The use of *Clausena anisata* in insect pest control in Africa: A review

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Abstract

Ethnopharmacological relevance

Clausena anisata is used traditionally by various communities across Africa against pests such as mosquitoes, flies and weevils among others. Pests are a major cause of disease and production losses in various crop and livestock production systems in Africa. This review discusses the available information on the occurrence, chemistry, biological activity and possible commercialization of *Clausena anisata* with a view to see the plant species being integrated in pest management.

Materials and methods

Information on the ethnomedical use, chemistry and biological activity of *C. anisata* published between 1980 and 2016 was accessed from various databases namely Science Direct, Springer Link and Wiley Online Library. In addition various relevant books were also consulted.

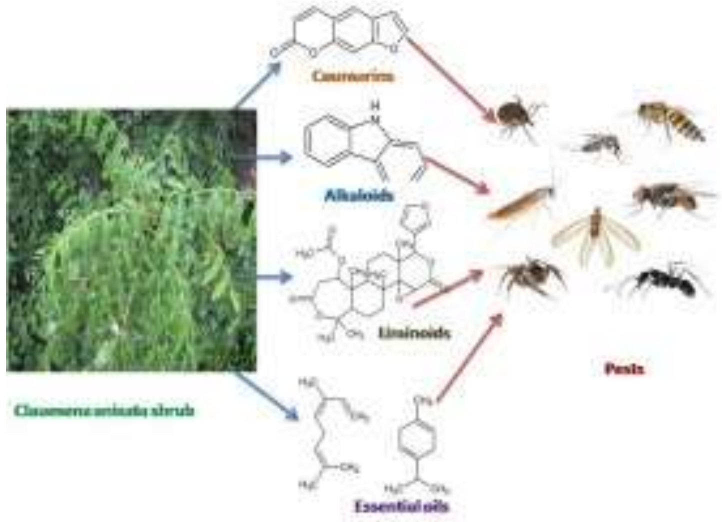
Results

The crude extracts as well as different fractions of *C. anisata* have been evaluated for activity against various insect pests and have been shown to be active. Furthermore, close to 50 compounds have been isolated and identified from *C. anisata*, which include coumarins, carbazole alkaloids, limonoids and essential oils (monoterpenes). Some of these compounds have been proven to exhibit pesticidal properties in both laboratory and field studies against various pests including mosquitoes, flies and weevils. The possible mechanisms of action of these compounds have been explored in this review.

Conclusion

The results of pesticidal and phytochemical screening of *C. anisata* strongly indicate that the species is endowed with pesticidal properties that can be harnessed into commercial products. However, one glaring challenge in the evaluation of this plant species for pesticidal activity has been the non-availability of standard testing systems. Researchers have used various methods which they developed based on their own circumstances and resources. Formulation, standard appropriate testing systems and agronomic research are key in unlocking the potential of this important African species.

Graphical abstract



Keywords: *Pest management; Botanical pesticide;* Clausena anisata*; Rutaceae*

1 Introduction

A pest is any living organism which is invasive or prolific, troublesome, noxious, destructive and detrimental to animals, humans or human activities such as crop or livestock production (Oerke, 2006). Pests often occur in large numbers, making the damage they cause even more detrimental (Maxmen, 2013). The most economically important pests are insects, mites, nematodes and gastropods. Pests are a major cause of disease and production losses in various crop and livestock production systems, frequently causing significant economic losses (McDermott and Coleman, 2001; Oerke, 2006; Smith, 2015).

Globally, arthropods (which include insects) destroy an estimated 18–26 % of annual crop production, at a value of more than $470 billion (Culliney, 2014). Previously, Pimentel (2009) estimated crop loss from insect pests to be around $280 billion, contributing 14% to total crop losses. Thus, the losses to insect pests seem to be on the increase. Losses in the livestock production sector resulting from diseases transmitted by pests are equally huge. For example, trypanosomosis which is transmitted by the tsetse fly accounts for an annual loss of close to US$ 5 billion in Sub-Saharan Africa alone, resulting from livestock mortality, reduced fertility,decreased milk yields, an inability of traction animals to work and the expenditure on controlling the disease (Shaw et al., 2014). Another major disease is rift valley fever (RVF), a mosquito-borne zoonotic viral disease that is characterized by the onset of abortions in 80 to 100% of pregnant animals, high neonatal mortality (up to 90% in new-born lambs and kids), and mortalities of 15–30% or higher in adult sheep and goats (Kusiluka and Kambarage, 1996). The disease has a serious negative impact on rural food security and household nutrition, as well as direct and indirect losses to livestock producers in affected countries. In a RVF outbreak in Kenya in 2007 the estimated losses to the economy was more than US$24.5 million, from a combination of direct losses in the agriculture sector and subsequent losses in downstream industries (Rich and Wanyoike, 2010).

Insect pests also affect humans directly by transmitting diseases. Examples of diseases transmitted by pests that cause mortality in humans are malaria, yellow fever and more recently zika virus. Malaria has the largest impact on human health, with an ongoing transmission in 106 countries and half of the world's population at risk of contracting the infection (WHO, 2015). According to WHO estimates, there were 214 million new cases of malaria worldwide in 2015 and an estimated 438 000 deaths. The greatest burden of malaria occurs across Africa with 90% of all malaria deaths occurring therein with the main victims being children under five years of age (WHO, 2015). Malaria is also a cause of poverty due to loss of ability to work and a major hindrance to economic development due to increased costs of health care, lost working days due to sickness, days lost in education, decreased productivity due to brain damage from cerebral malaria, and loss of investment and tourism (Gollin and Zimmermann, 2007; Worrall et al., 2005).

Control measures for pests are costly and often time-consuming and involve extensive use of chemical pesticides (Pimentel et al. 2005; Zalucki et al., 2012). While pesticides are in common use, most of them are toxic to the environment and pests are known to quickly acquire resistance to them, making them obsolete. The development of resistance frequently results in the need for several additional applications of the pesticides or the use of more expensive alternatives to control diseases and maintain crop yields, all at additional costs. In the US alone, the additional cost in pest control due to resistance is US$1.5 billion per year (Pimentel, 2009). Thus, there is a continuing need for the development of new products to address pest control problems but with better safety and ecotoxicity profiles.

The natural compounds found in plants offer one avenue for the discovery of safe novel pesticides since these compounds are typically biodegradable (Isman, 2005; Tripathi et al., 2009). Not surprisingly, there has been growing impetus worldwide to explore natural products particularly plants as a source of lead compounds for the development of new pest control agents. Natural products have long been used as pesticides and have broadly served as templates for some commercial synthetic pesticides that are on the market today (Gerwick and Sparks, 2014). For example, the pyrethroids, which constitute the majority of commercial household insecticides, are originally derived from pyrethrins isolated from flowers of *Tanacetum cinerariifolium (Trevir.) Sch.Bip. (Chrysanthemum cinerariifolium (Trevir.) Vis., Fl. Dalmat.* and *Tanacetum coccineum (Willd.) Grierson* (Casida, 1980). Examples of older botanical pesticides include nicotine which is derived from tobacco (Nicotiana spp.), rotenone an isoflavanoid obtained from the roots of tropical legumes in the genera *Derris, Lonchocarpus* and *Tephrosia* and ryanodine an alkaloid obtained from *Ryania speciosa* Vahl (Mann and Kaufman, 2012).This review focuses on *Clausena anisata* (Willd.) Hook.f. ex Benth., Rutaceae, a plant species that is widely used traditionally against various pests in Africa.

2 *Materials and Methods*

Information on the ethnomedical use, chemistry and pesticidal biological activity of *C. anisata* published between 1980 and 2016 was accessed from various databases namely Science Direct, Springer Link and Wiley Online Library. The species names "*Clausena anisata*" and other synonyms including "*Clausena dentata*", "*Clausena inaequalis*" and "*Amyris anisata"* were used as the keywords in searching the title, abstract and keywords of articles in the databases. In addition various relevant books were also consulted.

3 Results and Discussion

The search for '*Clausena anisata*" yielded 28 journal articles from ScienceDirect, 72 from Wiley Online Library and 82 from SpringerLink , while "*Clausena dendata"* yielded, 1, 0 and 20 from ScienceDirect, Wiley Online Library and SpringerLink respctively The articles from these databases were largely focused on the biological activity and/or chemical composition of *C. anisata* extracts and formed the basis of this discussion. Some of the articles obtained from this search were on the ecology of *C. anisata.*

3.1 Occurrence and Taxonomy

Clausena anisata belongs to the genus *Clausena* in the Rutaceae (or Citrus) family. The Medicinal Plant Names Services (MPNS) gives 34 scientific synonyms for this plant species (http://mpns.kew.org/mpns-portal/), which include *Clausena dentata* (Willd.) M.Roem., *Clausena inaequalis* (DC.) Benth. and *Amyris anisata* Willd. The *Clausena* genus comprises 15 species, and is distributed in Africa, southern Asia, Australia, and the Pacific Islands (Tchinda, 2011). The most distinctive morphological character of the genus that separates it from the species of other related genera is the gynophore, which is a large, well-developed, hourglass-shaped structure supporting the ovary (Swingle and Reece, 1967). *Clausena anisata* is the only representative of the *Clausena* genus in tropical Africa (Schmelzer, 2001). It is widespread across the continent occurring in forests and forest margins, riverine thickets and bushveld from Guinea and Sierra Leone eastwards to Ethiopia and the Sudan and southward down to the Cape in South Africa, only avoiding the driest regions. *Clausena anisata* is also found in tropical and Southeast Asia, growing in India, Nepal and Sri Lanka and extending as far as Queensland in north-eastern Australia and some Pacific islands. It is cultivated for its oil in Malaysia, Indonesia and the Philippines (Axtell and Fairman, 1992; Tchinda, 2011). The oil is used as a medicinal flavour and in the Philippino local brandy "Anisdos" (Axtell and Fairman, 1992). *Clausena anisata* is a deciduous shrub or small tree with pinnate compound leaves which are densely dotted with glands and have a strong aniseed – like scent, when crushed. The flowers are small and white with orange-yellow stamens. The inflorescence forms a branched axillary spray (Hyde and Wursten, 2011).

3.2 Ethnomedical Use

Clausena anisata is widely used against various pests and parasites in many parts of Africa. In Zimbabwe, the leaves are crushed and packed onto the wounds of animals to expel maggots (Chavunduka, 1976). It is used as a mosquito repellent in some parts of South Africa (Okunade and Olaifa, 1987; Mavundza et al., 2011). In Benin and Cameroon, *C. anisata* is used as an insecticide against stored-grain pests (Tapandjou et al., 2000; Boeke et al., 2004a). In Kenya and Ethiopia, the leaves of *C. anisata* are used against intestinal worms in animals or humans (Muthee et al., 2011; Firaol et al., 2013). The roots and leaf infusion are effective remedies against internal parasites especially flatworms infestations, such as schistosomiasis and taeniasis (Bryant, 1966).

The use of *C. anisata* in human traditional medicine is equally widespread throughout tropical Africa. Various parts of the plant are used to treat numerous disorders and infections which include diabetes, haemorrhoids, hypotension, hypertension, heart failure, indigestion, fever, pneumonia, headache, whooping cough, malaria, venereal diseases, sinusitis, wounds, and mouth infections (Hutchings et al., 1996) bilharzia (Adesina and Adewunmi, 1985), convulsions (Adesina and Ette, 1982; Makanju, 1983), mental disease and schizophrenia (Pujol, 1990; Watt and Breyer-Brandwijk, 1962) . Leaf and root decoctions are also taken as an aphrodisiac, as a tonic by pregnant women, to facilitate child birth and cleanse the uterus and as an antidote for snake-bites (Watt and Breyer-Brandwijk, 1962; Hutchings et al., 1996; Tchinda, 2011).

3.3 Chemistry of C. anisata

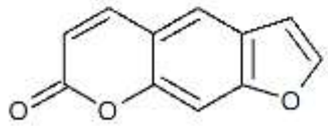
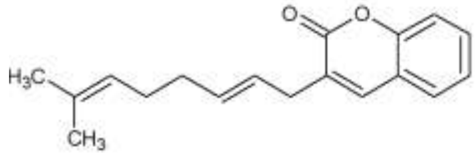
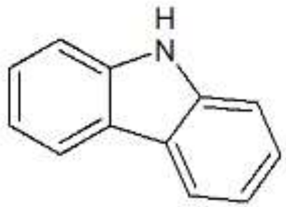
There have been extensive studies on the chemical composition of different plant parts of *C. anisata* and various compounds belonging to different chemical groups have been isolated and identified:

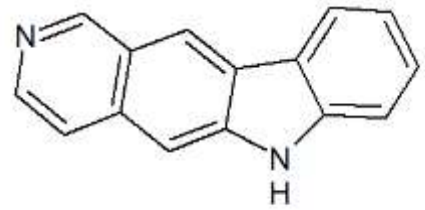
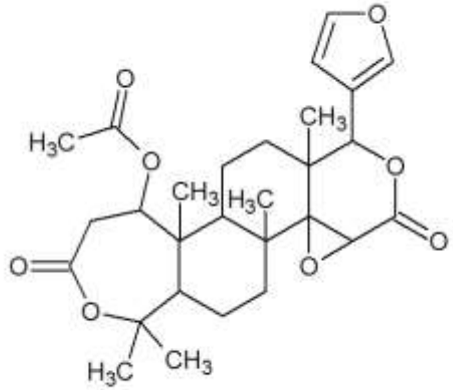
3.3.1 Coumarins

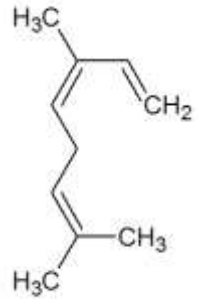
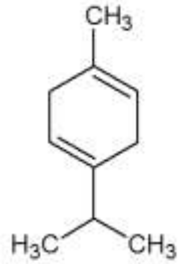
Coumarins are a class of heterocyclic compounds in which a benzene ring is fused with a pyrone ring (Table 1). Numerous coumarins have been isolated from *C. anisata* fruits, aerial parts and stem bark. They include the furanocoumarins, the geranyl coumarins and the furanocoumarin lactones (Table 1).

The abundance of coumarins in *C. anisata*, which are known for various biological activities such as insecticidal, feeding deterrence and repellency (Razavi, 2011; Tripathi et al., 2011), may in part, explain the ethnomedical use of *C. anisata* against pests and parasites. Bioenergetic disruption of muscle has been determined as a prominent mechanism underlying the insecticidal action of some coumarins (Zheng et al., 1998). Additionally, homology modelling and docking studies indicate that coumarins may also act as acetylcholinesterase inhibitors and can block the octopamine receptor pathway and thus be neurotoxic against insects such as mosquitoes (Khanikor et al., 2013). Furthermore, furanocoumarins can alter the detoxification capability of an organism, by reversibly or irreversibly inhibiting cytochrome P450 detoxification enzymes (Neal and Wu, 1994). A number of

Table 1: Classification and general structures of compounds isolated and identified from *Clausenaanisata*

Type	Subtype	Compound	Plant part	General Structure	Reference
Coumarins	Furanocoumarin	imperatorin isoimperatorin oxypeucedanine bergaptene xanthotoxin xanthotoxol chalepin xanthoxyletin osthol seselin	Fruits Stem bark Aerial parts Roots Leaves		Mester et al., 1977 Lakshmi et al., 1984 Gebreyesus and Chapya, 1983; Pitan et al., 2009 Mukandiwa et al., 201
	Geranylcoumarin	anisocoumarin A–J	Stem bark Roots		Ngadjui et al., 1989a, b; Ngadjui et al., 1991
	Furanocoumarin-lactone	indicolactone anisolactone 2′,3′-epoxyanisolactone	Stem bark		Lakshmi et al., 1984
Alkaloids	Carbazole alkaloids	atanisatin, clausanitin clausenol, clausenine girinimbine, heptaphylline 3-methylcarbazole, 1-methyl-3,4-dimethoxy-2-quinolone 3-formyl-1-hydroxycarbazole murrayamine-A ekeberginine clausamine A-H mukonal glycosinine,	Stem bark Roots Stem		Okorie, 1975 Chakraborty et al., 1995 Ngadjui et al., 1989c Songue et al., 2012 Ito et al.,1998; 2000; Tatsimo et al., 2015 Ito et al., 2009

		mukonidine clausine F			
	Lactone carbazole alkaloids	furanoclausamine A and B	Stem		Ito et al., 2009
	Pyranocarbazole alkaloid	mupamine	Root bark		Mester et al., 1977
Terpenes	Limonoids	limonin, zapoterin, clausenolide , clausenolide- 1-ethyl ether clausenarin	Stem bark Roots		Ngadjui et al., 1989d
Essential oils		-ocimenone Z-ocimenone gamma-terpinene germacrene D estragol γ-terpinene sabinene Z-β-ocimene	Leaves Seeds		Ngassoum et al., 1999 Gundidza et al., 1994 Innocent and Hassanali, 2015 Gundidza et al., 1994

		germacrene-B (E)-β-ocimene terpinen-4-ol		 	

coumarin compounds have also been identified as the antifeedant actives in *C. anisata* which include imperatorin and xanthoxyletin (Gebreyesus and Chapya, 1983), osthol [2H-1-Benzopyran-2-one, 7-methoxy-8-(3-methyl-2-butenyl)](Pitan et al., 2009) and seselin (2H,8H-Benzo[1,2-b:3,4-b']dipyran-2-one,8,8-dimethyl)(Mukandiwa et al., 2013).The exact mechanism of antifeedant action of coumarins is unknown.

3.3.2 Carbazole Alkaloids

Several carbazole alkaloids have also been isolated from the stem bark of *C. anisata* (Table 1). From the root bark, the pyranocarbazole alkaloid mupamine was isolated. The presence of carbazole alkaloids in *C. anisata* is typical of the *Clausena* genus. The alkaloids belong to the class of 1-oxygenated-3-methoxy-carbazoles possessing a prenyl side chain or an analogous moiety at C-4. Most carbazole alkaloids have been isolated from higher plants of the genus *Murraya*, *Clausena* and *Glycosmis* belonging to the family Rutaceae. Alkaloids are significant for the protection and survival of plants because they act as feeding deterrents against insects and herbivores (War et al., 2012).These alkaloids tend to be pharmacologically active in humans, animals and their pests. They inhibit feeding by inhibiting impulse generation in sugar sensitive cells in lepidopterans (Frazier, 1986; Simmonds et al., 1990) and competitively block sucrose responses in flesh flies (Morita et al., 1977). The sugar sensitive cells respond to compounds eliciting feeding, thus impulses generated from these cells would stimulate feeding. Alkaloids have also been shown to inhibit the pyranose and furanose receptor sites found in the sugar sensitive cells in flies (Wieczorek et al., 1988). The antiparasitic activity of various plants has been explained by the presence of various alkaloids therein (Aniszewski, 2007; Wink, 2012). The mode of action for the insecticidal activity of alkaloids varies with the structure of the molecule but many are reported to affect the acetylcholinesterase and sodium channels (Flattum and Shankland, 1971; Amar et al., 1991).

3.3.3 Limonoids

Limonoids have also been isolated from the stem bark and roots of *C. anisata*. These are modified triterpenes with, or derived from a precursor of a 4, 4, 8-trimethyl-17-furanylsteroid skeleton (Table 1). They characterize members of the *Meliaceae* family, where they are abundant and diverse (Connolly, 1983; Taylor, 1981; 1983). A limited range of limonoids is found in the *Rutaceae* family (Dreyer et al., 1972; Roy and Saraf, 2006). Limonoids are known to have insect antifeedant and growth regulating (IGR) activity (Champagne et al., 1992). The antifeedant mechanism of action of limonoids, as exemplified by the well-known azadirachtin, is different in different insect species, i.e., affecting more than one chemosensory cell type in more than one way (Koul, 2008). However, what is certain is that these terpenoids induce antifeedant effects in the various insect species by suppressing the phagostimulant receptor and stimulation of deterrent cells located in the medial sensillum of insects (Koul, 2008). The sensillum is an arthropod sensory organ protruding from the cuticle, or sometimes lying within or beneath it. As IGRs, limonoids adversely affect insect growth and development through their effects on enzymes and certain physiological regulatory processes essential to the normal development of insects or their progeny. Thus, the presence of this class of compounds in *C. anisata* may be an attributing factor to the reported use of *C. anisata* as a pest control agent.

3.3.4 Essential Oil

As with other species of the Rutaceae family, the leaves, fruits and stem bark of *C. anisata* are rich in aromatic essential oils. These essential oils have been implicated in the repellent and insecticidal activities of *C. anisata* against a number of pests. Several studies have been conducted on the chemical composition of the essential oil from *C. anisata* (Ekundayo et al., 1986; Ayedoun et al., 1997; Okunade and Olaifa, 1987; Gundidza et al., 1994; Ngassoum et al., 1999; Govindarajan, 2010; Usman et al., 2010; Yaouba et al., 2011). The composition of the essential oils determined from these studies varied widely with geographical regions as is expected. For instance, the main components obtained from essential oil from *C. anisata* collected from Cameroon were E-ocimenone (15.1%), Z-ocimenone (11.5%), gamma-terpinene (11.4%) and germacrene D (10.9%) (Ngassoum et al., 1999), while the oil from *C. anisata* collected in Tanzania contained mainly estragol (88.38%) and γ-terpinene (4.79%) (Innocent and Hassanali, 2015) and the oil from *C.anisata* collected from Zimbabwe contained mainly sabinene (33.0%), germacrene-D (17.0%), Z-β-ocimene (6.0%), germacrene-B (5.5%), (E)-β-ocimene (4.9%) and terpinen-4-ol (4.7%) (Gundidza et al., 1994). Essential oils are well known for pesticidal activity which is why many aromatic plants have been used for generations in traditional practice as insect repellents (Maia and Moore, 2011). Many commercial natural repellents contain a number of plant essential oils including those of *Cymbopogon citratus* (DC.) Stapf, *Pelargonium graveolens* L'Hér., *Cymbopogon nardus* (L.) Rendle, *Lippia javanica* (Burm.f.) Spreng., *Thymus vulgaris* L. and *Tagates minuta* L. (Maia and Moore, 2011).

4 *Biological Activity*

Clausena anisata contains a variety of compounds, which exhibit interesting biological activities. The plant and its compounds have been evaluated for various activities such as anticancer (Ito et al., 2000), anti-HIV-1 (Ayisi and Nyadedzor, 2003), anti-fungal (Hamza et al., 2006), anti-viral, anti-diabetic (Ojewole, 2002), antidiarrhoeal (Gundidza et al., 1994) anticonvulsant (Makanju, 1983), anti-inflammatory, immunomodulatory, anticoagulant antiplasmodial and analgesic (Okokon et al., 2012 a, b). This review, however, will focus on the pesticidal activity.

4.1 ***Activity against blowflies***

The effects of *C. anisata* on the behaviour and development of blowfly larvae have been investigated (Mukandiwa et al., 2012a, b; 2016a). In both laboratory and field studies, where fly larvae were exposed to meat or liver baited with the acetone leaf extracts (10mg/ml – 150mg/ml). Ivermectin was used as a positive control while acetone and water served as solvent controls. Developmental anomalies in the larvae, which included paralysis, prolongation of the larval stage, reduced pupation rates and pupae malformations (Mukandiwa et al., 2012a, b; 2016a) were reported. The authors have suggested that these effects indicate that the plant extracts interfere with neuroendocrine control of movement and moulting in the blowfly (Mukandiwa et al., 2012a, b).This may be due to the presence of coumarins and alkaloids within the plant species (as discussed earlier) which are known to inactivate the acetylcholinesterase enzyme (AChE) (Aniszewski, 2007; Khanikor et al., 2013). Such inactivation may result in the disruption of the cholinergic nervous system leading to paralysis and death (Coppage and Matthews, 1974). Higher concentrations of the acetone leaf extracts of *C. anisata* have also been shown to decrease ingestion by the larvae, pupae mass, adult emergence rates and adult sizes (Mukandiwa et al., 2012b). Seselin, a pyranocoumarin, was identified as one of the compounds in *C. anisata* that reduce feed intake and adult fly sizes (Mukandiwa et al., 2013).

4.2 ***Activity against mosquitoes***

4.2.1 Repellency

The activity of *C. anisata* leaf extracts against various mosquitoes species has been evaluated in a number of studies. In a study by Maharaj *et al*. (2010), the dichloromethane (DCM) / methanol (MeOH) leaf extracts of *C. anisata* had a repellency of 56% against *Anopheles arabiensis* in a 2-minute period. Innocent and Hassanali (2015), also reported that the crude essential oil of *C. anisata* had a protective efficacy ranging between 14 and 57% against *A. gambiae*. Mukandiwa et al. (2016b) evaluated leaf extracts of *C. anisata* against *A. aegypti* for their possible value as mosquito-net repellent agents, as topical repellents in a guinea pig model and as aerosol insecticide sprays. As mosquito-net treating agents, the acetone and hexane extracts of *C. anisata,* both at 15%, had average repellences of 46.9 ± 2.95 and 50.1 ± 2.02 %, respectively, 3 h after exposure. In the topical application assays using total bites as an indicator, repellency was dose-dependent, with the acetone extract having 93% repellency and the hexane fraction 67% repellency after 3 h in comparison to DEET, the positive control, which had 100% repellency. Thus, although the degree of repellency varies widely by method of application, *C. anisata* extracts repel mosquitoes. In addition, research findings by the various workers seem to suggest that the crude extract of *C. anisata* is more effective as a repellent than isolated single compounds. Innocent and Hassanali (2015) reported that estragole, which was the main component of the *C. anisata* oil tested, had a lower protective efficacy (between 10% and 38%) compared to the crude essential oil (between 13 and 57%). Similarly, Mukandiwa et al., (2016b) found that fractionation of the crude *C. anisata* extract resulted in a loss of total repellency. This loss of activity may imply that there is more than one bioactive compound acting synergistically to produce the observed activity.

The human forearm bait method is the WHO recommended method for evaluating mosquito repellents for use in humans as it utilizes the repellent end-user in the testing process and yields results that are relevant to the actual conditions of use (WHO, 2009). One ml of the treatments are applied to circa 600 cm^2 area of the forearm skin between the wrist and elbow and exposed to mosquitoes in a cage for 3 minutes.

Mosquito biting/feeding are recorded and data are presented as repellency/feeding deterrence. Only the work by Innocent and Hassanali (2015) evaluated *C. anisata* in this manner. The other researchers used guinea pigs as the bait for mosquitoes. Even within this animal model there are still a number of variations relating to the concentrations tested and the test period. Maharaj et al. (2010) evaluated the repellency of DCM/MeOH leaf extracts of *C. anisata* at 1% over a 2 minute-period. Mukandiwa et al. (2016b) evaluated the acetone and hexane extracts of *C. anisata* at15% and 7.5% respectively over a 3 hour period. All the bioassay systems used positive controls, commonly DEET and a negative control (solvent). It is noteworthy that the leaf extracts of C. *anisata* had repellent activity against mosquitoes in all the assays.

4.2.2 Larvicidal activity

The efficacy of *C. anisata* against mosquito larvae has been experimentally demonstrated (Mavundza et al., 2013; Mukandiwa et al., 2015). Mavundza et al., (2013) showed that the ethanol leaf extract of *C. anisata* caused larval mortality of the *Anopheles arabiensis* mosquito and had an LC_{50} value of 112.7 ppm. Similarly in a study by Mukandiwa et al. (2015), the *n*-hexane extract of *C. anisata* caused mortality of third instar larvae of the A. *eagypti* mosquito and had LC_{50} values of 68.3 and 59.7 ppm after 24h and 48h respectively. A pyranocoumarin, seselin, isolated from the same extract in the same study had LC_{50} values of 13.9 and 10 ppm at 24 and 48 h respectively. Larvicidal activity of *C. anisata* was evaluated according to the World Health Organization (WHO) guidelines for laboratory and field testing of mosquito larvicidal activity (WHO, 2005). The availability of the WHO guidelines has enabled an almost standard evaluation of plant extracts for larvicidal activity. The WHO guidelines stipulate the use of 25 mosquitoes larvae in each jar replicated four or more times for each concentration and that each test should be run three times on different days. It also stipulates for the use of a positive control, temephos. In both studies the number of dead larvae was recorded after 24 h and in Mukandiwa et al., (2015) also after 48 h of exposure and the percentage mortality was calculated as per the WHO protocol. The criterion for declaring the larvae dead was similar in both studies and also as per the WHO guidelines. The studies conducted to evaluate the larvicidal activity of *C. anisata* have largely followed this protocol and both studies conclude that *C. anisata* extracts are larvicidal against mosquitoes.

Reports from the Asian continent on *C. dentata (synonym of C. anisata.)* also confirm this larvicidal activity. Acetone, chloroform, ethyl acetate, methanol, and petroleum benzene leaf extracts of *C. dentata* were tested against the fourth instar larvae of *Anopheles stephensi*, *Culex quinquefasciatus*, and *Aedes aegypti* (Diptera: Culicidae) as per the WHO guidelines (Manjari et al., 2014). The highest larval mortality was found in acetone leaf extract, *C. quinquefasciatus* (LC_{50} = 0.150278 mg/ml; LC_{90} = 7.302613 mg/ml), *A. aegypti* (LC_{50} = 0.169495 mg/ml; LC_{90} = 1.10034 mg/ml), and *A. stephensi* (LC_{50} = 0.045684 mg/ml; LC_{90} = 0.045684 mg/ml).

4.2.3 Adulticidal activity

The adulticidal activity of *C. anisata* against mosquitoes has been reported by Mavundza et al. (2014) and Mukandiwa et al. (2016b). Of note again, is the fact that the different researchers used different methods to demonstrate the cidal effect of *C. anisata* extracts. In Mavundza et al. (2014) the adulticidal activity of the ethanol (EtOH) and dichloromethane (DCM) leaf extracts of *C. anisata* (1%) was evaluated in a contact assay following the WHO guidelines for testing mosquito adulticides for indoor residual spraying and treatment of mosquito nets with slight modifications. Plant extracts were impregnated into Whatman No 1 filter papers and the impregnated papers were air dried for 5 minutes and then inserted into an exposure tube in the

WHO testing kit. Twenty, 2–5 day old, blood-starved female mosquitoes were introduced into the test kit for 1 hour. They were then transferred to the holding tube to recover. At the end of the 24 hour recovery period, the number of dead mosquitoes was recorded and the percentage mortality was calculated. Adult mortality ranging from 4 to 10% after 24 hours of exposure was reported. A report from India further affirms this adulticidal activity (Ramkumar et al., 2015), in this work, *Clausena dentata* plant extracts were evaluated against *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes. The adult mortality was observed after 24 h of exposure. The highest mortality was found in acetone extracts against *Ae. aegypti* and *Cx. quinquefasciatus* with LC_{50} and LC_{90} values of 4.1783 mg/ml, 9.3884 mg/ml and 4.2451 mg/ml, 12.3214 mg/ml, respectively.

In the study by Mukandiwa et al. (2016b) the acetone and hexane leaf extracts of *C. anisata* were dissolved in a 1:1mixture of ethanol and sunflower oil. In each test, 1 ml of test materials was nebulised into the chamber using a medical ultrasonic nebuliser which already contained 60 mosquitoes at a rate of 0.2 ml/min. The numbers of mosquitoes knocked down at 15, 30, and 60 min were counted. The WHO classification of knockdown was used in this study (WHO, 2013). The acetone crude extract of *C.anisata* and its hexane fraction caused mosquito knockdown and eventually death with EC_{50} values of 78.9mg/ml (7.89%) and 71.6 mg/ml (7.16%) in the first 15 minutes after spraying. Similarly, Ramkumar et al. (2015) showed that C. dentata caused 85 ± 2 and 89 ± 1.5 % mortality against *Ae. aegypti* and *Cx. quinquefasciatus* respectively after 40 min in a smoke toxicity assay. A mortality of 100 % was recorded in the commercial mosquito control.

4.3 Activity against stored grain pests

4.3.1 Callosobruchus maculatus

The toxic and repellent effects of *C. anisata* leaf powder against the main insect pest of stored cowpea, *Callosobruchus maculatus,* were evaluated and the powder was shown to repel the female beetles from treated beans in a linear olfactometer (Boeke et al., 2004a). In a further study, by the same researchers, volatile oils of C. *anisata* were shown to repel the female beetles and caused significant lower numbers of eggs to be laid on treated beans and lower number of offspring (Boeke et al., 2004b).

4.3.2 *Sitophilus zeamais* and *Prostephanus truncatus*

Powdered leaves of *C. anisata* were evaluated on Cameroonian and German strains of the maize weevil, *Sitophilus zeamais,* and the less cosmopolitan larger grain borer, *Prostephanus truncatus* for their insecticidal activities, the effects on progeny production, grain damage and population increase by admixing powders and maize grains (Nukenine et al., 2010). The highest mortality induced was lower than 25%, irrespective of the insect species, powder content or exposure period. The *C. anisata* powder caused up to 39.3 ± 21.5% and 45.7 ± 24.5% reduction in adult emergence relative to control in the Cameroonian and German strains of the maize weevil respectively. The rate of increase of the populations of the three insect species was significantly reduced by the powder content of 1 g/100 g grain powder from *C. anisata* within 3 months of storage. The *C. anisata* powder also significantly reduced grain damage by the maize weevil as the levels of application increased (Nukenine et al., 2010).

4.3.3 *Acanthoscelides obtectus* (Say)

The efficacy of essential oil extracted from *C. anisata* was evaluated for insecticidal activity and its effect on progeny production of *Acanthoscelides obtectus* (Say), the main pest of stored common beans (Ndomo et al., 2008). The oil caused mortality of *A. obtectus* in both the contact and fumigation assays. The highest dosage (0.133 µl/g of grains) induced 37.3% mortality after 24 h of exposure in the contact assay which increased to 96% after 4 days. In the fumigation assay, the highest concentration of essential oil (0.118 µl/cm^3) caused 15% mortality after 6 h of exposure which also rose to 87.5% after 24 h of fumigation. The essential oil also evoked moderate repellent action at relatively low concentrations. In the same study, the oil also caused significant reduction in first generation (F1) progeny, with no progeny being produced in grains treated with the high dosage (0.133 µl/g) (Ndomo et al., 2008). The authors proposed that the activity of the oil was due to its high content of trans-anethol and isoeugenol methyl ether which are known to have insecticidal activities against several insect species of stored food products (Rice and Coats, 1994; Ho et al., 1997; Prates et al., 1998).

4.3.4 Tribolium castaneum

The efficacy of the essential oil of *C. anisata* has been compared to that of a synthetic insecticide, imidacloprid against the red flour weevil *Tribolium castaneum* (Coleoptera: Tenebrionidae) over 4 generations (F4) (Goudoum et al., 2010). The lethal dose of the insecticides that caused 80% of mortality (LD_{80}) at the first generation killed 60% of pest at the 3rd generation for the C. *anisata* essential oil and 25% of pest for imidacloprid. At the 4th generation, 25% of the pest populations were killed by the C. *anisata* essential oil and 5% by imidacloprid applications. The researchers from this study concluded that T. *castaneum* could acquire resistance to imidacloprid faster than to C. *anisata* essential oil. This is probably because oils are multiple component substances and there may be synergistic activity thus obviating the rapid onset of resistance.

The evaluation of *C. anisata* against stored pests has been mainly on the powdered leaves and the essential oil. Similar to the evaluations of pesticidal activity against other pests there has been no standard method followed for the evaluation of *C. anisata* against weevils. However the methods used in the aforementioned studies all mimicked exactly how the end-user would use a commercial product in the protection of grains. The confirmed activity against a range of stored grain pests warrants further research into the possible commercialisation of *C. anisata* for the protection of stored grain. Important research questions include the food-safe use of the extracts and also long-term toxicity.

4.4 Activity against other insects

The antifeedant activity of *C. anisata* against *Helicoverpa armigera* Hubner (Lepidoptera: Nuctuidae), the cotton bollworm, which is a major worldwide pest of many crops has been evaluated (Pitan et al., 2009). Antifeedant activity was confirmed for both the root and leaf extracts. A coumarin, osthol [2H-1-benzopyran-2-one, 7-methoxy-8-(3-methyl-2-butenyl)], was found to be the compound responsible for the antifeedant activity of C *anisata* against the cotton bollworm. The toxicity of the volatile oil of the leaves of *C. anisata* to the third nymphal instar of the variegated grasshopper, *Zonocerus variegatus* L. has also been reported. Estragole was identified as the component responsible for the toxicity (Okunade and Olaifa, 1987).

5 *General Discussion and Conclusion*

From the above information, it is clear that the chemistry of *C. anisata* has been extensively explored with a focus of validating the ethnomedical use of the species and identifying the compounds responsible for the observed pesticidal effects of the plant. The results strongly indicate that the species is endowed with pesticidal properties that can be harnessed into commercial products. Although a number of compounds, such as imperatorin, xanthoxyletin, and isoeugenol methyl ether, have been isolated and shown to have pesticidal activities none of the compounds or enriched extracts has been developed into commercial products. The possible reason for this could be that the performance of these natural compounds has not equalled or surpassed the current pesticides. There is a need to explore synthesis of analogues with enhanced activity or the possibility of using the known actives in combination. Alternatively, considering that crude extracts performed better than single compounds in some studies, standardisation and stabilisation of the extracts should be explored. The fact that the identified compounds belong to different compound classes that act via different mechanisms offers an opportunity for the development of a novel pesticide with reduced chances of resistance development. Combining various pesticidal compounds will also allow for the exploitation of synergistic activity which may result in enhanced activity and hence reduced applications of the pesticide. Studies by Innocent and Hassanali (2015), Lukwa et al. (2009) and Mukandiwa et al. (2016b) in which crude extracts had better activity compared to the isolated compounds supports this notion. Standardizing such extracts is a possible next step to their pharmaceutical development and commercialization. A drawback of many plant extracts and essential oils is that they volatilize quickly in the environment limiting persistency of protection against pests. To increase the attractiveness of using natural compounds there is therefore a need, to explore ways of enhancing their activities and stability possibly through novel formulation and delivery systems. It is also noteworthy that the concentrations used in the biological assays discussed are quite high and may be impractical to use in daily life. However as these are crude extracts, it is envisaged that enriching the extracts further or isolation of bioactive compounds will result in even lower concentrations.

The successful development of *C. anisata* into commercial products for the control of pests requires 3 major action points, namely: investigating safety to the end user and the environment; standardisation and building commercial value chains by either cultivation or sustainable wild harvesting. Toxicity testing is an important consideration for plant products. Naidoo and Seier (2010) suggested that single-dose acute, repeat-dose toxicity and reproductive toxicity testing should be conducted. Standardization can be used to, at a minimum, identify the marker compounds and determine the acceptance criteria (quantitative and qualitative) of a plant product thus ensuring batch uniformity and pharmaceutical stability. The major constituents in *C. anisata* have been identified and although they may not necessarily be the active ones they can allow for some level of consistency which ensures that batch variability is reduced and thus composition and biological activity are assured.

For commercialization, the availability of the plant material in large enough quantities to ensure constant year round supply is critical. Wild harvesting generally does not assure this and can inevitably lead to loss of habitats and negative environmental consequences for the target species as well as other organisms in its ecological niche (Briskin, 2000; Schippmann et al., 2002). Domestication and cultivation offers an opportunity for genetic modification to increase yield, reduce growing season or enhance biological activity (Katerere, 2014). The wide distribution of the plant species and its ability to grow well in most environments makes it a suitable candidate for cultivation for commercial purposes. The successful domestication and cultivation of *C. anisata* in Malaysia, Indonesia and the Philippines is encouraging.

From the foregoing, C. *anisata* has enormous potential as a source of novel pesticidal products. However formulation and agronomic research are key in unlocking the potential of this important African species.

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