from *C. anisata* leaf and stem extracts indicated a GOLD fitness score of 31.26 with only a single hydrogen bond appreciated with residue Glu208 in the active sites of lipase and hyaluronidase enzymes [157]. Analysis of the active site of the hyaluronidase enzyme indicated that its binding residues were Ala221, Leu222, Gly223, Thr224, Leu225, Lys226, Ile227, Thr271, Gly273, Lys274, and Leu275. Active site docking of compound 90 gave a GOLD fitness score of 17.20, which was lower than that of lipase [157].

Toxicity profile of C. anisata extracts and isolated compounds

Toxicological evaluation of medicinal plants, isolated pure compounds, and corresponding herbal products is pivotal for enhancing their approval and licensing as pharmaceutical products. As summarized in Table 7, it can be deduced that extracts from *C. anisata* are relatively non-toxic, which could also explain their widespread use in traditional medicine [223, 224]. Irungu et al. focused on the safety of extracts from *C. anisata* for specific solvents [51].

Table 7 Toxicity of *C. anisata* as reported in open literature

Toxicity [a]	Assay	Inferences	Reference (s)
LD_{50} = 948.68 mg/kg and 1,265 mg/kg (ethanol extract of leaves, root bark and stem bark)	Acute oral toxicity test	Moderate toxicity	[132]
LD_{50} = 27,172.5 mg/L (aqueous leaf extracts)	Brine shrimp lethality assay	Low toxicity	[37]
LC_{50} > 100 µg/mL (ethanol and ethyl acetate extracts of fruits, leaves, root and stem bark), LC_{50} = < 20 µg/mL (corresponding chloroform extracts)	Brine shrimp lethality assay	Nontoxic	[174]
Leaf ethanolic extracts (up to 5,000 mg/kg)	Acute oral toxicity test	Moderately toxic. No mortality up to 14th day but toxicity behaviors noted	[75]
LD_{50} = 393.7 mg/kg (leaf methanolic extract)	Acute oral toxicity test	Toxic as mortality was recorded	[6]
LD_{50} = 17,036.85 mg/kg bw (leaf ethanolic extract)	Acute oral toxicity test	Nontoxic to white mouse	[223]
LC_{50} = 71.9 and 60.5 µg/mL (roots DCM and ethanol extracts)	Brine shrimp lethality assay	Potentially toxic	[224]
LC_{50} = 533.0 µg/mL (leaves methanolic extract)	Brine shrimp lethality assay	Moderately toxic	[82]

LC_{50}, median lethal concentration; LD_{50}, median lethal dose; DCM, dichloromethane; *C. anisata*, *Clausena anisata* (Willd.) Hook.f. ex Benth.

The authors recorded no mortality in mice that received hexane extract of *C. anisata* stem bark, whereas the LD_{50} of animals administered chloroform extract was 4,166.70 mg/kg. This mild oral acute toxicity was speculated to be due to the abundance of cytotoxic carbazole alkaloids or coumarins (such as chalepin), which are known to be mutagenic and toxic, with an LD_{50} of 100 mg/kg in rats [88, 139, 140, 225]. This is substantiated by another report wherein the micromolar addition of chalepin led to the inhibition of rat liver mitochondrial respiration [149]. The authors inferred that chalepin (i) inhibits pyruvate/malate oxidation, (ii) does not interrupt succinate oxidation, (iii) inhibits site I phosphorylation, and (iv) is approximately one-tenth that of rotenone, a natural coumarin insecticide [149]. These results imply that further studies are required to provide sufficient evidence of the toxicity of the isolated compounds.

Clinical studies

Clinical validation of pharmaceutical and herbal products is mandatory and important as it promotes their acceptance and use. However, throughout this exhaustive review, only one clinical trial utilizing an herbal product from *C. anisata* was performed. This single-blind, randomized controlled trial of a Ghanaian herbal formulation (*C. anisata* and *Cassia sieberiana* D.C. roots) assessed the safety and effectiveness of the product in managing patients with osteoarthritis [226]. After 8 weeks of using the product by 44 subjects, the intensity of pain was reduced, and the disability score and days similarly declined. These observations support the use of herbal medicines. Toxicological assessments further indicated that the product was safe for human use. More clinical studies on this species are required to unravel its potential in the management of diseases across Africa.

Conclusion

C. anisata has a long history of use as an herbal medicine in Africa. Significant strides have been made over the last five decades to isolate, elucidate, and evaluate its phytoconstituents and the pharmacological activities of those constituents. Both crude extracts and isolated compounds from *C. anisata* possess anti-inflammatory, antimicrobial, antioxidant, antiplasmodial, antiproliferative, anti-human immunodeficiency virus 1, antimycobacterial, antihypertensive, and antidiabetic activities. The relevant bioactive compounds include alkaloids, coumarins, limonoids, and phenylpropanoids. Although some studies have indicated moderate toxicity of the extracts, some of the dominant compounds in this species, such as estragole and carbazole alkaloids, are mutagenic or cytotoxic. Accumulating evidence supports the traditional use of this species in herbal medicine. However, these studies do not explain the relationships between traditional uses, pharmacological activities, and underlying molecular mechanisms. Thus, future research studies should focus on the structure-bioactivity relationships and clinical potential of crude extracts and isolated compounds from *C. anisata.*

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