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PhD THESIS

BACK TO PLANTS FOR DRUG DISCOVERY: FROM ETHNOMEDICINE TO MORE CONVENTIONAL APPROACHES

by

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GENERAL INTRODUCTION

Over the last years, there is rekindling of interest in drug discovery from botanical resources. Indeed, plants have provided and continue to provide mankind with an inexhaustible and unique source of bioactive principles, some of which have been developed into drugs (Rates, 2001; Vannini et al., 2016). Many of these drugs are still in use so far and we often do not find them valuable synthetic alternatives in clinical practice (Fabricant and Farnsworth, 2001).

The plant chemicals used for medicinal purposes are largely secondary metabolites, which, biosynthetically, are produced from primary metabolites in the fight for survival. While primary metabolites play essential role in the normal growth, development, or reproduction of plants, secondary metabolites are involved in defence against herbivores (insects, vertebrates), microorganisms, and other plants competing for light, water and nutrients (Wink, 1999). Additionally, secondary metabolites serve as signalling compounds to attract pollinating and seed-dispersing animals, as well as for communication between plants and other living systems.

The potential for finding new and more drugs from vegetal resources is enormous, as a vast repertoire of plant species have not yet been screened for biologically active principles (Verpoorte, 2000). Despite the rise of combinatorial chemistry techniques in drug discovery, there are still reasons why plants remain an essential component in the search for novel medicines. In the first place, being metabolites of natural origin, plant-derived bioactive principles are more likely to succeed as drugs (Hert et al., 2009). In addition, clues from traditional medicinal uses of plant remedies may guide the endeavour to discover new medicaments from medicinal plants (Barlow et al., 2012; May et al., 2012). In contrast, the development of synthetic pharmaceuticals seems like “looking for a needle in a haystack”. In other words, it is rather a random process which, for the most part, requires a lot of time

(Reichert, 2003) and money (Dickson and Gagnon, 2004). Finally, plant-derived products exhibit over synthetic compounds a wider range of biologically relevant chemical space (Feher and Schmidt, 2003), and this characteristic accounts for their ability to yield more efficiently an unmatched diversity of therapeutically relevant chemicals (Drewry and Macarron, 2010). Of these, however, only a few have been assessed for their biological activities (Saklani and Kutty, 2008). Therefore, there should be an abundance of potential pharmaceuticals in plant extracts yet to be discovered.

This thesis proposes to explore various approaches to drug discovery from medicinal plants, identifying the areas of knowledge involved and addressing the challenges encountered, with the aim of enhancing the chance of success of the overarching process. At this end, the first part of my research program was dedicated to literature mining in support of drug discovery from medicinal plants. Here, we undertook a systematic review of literature with focus on *Tithonia diversifolia (Hemsl.) A. Gray* (fam. Asteraceae). *Tithonia diversifolia* (TD) was deliberately selected from a large array of plants because it is endemic to the tropics and, as such, we expected it to have a wide scope of therapeutically useful properties. Further, a preliminary consultation of online databases has indeed provided supporting literature on the medicinal virtues of this plant species. More interestingly, at the time of literature consultation, there was no TD-based drugs available on the marketplace, creating immense commercial interests for this plant. This work provided a comprehensive understanding on TD, pinpointing controversies and gaps in its current knowledge, and building a strong foundation for future drug development research. Beyond the scope of my research program, this step allowed me to acquire specific skills in the formulation of search terms for the retrieval of the appropriate literature, the critical appraisal of existing body of research and non-research literature, the analysis and synthesis of the results, as well as in the production of a comprehensive and informed literature review.

The second part of my PhD program was devoted to ethnomedical claims survey to select the best candidate plants to launch a drug discovery campaign. Thus, we carried out a 6-month cross-sectional questionnaire-based survey to explore the use of herbal remedies in People living with HIV (PLHIV) in Dschang (West Region, Cameroon). We focused our attention on PLHIV because AIDS represents a major concern in this part of the globe and a definitive cure or protective vaccine has not yet been developed. In addition, currently available disease-modifying antiretroviral drugs are still suffering from large unmet medical needs, which has led PLHIV in Cameroon to frequently seek alternative therapies from traditional health practitioners (THPs), including herbal remedies.

The third and last part of my research program focused on the use of clues derived either from the scientific literature or ethnomedicine, to design experimental models to characterize the biological activity of a selected plant. In this regard, we experimentally pursued cannabis (*Cannabis sativa L.*, fam. Cannabaceae) extract as a source of anti-inflammatory lead compounds which act through novel mechanisms. Our focus on this emerging research topic was supported by the fact that cannabis and its derivatives are currently generating a huge therapeutic interest as anti-inflammatory agents. Additionally, among their wide array of therapeutic potentials, their ability to stem inflammation do stand out. Noteworthily, this part of my research program saw the participation in terms of exchange of skills of LINNEA SA.

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CHAPTER 1: *Tithonia diversifolia (Hemsl.) A. Gray* as a Medicinal Plant: a Comprehensive Review of its Ethnopharmacology, Phytochemistry, Pharmacotoxicology and Clinical Relevance

(published in J. Ethnopharmacol. 220, 94–116. doi:10.1016/j.jep.2018.03.025)

Abstract

Ethnopharmacological relevance: *Tithonia diversifolia* (TD) is widely valued in several cultures for its medicinal properties. A comprehensive review of the current understanding of this plant species is required due to emerging concerns over its efficacy, toxicity and allergenic potential.

Aim of the review: We critically summarized the current evidence on the botany, traditional use, phytochemistry, pharmacology and safety of TD, with the view to provide perspectives for developing more attractive pharmaceuticals of plant origin, but also to lay a new foundation for further investigations on this plant.

Materials and methods: A preliminary consultation of search engines such as Web of Science, PubMed, ScienceDirect and other published/unpublished resources provided an overview of extant literature on TD. Then, we meticulously screened all titles, abstracts and full-texts to establish consistency in the application of inclusion criteria. Studies were considered for inclusion if they dealt with taxonomy, global distribution, local and traditional knowledge, phytochemistry, toxicity and biological effects.

Results: 1,856 articles were retrieved among which 168 were revised and included. Several studies conducted on cell lines and animals provided supporting evidence for some ethnomedicinal claims of extracts from TD. Short-term use of Tithonia extracts were effective and well-tolerated in animals when taken at lower doses. Both the toxic and therapeutic effects

were attributed to bioactive principles naturally occurring in this species including sesquiterpene lactones, chlorogenic acid and flavonoids.

Conclusions: T. diversifolia is a valuable source of bioactive compounds with significant therapeutic implications and favourable safety index. However, more rigorously designed investigations are needed to recommend the whole plant or its active ingredients as a medication and should focus on understanding the multi-target network pharmacology of the plant, clarifying the effective doses as well as identifying the potential interactions with prescribed drugs or other chemicals.

Abbreviations: ABTS, 2,2′-Azinobis (3-ethylbenzothiazoline-6-sulphonate); Ach, acetyl choline; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AMPK, 5' adenosine monophosphate-activated protein kinase; AST, Aspartate transaminase; CAs, chlorogenic acids; CC50, half-maximal cytotoxic concentration; COX, cyclooxygenase; DM, dichloromethane; DPPH, 2,2-diphenyl-1- picrylhydrazyl; DRC, democratic republic of Congo; EO, essential oils; FXR, farnesoid X receptor; GGT, Gamma-glutamyl transferase; GI50, concentration of extract that inhibited the growth of leukaemia cells; GOT, glutamic oxaloacetic transaminase; GPT, glutamate pyruvate transaminase; HDL, high density lipoprotein; HIV, human immunodeficiency syndrome; hMSC, human mesenchymal stem cells; HSV, herpes simplex virus; IC50, half-maximal inhibitory concentration; IL, interleukin; IR, Infrared spectroscopy; LC50: Lethal concentration 50; LD50 Lethal dose 50; LDH, Lactate Dehydrogenase; LDL, Low Density Lipoprotein; LO, lipoxygenase; LRE, leaf rinse extract; LPS, Lipopolysaccharide; LXR, liver X receptor; MAC, minimum amoebicidal concentration; MIC, minimum inhibitory concentration; MPO, myeloperoxidase; MTD, maximum tolerated dosage; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide; NAC, N-Acetyl-L-Cysteine; NadD, NaMN adenylyltransferase, NF-κB, Nuclear Factor-κB; NMR, Nuclear Magnetic Resonance Spectroscopy; NO, nitric oxide; PBMCs, peripheral blood mononuclear cells; PCV, packed cell volume; PE, polar extract; PHA, phytohemagglutinin; PPAR, Peroxisome Proliferator-Activated Receptor; PRISMA, Preferred Reporting Items for Systematic reviews and Meta-Analyses; ROS, Reactive Oxygen Species; SI, Selectivity index; STLs, sesquiterpene lactones; TEAC, Trolox Equivalent Antioxidant Capacity; TD, Tithonia diversifolia; TNF, tumour necrotic factor; WBC, white blood cells; ZI, zone of inhibition.

1. Introduction

Tithonia diversifolia (TD; Asteraceae) is widespread in tropical and subtropical climes. The plant was named after the botanist René Desfontaines from "*Tithonus*", consort of "*Aurora*" in Greek mythology, in allusion to the glowing orange ray corollas of the flowers of *T. rotundifolia*, which French people called "couleur aurore" (Musée national d'histoire naturelle (France), 1802). On the other hand, the specific name diversifolia (separated leaves) was recovered from the Latin words "diversus" (divergent) and "folium" (leaf). According to theplantlist.org, *Tithonia diversifolia (Hemsl.) A. Gray* is the accepted name to refer to this plant, and other scientific names such as *Helianthus quinquelobus Sessé & Moc., Mirasolia diversifolia Hemsl., Tithonia diversifolia var. diversifolia, Tithonia diversifolia subsp. diversifolia, Tithonia diversifolia var. glabriuscula S.F.Blake, Urbanisol tagetiflora var. diversifolius (Hemsl.) Kuntze, Urbanisol tagetiflora var. flavus Kuntze and Urbanisol tagetifolius f. grandiflorus Kuntze*, are relegated to synonymy. Commonly, the plant is known under many different names including the tree marigold, Japanese sunflower, and wild sunflower (Itis.gov, 2017).

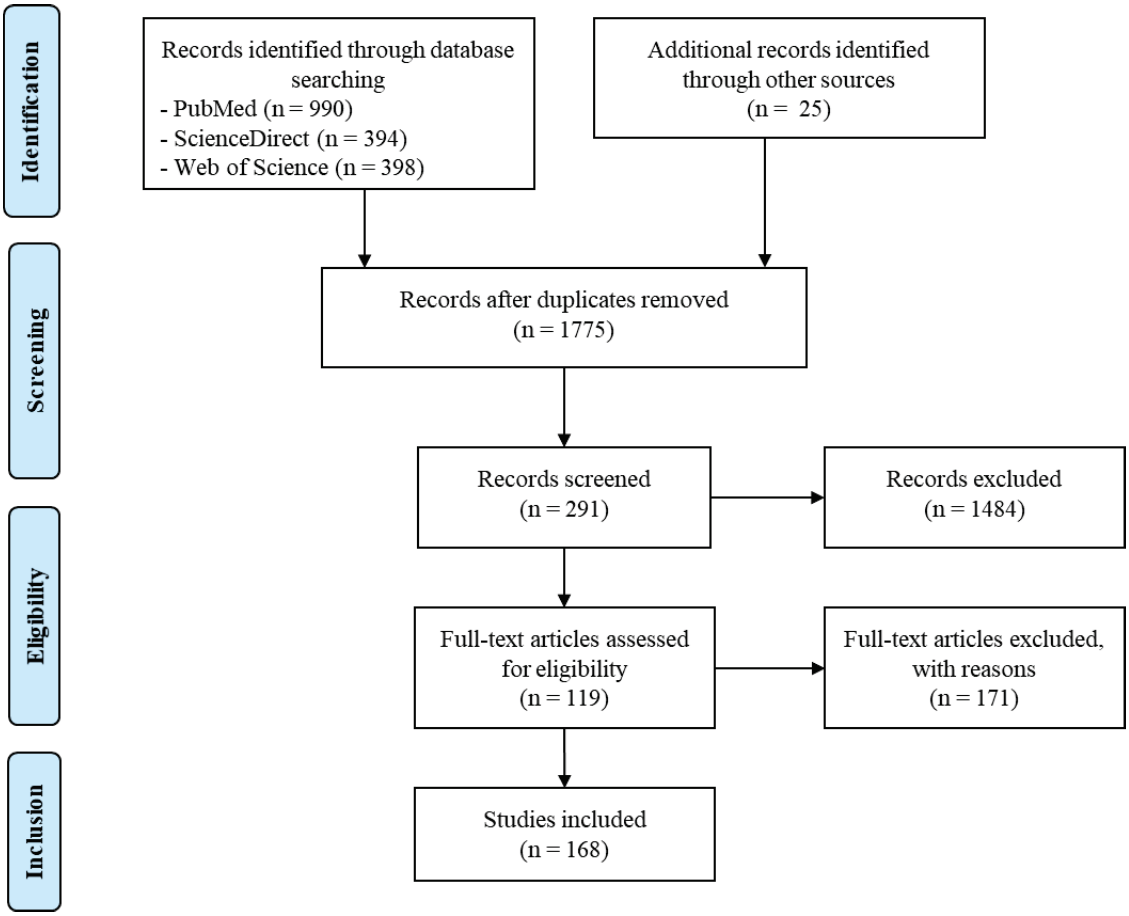
Traditionally, all parts of the plant especially the leaves, are widely used by indigenous people for treating a wide spectrum of ailments and diseases ranging from topical application —to treat wounds, skeleto-muscular disorders, abscesses, dermatological conditions, and stomach pains— to oral administration for diabetes, malaria, fever, hepatitis and infectious diseases. Several in vitro and in vivo studies have provided heterogenous evidence supporting most of the traditional therapeutic claims of TD. Bioassay-guided phytochemical screening of TD extracts led to the identification of a wealth of bioactive principles with significant therapeutic implications and favourable safety index. However, besides extensive use in folk medicine, there are numerous other possible applications of *Tithonia diversifolia*, the most investigated being as ornamental, fuelwood, fodder, green manure, biopesticide, living fence and boundary demarcation (Ng'inja et al., 1998; Otuma et al., 1998).

With the upward popularity of TD, there are major concerns about its efficacy, toxicity and allergenicity. Therefore, a comprehensive review of the current understanding of this plant is needed to provide a source of information for anyone potentially interested in, but also to pinpoint research gaps for future investigation. To this end, we systematically retrieved and critically summarized extant literature pertaining to the pharmacotoxicology of TD and its relationship with local/traditional use and phytochemistry. The botanical characterization of TD was performed as well. Moreover, we provided relevant insights into the potential applications of TD in clinical practice.

2. Search strategy

The search strategy adopted was in keeping with PRISMA guidelines (Moher et al., 2009). Knowledge about TD was collected from both online databases and non-electronic resources. In this search, the many other names attributable to TD including synonyms and common/local names were used to retrieve - without language nor year restrictions - several reports issued in the period up to and including July 31, 2017. Overall, a total of 1,804 reports have been collected, including 990 from PubMed, 398 from Web of Science, 394 from ScienceDirect, and 22 from other sources (books, PhD/MSc dissertations and website resources). After subsequent duplicates removal (Supporting information SI. 1) and screening for relevant titles and abstracts (Supporting information SI. 2), a total of 119 text articles were obtained and assessed for eligibility (Supporting information SI. 3). Text articles were considered for inclusion if they dealt with taxonomy, global distribution, local and traditional knowledge, phytochemistry, toxicity and biological effects. Finally, 168 articles were selected, of which forty-nine were added after analyses of the reference lists of the included papers (Supporting information SI. 4).

Fig. 1. Flow diagram depicting literature search strategy.

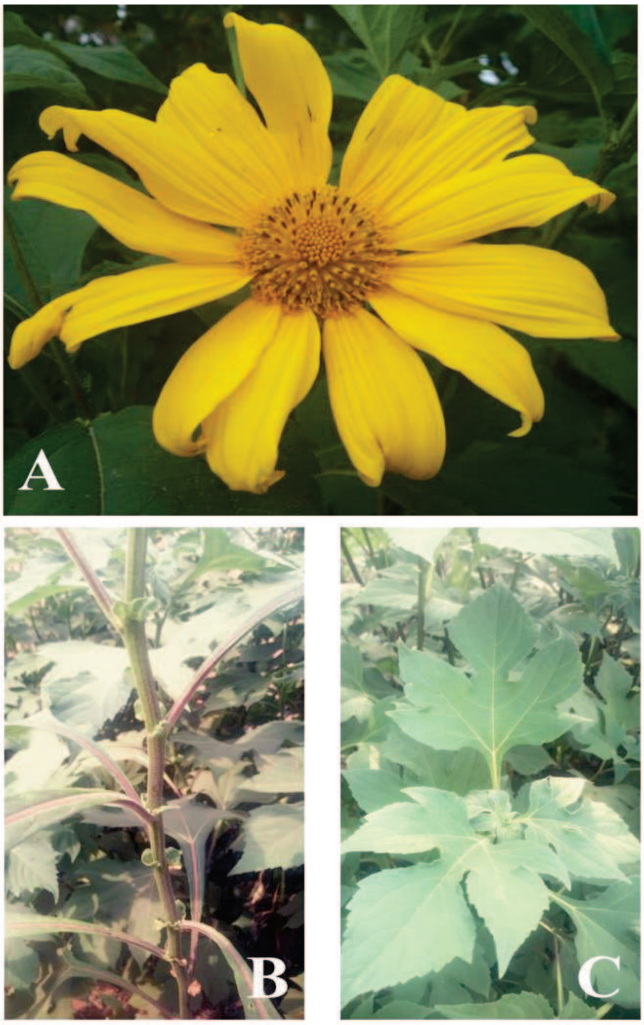


3. Botanical characterization

Initially, La Duke (1982) has enumerated only thirteen taxa divided into two taxonomic sections: Tithonia and Mirasolia (Duke, 1982). Nowadays, ten newfound species have been added, bringing the total to twenty-three accepted taxa among which *Tithonia diversifolia* is by far the most popular species of this genus (theplantlist.org, 2017).

TD can be vividly depicted as a flowering shrub-like species, growing up to over 2-3 m (6.6-9.8 ft.) high. The flowers are 5-15 cm wide and shaped like a daisy (Figure 1.A). The head (capitulum) is 10-30 cm long and bears small individual yellow flowers (florets) crowded together and subtended by green bracts. In the outermost portion of the capitulum, 7-15 petal-like florets (ray florets) frame 80-120 tiny florets (disc florets). Flowering occurred in October, and each mature stem can bear several flowers (Muoghalu and Chuba, 2005). The stem is striated and usually leafless on its lower parts (Figure 1.B). When young, stem surface is hairy and green in colour, but it turns woody with a hollow core as TD matures. TD leaves (15-30 cm long) can be described as sub-ovate, petiolate, 3- to 7-lobed (often unlobed in new shoots and upper leaves), delicately hairy, and alternately to oppositely arranged (Figure 1.C). The blade is green coloured, palmately veined, cuneate at base, acute to acuminate at top, and crenate to serrate at the edge. The light green petiole of 2-10 cm long, fringes with the blade at base. Seeds are achenes of 4-8 mm long, greyish coloured, somewhat 4-angled, and enclosed in tight appressed pubescent hairs. Typical mature TD produces 80,000 to 160,000 seeds/m2 annually, whose 70% can fully germinate (Yang et al., 2012).

Fig. 2. Parts of *Tithonia diversifolia (Hemsl.) A. Gray*; (A) flower; **(B)** stem; **(C)** leaf. Pictures have been taken by AMT in the Campus of the University of Dschang (Cameroon) in February 2017

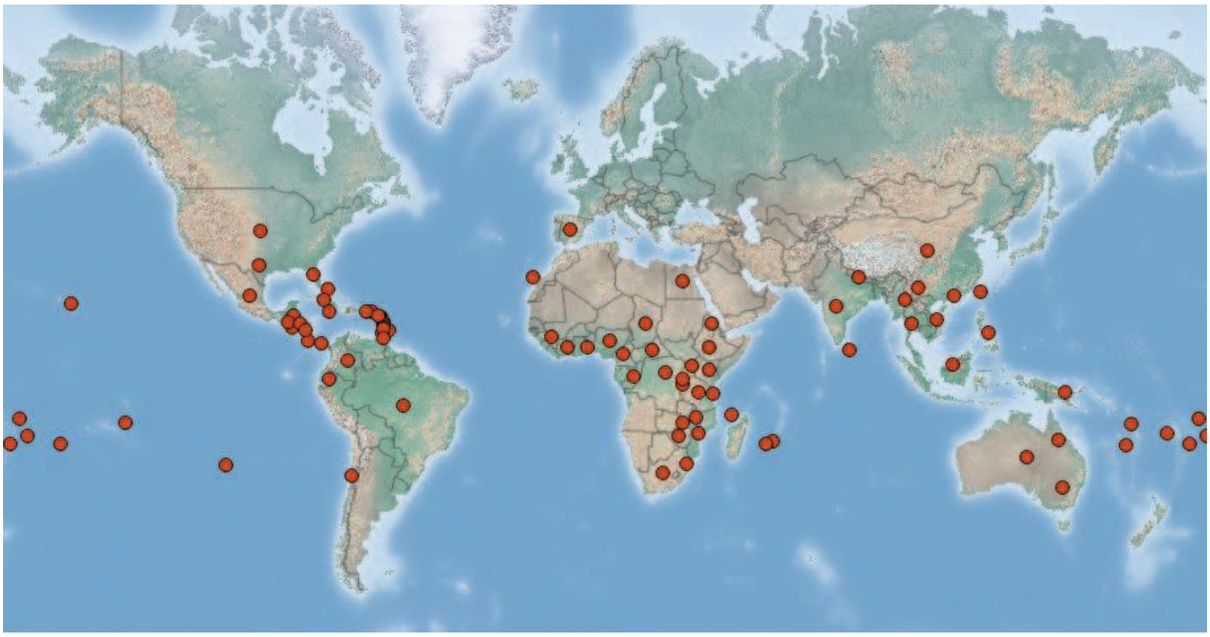


4. Distribution and habitat

Tithonia diversifolia is currently distributed in up to 64 countries around the world (Figure 3), but its precise origin remains controversial. Reportedly, the plant is native to Mexico and then spread to other parts of America including British Columbia, USA, Belize, Costa Rica, Guatemala, Honduras, Jamaica, Nicaragua, and Panama. Introduced as ornamental and/or green manure in Africa, Asia, and Oceania (Anon, 2017), TD has since become naturalized in tropical and subtropical areas where it grows wild as a weed in roadsides, wastelands, crop fields, and homesteads (Jex-Blake, 1957; Blake, 1921).

In introduced areas, TD can quickly form dense stands that aggressively invade well-established cultivated lands. In addition, it can release allelochemicals which deeply affect the nutrient uptake and growth of young native plants (Oyerinde et al., 2009). Future directions as regards to the introduction and subsequent establishment of TD generate massive debate and controversy. In many countries, this species is considered a great threat to agropastoral activities. However, it may be of particular interest for the sustainable development of new drugs or other derived products, since adequate amounts of raw material are always available.

Fig. 3. Distribution map of *Tithonia diversifolia* (Reproduced from www.cabi.org/isc. under a Creative Commons Attribution Licence on 12th January 2017).



5. Ethnobotany

5.1. Ethnomedicinal use

TD is among the most frequently mentioned medicinal plant species in its native region of lowland Mesoamerica (Heinrich et al., 1998; Geck et al., 2016). Leonti et al. (2003) have established that this species has been of cultural importance for millennia in this region. This claim has been further substantiated in a recent PhD thesis (Geck, 2017). Likewise, the fact that the leaves of TD are used in Ayurveda practice reflects the historical/cultural depth of this species (Instituteofayurveda.org, 2017). Such a cultural component could provide the rationale for the ethnomedicinal uses of TD and it includes the plant's organoleptic properties as well as criteria related to humoral medicine (Geck et al. 2017). Specifically, this plant species is considered of outstanding medicinal value by local healers because of its intensely bitter flavour and the correlated hot humoral quality. In addition, TD is known as árnica in its native range of Mexico, with significant implications to the rationale behind its medicinal uses, as it may be considered by local people as being related to the European Arnica montana or the "Mexican arnica" Heterotheca inuloides, which are both officially recognized in the herbal pharmacopoeia of Mexico (Heinrich et al., 1998; FEUM, 2013; Heinrich and Booker, 2015).

T. diversifolia is collected all year-round and used fresh or dry as traditional remedies in several cultures and sometimes for similar purposes (Table 1). The commonly used plant parts include the leaves, but also flowers, stems and roots are mentioned in some ethnobotanical reports (Játem-Lásser et al., 1998; Radol et al., 2016). Most frequently, infusions (Kamdem et al., 1986), decoctions (do Céu de Madureira et al., 2002), and poultice of TD, alone or in multi-ingredient preparation (Stangeland et al., 2011), are used as needed and without any specific dosage to treat and prevent a large number of ailments and diseases in humans (Leonti et al., 2003) and animals (Okombe Embeya et al., 2014). For instance, TD is administered orally for treating diabetes, malaria, fever, pains, diarrhoea, hepatitis, infectious diseases and other conditions (Baerts and Lehmann, 1989; Rwangabo, 1993; Masakiyo Takahashi, 1995; do Céu de Madureira et al., 2002). Oral administration implies that the plant part used is either pounded or macerated/infused and drunk (Maregesi et al., 2007). Besides, this plant species is applied topically as poultice or bath to wounds, bruises, skeleto-muscular disorders, abscesses, dermatological conditions, and stomach pains (Del Amo Rodriguez, 1979; Weimann and Heinrich, 1997; Heinrich et al., 1998; Leonti, 2002).

Table 1: Ethnomedicinal uses of *Tithonia diversifolia* in different ethnic groups.

ethnic groups (province/country)	vernacular/local names	part used	dosage form	ethnomedicinal use/disease treated	references
Sao Tomé and Principé Islands	Girassol	aerial parts	Decoction	malaria and fever	(do Céu de Madureira et al., 2002)
Taiwan	Nitobegiku	leaves	aqueous extract, infusion	diabetes, improve liver function	(Takahashi, 1998)
Japan	Nitobe chrysanthemum	unstated	decoction or infusion	anti-poison	
Rwanda	Ikicamahirwe	unstated	Unstated	ascariasis and diarrhoea	(Baerts and Lehmann, 1989; Rwangabo, 1993)
Bunda (Tanzania)	Maua	leaves	pounded leaves were either soaked in water or macerated	skin infections, stomach pains	(Maregesi et al., 2007)

Buhozi; Katanga (Democratic Republic of Congo)	Cilula; Kilulu nkundja	leaves	crush or maceration	intestinal worms in goats, cholera, boost immune functions; anthelminthic for livestock	(Karhagomba et al., 2013; Okombe Embeya et al., 2014)
Venezuelan Andes (Venezuela)	Arnica	leaves and stems	juice	Abscesses	(Játem-Lásser et al., 1998)
Karen; Lahu (Thailand)	Por kae ro; Sub-ve^ hk'a~	leaf	decoction or powder	itching, ringworm, muscular pain, urethral stones, stomach pains and indigestion	(Anderson, 1986; Tangjitman et al., 2013)
Southern China	Zhong Bing Ju	unstated	Unstated	diuretic, athlete's foot, night sweats, hepatitis, jaundice and cystitis	(Wanzala et al., 2016)
Nyakayojo; Mpigi (Uganda)	Ngaro itano; Ngar'itaano Komanyoko; Ekimyula	leaves and flowers	dried and mixed with five other plants	malaria, HIV/AIDS, fungal and bacterial infections, deworming, and boosting of immunity and energy	(Nyamukuru et al., 2017; Kamatenesi-Mugisha et al., 2008; Kamatenesi Mugisha et al., 2014; Stangeland et al., 2011)

Samaya; Cobán (Guatemala)	Unstated	leaves	decoctions	wounds, fungal diseases, malaria, dermatological diseases in domestic animals	(Tejeda Marroquin, 2003)
Rodrigues (Republic of Mauritius)	Unstated	unstated	Unstated	eczema	(Gurib-Fakim et al., 1996)
Cameroon	Fleur jalousie; Satan's flower; Ondondon Si	leaves	maceration	measles, HIV, malaria	(Bouberte et al., 2006a; Kamdem et al., 1986)
Yoruba; Omu-Aran; Ogbomoso (Nigeria)	Sepeleba	leaves, flowers	mixed with other plants; flowers and leaves are squeezed	dysmenorrhea, febrile illness, malaria	(Ajaiyeoba et al., 2006; Olorunnisola et al., 2013; Owoyele et al., 2004)
Chungtia (India)	Zoninaro	fresh leaves	infusion, paste	hypertension, malaria, abscesses and body pain	(Kichu et al., 2015)

Sri Lanka	Padimella/Wal suriyakantha	leaves		constipation, sore throat and reducing fever	(Instituteofayurveda.org, 2017)
Kikuyu; Luo; Luhya; Mukhwa; Mbeere and Embu (Kenya)	Marûrû; Maua Madongo; Maua makech; Masambu malulu; Libinzo; Kirurite	leaves and/or roots	decoction; water is added to pounded leaves	ear, nose and throat diseases, Herpes zoster, appetite stimulant, anti-ectoparasites for cattle, snake antivenin, malaria, stomach pains, gastrointestinal complaints and typhoid fever	(Johns et al., 1995; Mukungu et al., 2016; Njoroge and Bussmann, 2006; Owuor and Kisangau, 2006; Radol et al., 2016)
Zapotec; Zoque; Nahua; Mixe Highland Maya of Chiapas; Lowland Mixe; Popoluca**;** Yucatan; Veracruz (Mexico)	Rula'a: Tam chich, tapungäsy jäyä, tapkuy, tatkuy, tan tzitzi, tabkuy, tam tzitzi	leaves or shoots	powder, creams, liniment, poultices, shower bath, teas, tinctures, syrup, scabies, wounds	folk illness, wounds, bruises, skin infections, musculoskeletal disorders, malaria and other forms of fever, hematoma, gastrointestinal disorders, dermatological conditions, muscular cramps, skin eruptions, asthma, bronchitis, chills, nicotine addiction, diabetes, stomach pain, skin sores,	(Berlin et al., 1996; Del Amo Rodriguez, 1979; Morton, 1981; Frei et al., 1998; Heinrich et al., 1998; Marco Leonti et al., 2001; Marco Leonti et al., 2002; Méndez González et al., 2014;

	pimples, haemorrhoids, lymphadenitis, rheumatism, cough, smallpox, pains and inflammatory conditions	Geck et al., 2016; Geck et al., 2017)

5.2. Nonmedical uses

Leaves and flowers of TD showed a high nutritive-quality index (Osuga et al., 2012), suggesting that they can be used as fodder for chicken, fish, goats and cows (Pathoummalangsy and Preston, 2008). Indeed, TD supplementation of cattle provided several beneficial effects including the weight gain (Wambui et al., 2006; Premartane et al., 1998) and increase in milk yield (Katongole et al., 2016). Additionally, its flowers attract bees which in turn yield honey (Ingram, 2011). Interestingly, the tea from the fresh leaves or ash were applied to crops to provide protection against insects including termites (Adoyo et al., 1997). This protective action of TD against insects occurred through its feeding deterrent and insecticidal effects (Dutta et al., 1993; Flerida A. Carino and Morallo-Rejesus, 1982). In many countries such as Uganda and Kenya, this plant species is employed by farmers as a biopesticide to replace hazardous and expensive synthetic pesticides (Mwine et al., 2011). Moreover, the leafy dry matter of TD is spread over the soil or buried underground to improve the soil fertility, enhance the availability of minerals/nutrients, and increase the crop yields (Jama et al., 2000; van Sao et al., 2010; Kaho et al., 2011). However, as green manure, TD requires a high workforce, so its use should be preferred in high-value crops such as tomato, kale, carrot, and maize (Jama et al., 2000). More interestingly, TD can form dense stands that reduce rain impact on the soil, thus limiting erosion (Ng'inja et al., 1998). On the other hand, dried stumps are burnt as firewood and fuelwood.

6. Phytochemistry

Olayinka et al. (2015) reported the presence of alkaloids, tannins, flavonoids, saponins, terpenoids and phenols in the leaves, roots and stems of TD. Likewise, glycosides were detected in appreciable proportions in aqueous and methanolic extracts of shoots of TD (Otusanya and Ilori, 2012). However, it is worth mentioning that the synthesis of secondary metabolites in various parts of TD is influenced by temperature, rainfall, humidity, solar radiation and soil nutrient uptake (Sampaio et al., 2016). This might make it difficult to identify the bioactive components of this species.

To date, more than a hundred secondary metabolites have been isolated from various TD extracts (Table 3), and their structures have been assigned based on their IR, NMR and mass spectra (Baruah et al., 1979). The sesquiterpenoids, diterpenoids and flavonoids are considered the most prominent family of components occurring in TD (Chagas-Paula et al., 2012). The major sesquiterpenoids that have been isolated are sesquiterpene lactones (STLs) and they include germacranolides, eudesmanolides and guaianolides. Hitherto, the most studied germacranolides isolated from TD were tagitinins. Nine classes of tagitinins have been isolated so far and they differ from each other according to oxygenation and unsaturation patterns.

Described as bitter-tasting compounds, tagitinins A (1), C (2) and F (3) were first isolated along with tirotundin (4) and hispidulin (5), from the aerial parts of the plant (Baruah et al., 1979). Interestingly, (3) could be afforded from the irradiation of (2) by Hg lamp (Chowdhury et al., 1983). Next, compounds (1), (2), (3) as well as tagitinin C methylbutyrate (6) and tirotundin 3-O-methyl ether (7) were isolated from the glandular trichomes on the abaxial surface of the leaves and inflorescences (Ambrósio et al., 2008). Importantly, (2) was recorded as the major STLs, and its highest content was observed in September–November. Tagitinin D (4) was previously isolated from *Tithonia tagitiflora* (Pal et al., 1976) and thereafter, its name was substituted by tirotundin (4) as both were structurally identical (Baruah et al., 1979). Recently, tagitinins G (8), H (9) and I (10) have been isolated from the aerial parts of TD together with 1β-hydroxydiversifolin-3-O-methyl ether (11), and tagitinin F 3-O-methyl ether (12) (G. Zhao et al., 2012). Other germacranolide STLs isolated from the aerial parts include 2α-hydroxytirotundin (13), 1β,2α-epoxytagitinin C (14), 1α-hydroxytirotundin 3-O-methyl ether (15), 1β-methoxydiversifolin (16), 1β-methoxydiversifolin 3-O-methyl ether (17), 1α-hydroxydiversifolin 3-O-methyl ether (18) and 2-O-methyl derivative of tagitinin B (19) (Gu et al., 2002; Kuroda et al., 2007; Sergio Pereira et al., 1997). Moreover, the leaves provided 1-acetyltagitinin A (20), acetyltagitinin E (21) (Wu et al., 2001), as well as four furanoheliangolides including 1,3-dihydroxy-3,10-epoxy-8(-2-methylpropanoyloxy)-germacra-11(13)-ene-6,12-olide (22), 1,3-dihydroxy-3,10-epoxy-8-(2-methylpropanoyloxy)-germacra-4,11(13)-diene-6,12-olide (23), 1,3-dimethoxy-3,10-epoxy-8-(2-methylpropanoyloxy)-germacra-4,11(13)-diene-6,12-olide (24) and 1-hydroxy-3-methoxy-3,10-epoxy-8-(2-methylpropanoyloxy)-germacra-4,11(13)-diene-6,12-olide (25) (Herrera et al., 2007). The ethyl acetate extract of the leaves was source of 8β-O-(2-methylbutyroyl)-tirotundin (26), 8β-O-(isovaleroyl)tirotundin (27), 3β-acetoxytithifolin (28), 3α-acetoxycostunolide (29), 3-methoxytirotundin (30), 2-formyl-4-hydroxy-4α-methyl-3-(3-oxobutyl)cyclohexaneacetic acid (31), and (2E,6E10E)-3-(hydroxymethyl)-7,11,15-trimethylhexadeca-2,6,10,15-tetraene-1,14-diol (32) (Miranda et al., 2015).

Eudesmanolide STLs such as 3α-(acetoxy)diversifolol (33), methyl 3α-acetoxy-4α-hydroxy-11(13)-eudesmen-12-oate (34) and diversifolol (35) were isolated from the roots (Kuo and Chen, 1998), while the aerial parts afforded tithofolinolide (36) and its derivative 3β-acetoxy-8β-isobutyryloxyreynosin (37) (Gu et al., 2002). Guaianolides including 8β-(Isobutyroyloxy)-4-oxo-3,4-secoguai-11(13)-ene-12,6α;3,10α-diolide (38), 4α,10α-dihydroxy-3-oxo-8β-isobutyryloxyguai-11(13)-en-6α,12-olide (39) and 3-hydroxy-8β-(isobutyroyloxy)leucodin-11(13)-ene (40) were found in the glandular trichomes (Ambrósio et al., 2008), whereas 8β-isobutyryloxycumambranolide (41) was provided by the leaves (Kuo and Chen, 1998).

Other sesquiterpenoid compounds including the dinorxanthane sesquiterpene 4,15-dinor-3-hydroxy-1(5)-xanthen-12,8-olide (42) ¬-also known as diversifolide, the chromene 2-deacetyl-11β,13-dihydroxyxanthinin (43) along with 6-acetyl-7-hydroxy-2,3-dimethylchromone (44), 6-acetyl-2,2-dimethylchromene (45), 6-acetyl-7-hydroxy-2,2-dimethylchromene (46) and 6-acetyl-7-methoxy-2,2-dimethylchromene (47), were isolated from the roots (Kuo and Lin, 1999). The aerial parts provided 6-acetyl-2,2-dimethylchromene-8-O-β-D-glucoside (48) and 6-acetyl-8-hydroxy-2,2-dimethylchromene (49) (Zhai et al., 2010). Demethylacetovanillochromene (50) was isolated from the stem (Shamsuddin et al., 2001). Ethyl acetate extract of the leaves also afforded (31) and (32) (Miranda et al., 2015), while two cerebrosides including (2R)-N-{(1S,2S,3R,8E)-1-[(β-D-glucopyranosyloxy)methyl]-2,3-dihydroxyheptadec- 8-en-1-yl}-2-hydroxyhexadecanamide (51) and (2R)-N-{(1S,2R,8E)-1-[(β-D-glucopyranosyloxy)-methyl]-2-hydroxyheptadec-8-en-1-yl}2-hydroxyhexadecanamide (52) have been isolated from the aerial parts (G. J. Zhao et al., 2012b).

Flavonoids were found in the leaf trichomes and they include (5), luteolin (53), and nepetin (54) (Ambrósio et al., 2008). Four phenolic compounds such as (E)-3-(((3-(3,4-dihydroxyphenyl)acryloyl)oxy)methyl)-2-methyloxyrane-2-carboxylic acid (55), (1S,3S,4S)-dicaffeoylquinic acid (56), (1S,3R,4S)-dicaffeoylquinic acid (57) and (1R,3S,5S)-dicaffeoylquinic acid (58) have been isolated from the leaves (Pantoja Pulido et al., 2017) along with the anthraquinone tithoniaquinone A (59), the ceramide tithoniamide B (60), psoralen (61) and l-quebrachitol (62) (Bouberte et al., 2006b). Chlorogenic acids (63) were found in the polar fraction of TD leaf extracts (Chagas-Paula et al., 2011). Flowers afforded the isocoumarin tithoniamarin (64) and β-sitosterol glucopyranoside (65) (Bouberte et al., 2006a). The aerial parts provided 6"-O-β-D-apiofuranosyl-trichocarpin (66) and 1-heptade-4,6-diyne-3,10,16,17-tetraol-3-O-β-D-glucopyranoside (67), which can serve as chemotaxonomic fingerprints of TD (G. J. Zhao et al., 2012a). Esters related to artemisinic acid (68) (Bordoloi et al., 1996), along with phytosterols such as esters of faradiol (69), stigmasterol (70), β-sitosterol (71) and squalene (72) (Ragasa et al., 2008) were afforded by mature stem and flowers. The pentacyclic diterpene, namely ent-kaur-16-en-19-oic acid (73) was found in the glandular trichomes of the leaves (Ambrósio et al., 2008).

Essential oil (EO) can be afforded from flowers, leaves, stems and roots of Tithonia diversifolia (Agboola et al., 2016) and the yield is relatively low (0.019-0.1% w/w) compared to other Asteraceae plant family members. EO is pale yellow-coloured, and it is composed mainly of monoterpenes (44.44%) and sesquiterpenes (26.67%) (Agboola et al., 2016). Volatiles represented 96.7%, 93.7%, 88.9% and 93.7% of content of EO afforded from flowers, leaves, stems and roots, respectively. Leaf and flower EO are so far, the most studied EO. Leaf essential oil has a characteristic woody odour and its main volatiles include α-pinene (74) (32.9%), β-caryophyllene (75) (20.8%), germacrene D (76) (12.6%), β-pinene (77) (10.9%) and 1,8-cineole (78) (9.1%) (Moronkola et al., 2007). In addition, isocaryophyllene (79), nerolidol (80), 1-tridecanol (81), sabinene (82), α-copaene (83), α-gurjunene (84) and cyclodecene (85) were found in appreciable amounts in fresh leaves EO (Wanzala et al., 2016). On the other hand, compounds (76) (20.3%), (75) (20.1%) and bicyclogermacrene (86) (8.0%) were representative of flower EO, with its strong characteristic sweet smell. Importantly, (74) (60.9–75.7%), δ-pinene (87) (7.2–11.0%) and limonene (88) (0.9–4.3%) were common to all essential oils, with (74) being considered the most abundant volatile (Lawal et al., 2012)

7. Pharmacology

Studies conducted on cell lines, microorganisms, and model animals of human diseases, showed a broad spectrum of bioactivities for different parts and varying extracts of TD. The anti-inflammatory, antimalarial, antidiabetic, antioxidant and anticancer effects do stand out but there is also a stunning array of other relevant biological effects (Table 3). At the molecular level, the pharmacological effects of TD may be ascribed mainly to STLs, saponins, chlorogenic acids and flavonoids whose structures are illustrated in figure 4. Below are critically summarized the main findings of the pharmacological activities of TD retrieved from the literature.

Table 2. Compounds isolated from *T. diversifolia*, their sources and corresponding references. The numbers assigned to the compounds do not correspond to those used in Figure 4.

Nº	Nomenclature	Source	References
1	tagitinin A	aerial parts; glandular trichomes of the leaves and inflorescences	(Ambrósio et al., 2008; Glaser et al., 2005; Baruah et al., 1979)
2	tagitinin C	aerial parts glandular trichomes on the abaxial surface of the leaves and inflorescences	(Ambrósio et al., 2008; Baruah et al., 1979)
3	tagitinin F	glandular trichomes on the abaxial surface of the leaves and inflorescences; aerial parts	(Baruah et al., 1979; Sergio Pereira et al., 1997; Gu et al., 2002; Kuroda et al., 2007; Ambrósio et al., 2008)
4	tirotundin = tagitinin D	aerial parts	(Baruah et al., 1979)
5	hispidulin	aerial parts; leaf trichomes	(Baruah et al., 1979)
6	tagitinin C methylbutyrate	glandular trichomes on the abaxial surface of the leaves and inflorescences	(Ambrósio et al., 2008)

7	tirotundin 3-O-methyl ether	glandular trichomes of the leaves and inflorescences	(Ambrósio et al., 2008)
8	tagitinin G	aerial parts	(Zhao et al., 2012)
9	tagitinin H	aerial parts	(Zhao et al., 2012)
10	tagitinin I	aerial parts	(Zhao et al., 2012)
11	1β-hydroxydiversifolin-3-O-methyl ether	aerial parts	(Zhao et al., 2012)
12	tagitinin F 3-O-methyl ether	aerial parts	(Zhao et al., 2012)
13	2α-hydroxytirotundin	aerial parts	(Gu et al., 2002)
14	1β,2α-epoxytagitinin C	aerial parts	(Gu et al., 2002)
15	1α-hydroxytirotundin 3-O-methyl ether	aerial parts	(Gu et al., 2002; Kuroda et al., 2007; Sergio Pereira et al., 1997)
16	1β-methoxydiversifolin	aerial parts	(Gu et al., 2002; Kuroda et al., 2007; Sergio Pereira et al., 1997)
17	1β-methoxydiversifolin 3-O-methyl ether	aerial parts	(Gu et al., 2002; Kuroda et al., 2007; Sergio Pereira et al., 1997)

18	1α-hydroxydiversifolin 3-O-methyl ether	aerial parts	(Gu et al., 2002; Kuroda et al., 2007; Sergio Pereira et al., 1997)
19	2-O-methyl derivative of tagitinin B	aerial parts	(Gu et al., 2002; Kuroda et al., 2007; Sergio Pereira et al., 1997)
20	1-acetyltagitinin A	Leaves	(Kuo and Chen, 1998)
21	acetyltagitinin E	Leaves	(Wu et al., 2001)
22	1,3-dihydroxy-3,10-epoxy-8(-2-methylpropanoyloxy)-germacra-11(13)-ene-6,12-olide	leaves	(Herrera et al., 2007)
23	1,3-dihydroxy-3,10-epoxy-8-(2-methylpropanoyloxy)-germacra-4,11(13)-diene-6,12-olide	leaves	(Herrera et al., 2007)
24	1,3-dimethoxy-3,10-epoxy-8-(2-methylpropanoyloxy)-germacra-4,11(13)-diene-6,12-olide	leaves	(Herrera et al., 2007)

25	1-hydroxy-3-methoxy-3,10-epoxy-8-(2-methylpropanoyloxy)-germacra-4,11(13)-diene-6,12-olide	leaves	(Herrera et al., 2007)
26	8β-O-(2-methylbutyroyl)-tirotundin	Leaves	(Miranda et al., 2015)
27	8β-O-(isovaleroyl)tirotundin	Leaves	(Miranda et al., 2015)
28	3β-acetoxytithifolin	Leaves	(Miranda et al., 2015)
29	3α-acetoxycostunolide	Leaves	(Miranda et al., 2015)
30	3-methoxytirotundin	Leaves	(Miranda et al., 2015)
31	2-formyl-4-hydroxy-4α-methyl-3-(3-oxobutyl)cyclohexaneacetic acid	Leaves	(Miranda et al., 2015)
32	(2E,6E10E)-3-(hydroxymethyl)-7,11,15-trimethylhexadeca-2,6,10,15-tetraene-1,14-diol	Leaves	(Miranda et al., 2015)
33	3α-(acetoxy)diversifolol	roots	(Kuo and Chen, 1998)
34	methyl 3α-acetoxy-4α-hydroxy-11(13)-eudesmen-12-oate	roots	(Kuo and Chen, 1998)
35	diversifolol	roots	(Kuo and Chen, 1998)

36	tithofolinolide	aerial parts	(Gu et al., 2002)
37	3β-acetoxy-8β-isobutyryloxyreynosin	aerial parts	(Gu et al., 2002)
38	8β-(Isobutyroyloxy)-4-oxo-3,4-secoguai-11(13)-ene-12,6α;3,10α-diolide	glandular trichomes of leaf and inflorescence	(Ambrósio et al., 2008)
39	4β,10α-dihydroxy-3-oxo-8β-isobutyryloxyguai-11(13)-en-6α,12-olide	glandular trichomes of leaf and inflorescence	(Ambrósio et al., 2008)
40	3-Hydroxy-8β-(isobutyroyloxy)leucodin-11(13)-ene	the glandular trichomes of the leaves and inflorescences	(Ambrósio et al., 2008)
41	8β-isobutyryloxycumambranolide	Leaves	(Kuo and Chen, 1998)
42	diversifolide = 4,15-dinor-3-hydroxy-1(5)-xanthen-12,8-olide	Roots	(Kuo and Lin, 1999)
43	2-deacetyl-11β,13-dihydroxyxanthinin	Roots	(Kuo and Lin, 1999)
44	6-acetyl-7-hydroxy-2,3-dimethylchromone	Roots	(Kuo and Lin, 1999)
45	6-acetyl-2,2-dimethylchromene	Roots	(Kuo and Lin, 1999)
46	6-acetyl-7-hydroxy-2,2-dimethylchromene	roots	(Kuo and Lin, 1999)
47	6-acetyl-7-methoxy-2,2-dimethylchromene	roots	(Kuo and Lin, 1999)

48	6-acetyl-2,2-dimethylchromene-8-O-β-D-glucoside	aerial parts	(Zhai et al., 2010)
49	6-acetyl-8-hydroxy-2,2-dimethylchromene	aerial parts	(Zhai et al., 2010)
50	Demethylacetovanillochromene	Stem	(Shamsuddin et al., 2001)
51	(2R)-N-{(1S,2S,3R,8E)-1-[(β-D-glucopyranosyloxy)methyl]-2,3-dihydroxyheptadec- 8-en-1-yl}-2-hydroxyhexadecanamide	aerial parts	(Zhao et al., 2012)
52	(2R)-N-{(1S,2R,8E)-1-[(β-D-glucopyranosyloxy)-methyl]-2-hydroxyheptadec-8-en-1-yl}2-hydroxyhexadecanamide	aerial parts	(Zhao et al., 2012)
53	luteolin	leaf trichomes	(Ambrósio et al., 2008)
54	nepetin	leaf trichomes	(Ambrósio et al., 2008)
55	(E)-3-(((3-(3,4-dihydroxyphenyl)acryloyl)oxy)methyl)-2-methyloxyrane-2-carboxylic acid	leaves	(Pantoja Pulido et al., 2017)
56	(1S,3S,4S)-dicaffeoylquinic acid	leaves	(Pantoja Pulido et al., 2017)

57	(1S,3R,4S)-dicaffeoylquinic acid	Leaves	(Pantoja Pulido et al., 2017)
58	(1R,3S,5S)-dicaffeoylquinic acid	leaves, aerial parts	(Pantoja Pulido et al., 2017)
59	tithoniaquinone A	leaves	(Bouberte et al., 2006b)
60	tithoniamide B	leaves	(Bouberte et al., 2006b)
61	psoralen	leaves	(Bouberte et al., 2006b)
62	*l*-quebrachitol	Leaves	(Bouberte et al., 2006b)
63	chlorogenic acid	leaves	(Chagas-Paula et al., 2011)
64	tithoniamarin	Flowers	(Bouberte et al., 2006a)
65	β-sitosterol glucopyranoside	Flowers	(Bouberte et al., 2006a)
66	6"-O-β-D-apiofuranosyl-trichocarpin	aerial parts	(Zhao et al., 2012)
67	1-heptade-4,6-diyne-3,10,16,17-tetraol-3-O-β-D-glucopyranoside	aerial parts	(Zhao et al., 2012)
68	artemisinic analogues	mature stem and flowers	(Bordoloi et al., 1996)
69	esters of faradiol	mature stem and flowers	(Ragasa et al., 2008)
70	Stigmasterol	mature stem and flowers	(Ragasa et al., 2008)
71	β-sitosterol	mature stem and flowers	(Ragasa et al., 2008)

72	squalene	mature stem and flowers	(Ragasa et al., 2008)
73	ent-kaur-16-en-19-oic acid	glandular trichomes of leaf and flowers	(Ambrósio et al., 2008)
74	α-pinene	essential oil	(Moronkola et al., 2007)
75	β-caryophyllene	essential oil	(Moronkola et al., 2007)
76	germacrene D	flower essential oil	(Moronkola et al., 2007)
77	β-pinene	essential oil	(Moronkola et al., 2007)
78	1,8-cineole	leaf essential oil	(Moronkola et al., 2007)
79	Isocaryophyllene	essential oil	(Wanzala et al., 2016)
80	Nerolidol	essential oil	(Wanzala et al., 2016)
81	1-tridecanol	aerial parts	(Wanzala et al., 2016)
82	sabinene	essential oil	(Wanzala et al., 2016)
83	α-copaene	essential oil	(Wanzala et al., 2016)
84	α-gurjunene	essential oil	(Wanzala et al., 2016)
85	cyclodecene	essential oil	(Wanzala et al., 2016)
86	bicyclogermacrene	flower oil	(Moronkola et al., 2007)
87	δ-pinene	essential oil	(Moronkola et al., 2007)

88	Limonene	essential oil	(Moronkola et al., 2007)
89	2-hydroxy-5-acetylbenzoic acid	aerial parts	(Zhao et al., 2012)
90	2-mercaptobenzothiazole	aerial parts	(Zhao et al., 2012)
91	3-(4-hydroxyphenyl)-3-oxopropyl-*β*-d-glucopyranoside	aerial parts	(Zhao et al., 2012)
92	3-indolecarboxylic acid	aerial parts	(Zhao et al., 2012)
93	Arbutin	aerial parts	(Zhao et al., 2012)
94	harman-3-carboxylic acid	aerial parts	(Zhao et al., 2012)
95	ilicic acid	aerial parts	(Gu et al., 2002)
96	phloroglucinol trimethyl ether	aerial parts	(Zhao et al., 2012)
97	Pinoresinol	aerial parts	(Zhao et al., 2012)
98	protocatechuic acid	aerial parts	(Zhao et al., 2012)
99	tagitinin B	aerial parts	(Baruah et al., 1979)
100	tagitinin E	aerial parts	(Baruah et al., 1979)
101	Uracil	aerial parts	(Zhao et al., 2012)
102	Vanilloloside	aerial parts	(Zhao et al., 2012)

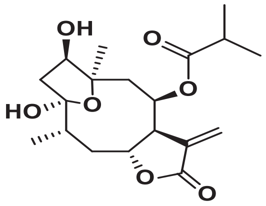
103	methyl 3,5-dicaffeoyl quinate	aerial parts	(Zhao et al., 2012)
104	(−)-isolariciresinol-3*α*-O-*β*-d-glucopyranoside	aerial parts	(Zhao et al., 2012)
105	Diversifolin	aerial parts	(Rüngeler et al., 1998)
106	diversifolin 3-O-methyl ether	aerial parts	(Rüngeler et al., 1998)
107	1β-hydroxytirotundin-3-O-methyl ether	aerial parts	(G. Zhao et al., 2012)
108	4α,10α-dihydroxy-3-oxo-8β-(isobutyryloxy)guaia-11(13)-en-6α,12-olide	aerial parts	Gu et al., 2002

7.1. Anti-inflammatory activity

TD has demonstrated interesting anti-inflammatory effects as evidenced by the following in vitro and in vivo studies. In in vitro, the leaf extract of TD caused inhibition of lymphocyte proliferation (Lasure et al., 1995). This inhibitory effect occurred dose-dependently at concentrations ranging from 0.66 to 25.00 µg/mL (IC_{50} = 4.42 µg/mL) (Hiransai et al., 2016). Moreover, at concentrations of 0.94-30 µg/mL, the aqueous leaf extract significantly reduced (IC_{50} = 11.63 µg/mL) NO generation by LPS-activated RAW264.7 cells in a concentration-dependent way. In rats, the methanolic leaf extract was found to prevent oedema and granuloma in a dose-dependent manner (Owoyele et al., 2004). Interestingly, at the dose of 100 mg/kg, the anti-inflammatory effect of the methanolic extract was higher than that produced by indomethacin (5 mg/kg) used as a positive control. On the other hand, the carrageenan-induced oedema was significantly inhibited in mice at doses of 150 and 300 mg/kg (Sijuade et al., 2016).

To gain insight into the contribution of various TD constituents to the observed anti-inflammatory activity, three chemically different leaf extracts were investigated, including a leaf rinse extract (LRE), a polar extract (PE), and an infusion. LRE, PE and infusion were described as main source of STLs, chlorogenic acids (CAs) and flavonoids, respectively (Chagas-Paula et al., 2011). At oral doses of 10-150 mg/kg, LRE and PE produced an evident and dose-dependent antiedema effect in mice. Surprinsingly, the infusion was inactive although it is known to be chemically close to PE. However, this contrasts with a previous study in which the carrageenan-induced oedema was inhibited in rats pre-treated with 10 mL/kg of aqueous extract (Lin et al., 1993). Perhaps, this loss of activity of the infusion is owed to variation in the composition of various extracts of TD. Importantly, at doses of 10 and 50 mg/kg, PE exhibited a better and faster anti-inflammatory effect than LRE and indomethacin (10 mg/kg). This suggests that CAs from PE may represent a promising candidate for future anti-inflammatory drugs. However, pure CAs provided a modest anti-inflammatory activity in prior research (dos Santos et al., 2006; Huang et al., 1991) suggesting that CAs present in PE may act in synergy with other constituents of the extract for a better anti-inflammatory effect. Chagas-Paula et al. (2011) have also found that when applied topically (0.05 and 0.5 mg/ear) to the inflamed ears of the mice, all these three extracts produced significant inhibition of neutrophil recruitment. Importantly, such an effect was achieved only at low doses. Taken together, these findings indicate that the anti-inflammatory activity of TD might be ascribed mainly to three different classes of secondary metabolites including STLs, CAs and flavonoids. Among STLs, tagitinins **1**, **2** and **3** isolated from the leaves, were found to alter the neutrophil functions (Abe et al., 2015). Indeed, at the dose of 100 μM, all the investigated tagitinins significantly inhibited IL-6, IL-8 and TNF-α production by human neutrophils. Interestingly, **1** induced TNF-α secretion in the absence of inflammatory stimuli, indicating that it may be endowed with immunomodulatory effect. In addition, all three tagitinins decreased the survival rate of the activated neutrophils. However, **1** was the only investigated tagitinin that induced apoptosis of neutrophils in the absence of inflammatory stimuli. Such an effect, though considered potentially beneficial for treating and preventing diseases such as cancers, could unfortunately lead to harmful levels of immunodeficiency (Kolaczkowska and Kubes, 2013) which may jeopardize the anti-inflammatory use of TD. Abe et al. (2015) have also shown that **3** produced a significant decrease of myeloperoxidase generation by human neutrophils at a concentration of 100 μM. In mice, tagitinins **1** and **2** provided modest anti-inflammatory effects when compared to indomethacin (García et al., 2006) indicating that they may be applied to mild to moderate inflammatory conditions. Other STLs including furanoheliangolides **22-25** (0.6–10 μM) significantly caused a dose-dependent decrease of superoxide anion generation by human neutrophils (Herrera et al., 2007). Further, saponins isolated from the leaves of TD produced at doses of 40–80 mg/kg, a significant increased WBC count in normal rats after 21 days of treatment (Ejelonu et al., 2017). This suggests that TD may also enhance cell-mediated immunity (Rajput et al., 2007).

Fig. 4. Structure of bioactive principles isolated from TD extracts: **1.** tagitinin A; **2.** tagitinin C; **3.** tagitinin F; **4.** tagitinin G; **5.** tagitinin I; **6.** tirotundin 3-O-methylether; **7.** tithofolinolide; **8.** 1β,2α-epoxytagitinin C; **9.** 4α,10α-dihydroxy-3-oxo-8β-(isobutyryloxy)guaia-11(13)-en-6α,12-olide; **10.** 3β-acetoxy-8β-isobutyryloxyreynosin; **11.** 1β-methoxydiversifolin-3-O-methyl ether; **12.** (E)-3-(((3-(3,4-dihydroxyphenyl)acryloyl)oxy)methyl)-2-methyloxyrane-2-carboxylic acid; **13.** 4,5-dicaffeoylquinic acid; **14.** 3,4-dicaffeoylquinic acid; **15.** 3,5-dicaffeoylquinic acid; **16.** 4,7,8-trihydroxy-4,7-dimethyldecahydronaphthalen-1-yl)propanoic acid; **17.** 8-acetoxy-4,7-dihydroxy-4,7-dimethyldecahydronaphthalen-1-yl)acrylic acid; **18.** 1β-hydroxytirotundin-3-O-methyl ether; **19.** 1,3-dihydroxy-3,10-epoxy-8-(-2-methylpropanoyloxy)-germacra-11(13)-ene-6,12-olide; **20.** 1,3-dimethoxy-3,10-epoxy-8-(2-methylpropanoyloxy)-germacra-4,11(13)-diene-6,12-olide; **21.** 1-hydroxy-3-methoxy-3,10-epoxy-8-(2-methylpropanoyloxy)-germacra-4,11(13)-diene-6,12-olide; **22.** 1,3-dihydroxy-3,10-epoxy-8-(2-methylpropanoyloxy)-germacra-4,11(13)-diene-6,12-olide; **23.** 1β-hydroxydiversifolin-3-O-methyl ether; **24.** diversifolin; **25.** diversifolin 3-O-methyl ether; **26.** 4β,10α-dihydroxy-3-oxo-8β-isobutyryloxyguai-11(13)-en-6α,12-olide; **27.** tirotundin.



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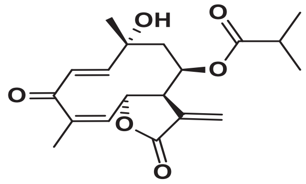

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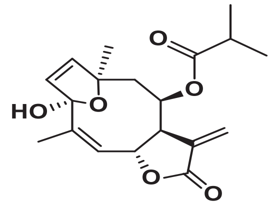

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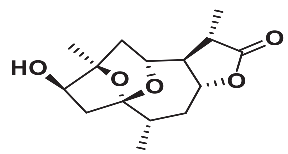

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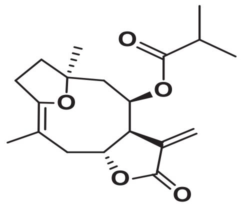

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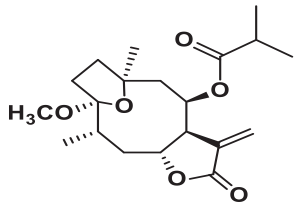

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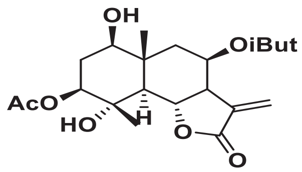

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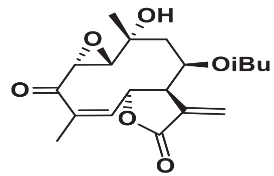

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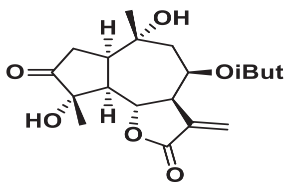

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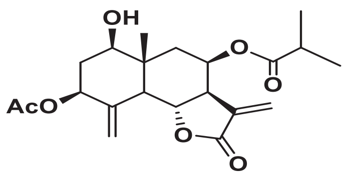

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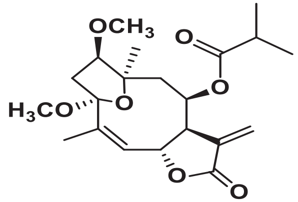

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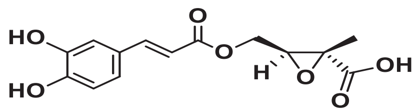

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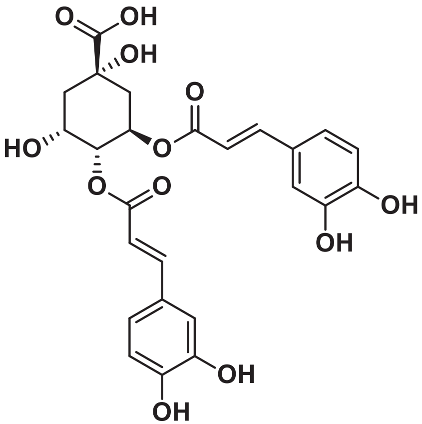

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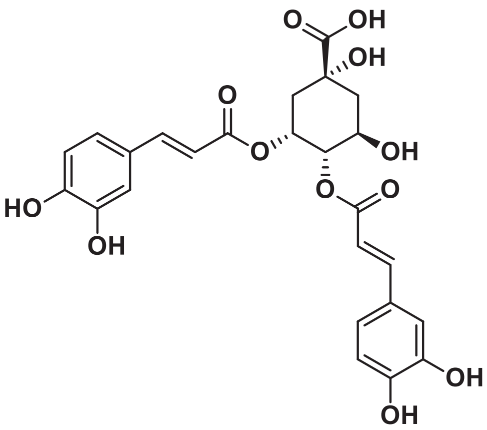

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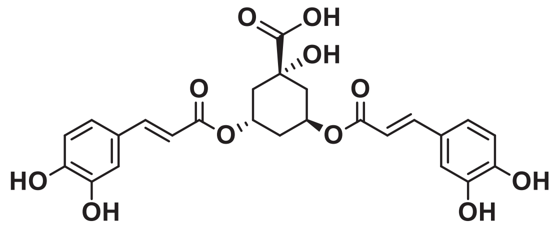

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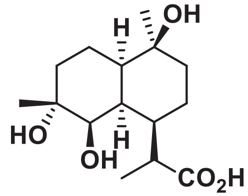

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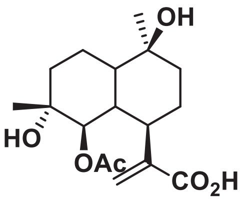

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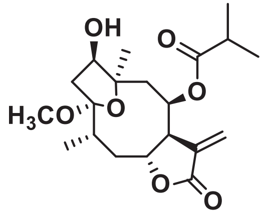

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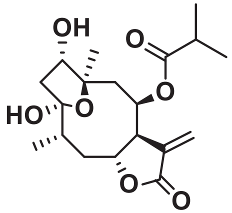

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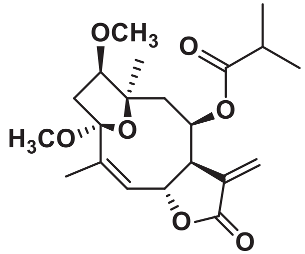

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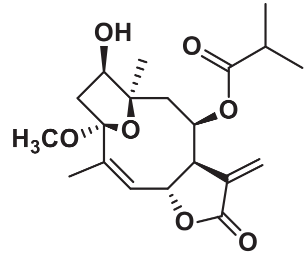

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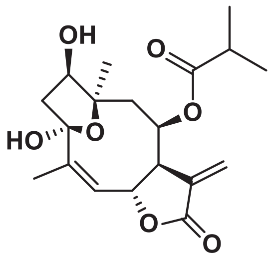

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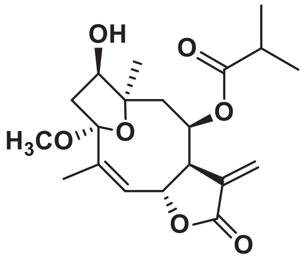

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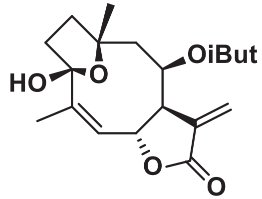

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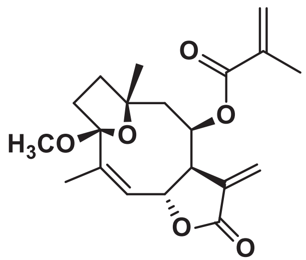

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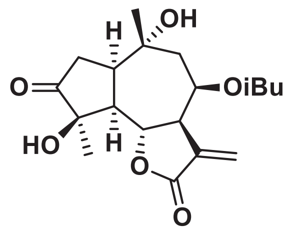

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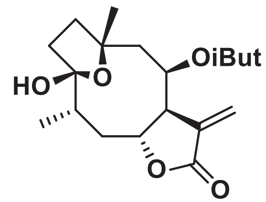

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Deciphering the mechanism behind the anti-inflammatory action of TD, Chagas-Paula et al. (2015) reported that the ethanolic leaf extract produced dual inhibition of COX-1 and 5-LOX. This extract, at the dose of 50 µg/mL caused significant inhibition of NF-κB activity, thereby indicating that the anti-inflammatory effect of TD was downstream from COX and 5-LOX in the signalling cascade (Bork et al., 1996). Importantly, STLs **4**, **105** and **106** were identified as the major NF-κB inhibitors occurring in TD extracts (Bork et al., 1997; Rüngeler et al., 1998). According to Rüngeler et al. (1999) these compounds may act through alkylation of cysteine residues in DNA binding loop of NF-kB, thereby making impossible the specific interaction of NF-kB with DNA (Rüngeler et al., 1999). Another important mechanism of action of TD leaf extracts include the dose-dependent inhibition of (^{3}H)-thymidine uptake of lymphocytes (Lasure et al., 1995). This finding may explain, at least in part, the antiproliferative effect of TD against lymphocytes.

7.2. Analgesic effect

Pre-treatment of rats with 50-200 mg/kg of methanolic extract of TD leaves produced a significant decrease of the nociceptive and inflammatory pains (Owoyele et al., 2004). Importantly, at the dose of 100 mg/kg, the observed painkilling effect was higher than that provided by indomethacin (5 mg/kg), suggesting that TD can be a good alternative to nonsteroidal anti-inflammatory drugs (NSAIDs). Likewise, at doses of 150 and 300 mg/kg, the methanolic extract induced antinociceptive effects in mice (Sijuade et al., 2016). Interestingly, the highest activity was achieved half-hour after the administration of a dose of 300 mg/kg of the extract. Together, these findings suggest that TD can afford effective drug candidates for the management of neuropathic and inflammatory pains. However, the molecular mechanism behind the analgesic effect of TD awaits further research.

7.3. Antiprotozoal effects

7.3.1. Antimalarial activity

Goffin et al. (2002) have found that ether and methanol extracts from the aerial parts of TD were moderately active against *Plasmodium falciparum* including the chloroquine-sensitive (FCA) and chloroquine-resistant (FCB1) strains. Importantly, the ether extract exhibited the highest antiplasmodial effect with IC_{50} values of 0.75 and 0.83 μg/mL for FCA and FCB1, respectively. The authors also found that the aqueous extract was inactive. This observation is somewhat surprising as TD decoction or infusion is frequently reported to treat malaria in folk medicine. However, in a more recent study, the aqueous extract from the leaves and flowers was active against chloroquine-sensitive strains of *Plasmodium* with IC_{50} values of 15.6 and 24.5 μg/mL, respectively for leaf and flower extracts (Muganga et al., 2010). The diverse bioactivity of TD can be explained by the aforementioned variations in the composition of TD extracts. Next, the bioassay-guided fractionation of the aqueous and ether extracts led to isolation and identification of **2** as the major responsible for the antiplasmodial effect of TD (Goffin et al., 2002) along with artemisinic acid analogues (Bordoloi et al., 1996), whose effect has yet to be characterized. Goffin et al. (2002) have found that **2** accounted for 30.5% and 0.5% of ether and aqueous extracts respectively, suggesting that the antiplasmodial action of TD extracts lie mainly on tagitinin C. Indeed, purified **2** was active against various strains of *Plasmodium falciparum* including the chloroquine-sensitive (IC_{50} of 0.33 μg/mL), and chloroquine-resistant strains (IC_{50} of 0.24 and 0.25 μg/mL for FCB1 and W2, respectively). Later, Maregesi et al. (2010) have shown that methanol leaf extract was also effective, even moderately, against chloroquine-sensitive strains (IC_{50} values of 31.25-62.5 μg/mL). In another set of experiments, methanol and dichloromethane leaf extracts were highly active against chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum* ($IC_{50} < 2.0$ μg/mL). Interestingly, the highest antiplasmodial effect ($IC_{50} < 1.5$ μg/mL) was achieved with dichloromethane extracts from the leaves and flowers (Muganga et al., 2010). In mice, the aqueous and methanolic extracts of TD cleared *Plasmodium* at 50% and 74%, respectively (Oyewole et al., 2008). Both were more effective in clearing parasites when administered before the onset of the disease, indicating the time-dependency of their antiplasmodial action. Accordingly, earlier administration of TD extracts would reduce parasitaemia within a few days with less cytotoxic side effects.

do Céu de Madureira et al. (2002) have reported that ethanolic extract of the aerial parts of TD also showed evident antiplasmodial effect (IC_{50} = 15 μg/mL) against chloroquine resistant strains of *P. falciparum*. Importantly, this effect was also observed for petroleum ether (PE) and dichloromethane (DM) fractions (IC_{50} < 10 μg/mL). Additionally, PE fraction showed significant schizontocidal activity (IC_{50} = 18 μg/mL) in *in vitro* assays. Interestingly, these extracts and fractions were moderately effective in mice infected with *P. berghei*. Specifically, oral administration of 200-600 mg/kg of ethanolic leaf extract to mice reduced the parasitaemia in a dose-dependent manner (Dada and Oloruntola, 2016). Interestingly, the highest antiplasmodial effect was achieved at the dose of 600 mg/kg and no change in mice weight was observed. Moreover, Elufioye and Agbedahunsi (2004) have found that ethanolic extract of the aerial parts of TD (50–400 mg/kg daily) was active dose-dependently on early, residual and established malaria infections in mice. Importantly, such effects were comparable to that provided by pyrimethamine (1.2 mg/kg per day) and chloroquine (5 mg/kg per day), used as the positive controls. This finding suggests that TD may be medicinally used for both curative and preventive purposes. However, the survival period of mice treated with ethanolic extract for 28 days in established infection reduced as the dose increased. This indicates a possible occurrence of toxicity during subchronic administration of TD. On the other hand, combination of TD leaves with other plants such as *Lawsonia inermis* caused a synergic chemosuppressive effect against both the chloroquine-sensitive (IC_{50} = 0.43±0.02 μg/mL) and chloroquine-resistant (IC_{50} = 2.55±0.19 µg/mL) strains of *P. falciparum* (Afolayan et al., 2016). Applied to mice, this preparation resulted in an 83.6% reduction of the parasitaemia. However, the addition of *Chromolaena odorata* to the aforementioned combination canceled the *in vitro* antiplasmodial effect, although some degree of synergy occurred in mice.

7.3.2. Other antiprotozoal effects

De Toledo et al. (2014) have reported that DM leaf rinse extract of TD produced a significant leishmanicidal effect (LD_{50} = 1.5±0.50 µg/mL) after 6 hours of incubation with promastigote forms of *Leishmania braziliensis*. This effect was related to STLs (LD_{50} range of 6.0±2.5-37.4±7.1 µM) including **1**, **2**, **3**, **4**, **7**, **14** and **39**. Interestingly, **2** showed significant cytotoxic effect (SI=1.4) against infected macrophages while **3**, **7** and **39** significantly reduced the internalization of parasites. This suggests that TD can be active against both forms of *Leishmania braziliensis*.

Olukunle et al. (2010) have found that a 3-day administration of aqueous leaf extract of TD (400 mg/kg per day) to rats infested with *Trypanosoma brucei brucei* resulted in a significant reduction of parasitaemia from 5.40±0.3 to 2.60±1.1 x 10^6 (per microscope field). This suggests that TD is endowed with antitrypanosomial effects.

7.4. Repellent activity

Oyewole et al. (2008) have found that essential oil of TD increased significantly the protection time against mosquito bites such as *Anopheles gambiae*, *Aedes aegypti*, and *Culex quinquefasciatus*. This suggests that TD may be used as repellent for protection against malaria and other diseases including chikungunya, dengue, yellow fever and Zika. Importantly, Wachira et al. (2014) have shown that methanolic leaf extract of TD was toxic against adult females of *A. gambiae*, and the highest effect was achieved at day 7 of mosquito feeding (LC_{50} = 1.52 mg/mL). Authors have also found that such an extract produced a weak larvicidal activity (LC_{50} after 72 h of exposure = 0.33 mg/mL), which however increased with the exposure time.

7.5. Antidiabetic effect

At doses of 500 and 1500 mg/kg, TD ethanolic extract caused a significant reduction of glucose levels in KK-Ay mice within 7 hours of treatment (Miura et al., 2005). Moreover, after 3 weeks of treatment with 500 mg/kg of such an extract, the plasma insulin and blood glucose were significantly decreased in diabetic mice. This observation indicates that TD may improve insulin resistance in type II diabetes. Likewise, the aqueous leaf extract at the oral dose of 400 mg/kg produced a time-dependent decrease of blood glucose in alloxan-induced diabetic rats (Olukunle et al., 2014). More precisely, such an extract produced a 36% and 82.3% reduction of glycaemia at days 1 and 21 of the treatment, and this effect was comparable to that produced by glibenclamide (10 mg/kg), used as a positive control. This finding suggests that TD may improve insulin release by the remnant β pancreatic cells of the diabetic rats so that it may be applied to treat type I diabetes. Interestingly, Thongsom et al. (2013) have reported that a 30-day administration of 500 mg/kg of aqueous leaf extract produced a significant reduction of glucose level in both diabetic and non-diabetic mice (Thongsom et al., 2013). Importantly, the observed effect was higher than that produced by glibenclamide (60 mg/kg), indicating that 500 mg/kg of aqueous extract can be an effective dose for diabetes. TD aqueous extract was also found to prevent oxidative damage in the pancreas and liver, as evidenced by the reduction of the malondialdehyde following treatment of mice. This observation suggests that the antidiabetic effect of TD may lie on its free radical scavenging potential. In addition, the dipeptidyl peptidase IV was inhibited (IC_{50} = 15,385.27 mg/mL) by ethanolic extract (Purnomo et al., 2014). As a result, the incretin levels were increased, and this may help regulate blood glucose by stimulating insulin secretion and β-pancreatic cell proliferation as well as by inhibiting glucagon secretion. At the molecular level, 10 μg/mL of STLs including **8**, **10**, **11** and **107** produced increased glucose uptake in differentiated 3T3-L1 adipocytes without any significant toxic effects (Zhao et al., 2012)~~;~~ This suggests that STLs may be responsible for the antidiabetic properties of TD. Importantly, the highest effect was achieved by **10** which increased the glucose uptake by 3.1 fold compared to the basal level. Other STLs such as **1** and **4** were found to act as dual LXR/FXR agonists at a concentration of 10 μM (Lin, 2013). Interestingly, both have been identified as PPARγ agonists (Lin, 2012), so they may improve insulin resistance and glucose uptake in diabetic patients.

7.6. Antibacterial and antifungal activities

Various extracts from TD leaves have been assayed for their activity against fourteen strains of bacteria including *Bacillus anthracis, Bacillus cereus, Bacillus polymyxa, Bacillus stearothermophilus, Bacillus subtilis, Clostridium sporogenes, Corynebacterium pyogenes, Staphylococcus aureus, Streptococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Pseudomonas fluorescens,* and *Shigella dysenteriae* (Obafemi et al., 2006). Of these, the ethyl acetate leaf extract was the most active, showing inhibitory activity against five bacteria Gram-positive and two Gram-negative. It was followed by the hexane and methanolic extracts, respectively. All the investigated extracts were also active against *Candida albicans*. Maregesi et al. (2008) have also found that methanolic leaf extract was active against *B. cereus* and *S. aureus* with MIC values of 500 and 1000 μg/mL, respectively (Maregesi et al., 2008). Moreover, at the dose of 80 mg/mL, such an extract was very active against *E. coli* and *B. subtilis*, with average diameter of zones of inhibition (ZI) of 20.33 mm and 23 mm, respectively (Gutierrez et al., 2013). However, it was moderately active against *P. vulgaris* and *S. aureus*. Likewise, the ethanolic leaf extract was active against *S. aureus* at concentration of 10 μg/spot (Bork et al., 1996).

In another study, the antibacterial activity of DM, ethyl acetate and methanol extracts from TD leaves were evaluated for their effect against *Pseudomonas aeruginosa*, *staphylococcus* *aureus*, *Bacillus subtilis* and *Escherichia coli*, using micro-broth diffusion method (Douglas and Jeruto, 2016). As a result, the test pathogens were more sensitive to DM leaf extract. Specifically, at a concentration of 25 mg/mL, DM extract was very active against *S. aureus* (ZI = 18 mm) and *P. aeruginosa* (ZI = 14 mm), but it was least active against *E. coli*. However, the soap based on aqueous leaf extract of TD (0.0-15.0% w/w) significantly inhibited the growth of *E. coli* and *C. albicans* (Kareru et al., 2010). At concentrations higher than 9.0% w/w, this soap provided a dose-dependent bactericidal effect and was active mostly against *E. coli*. Interestingly, the aqueous leaf extract of TD was inactive against *P. aeruginosa*, *Microbacterium foliorum*, *B. subtilis*, and *Rhodococcus equi* (Tran et al., 2013). However, when turned into silver nanoparticles, such an extract became highly active against both Gram-positive (*M. foliorum*, *B. subtilis*, and *R. equi*) and Gram-negative bacteria (*P. aeruginosa*) with ZI of 13, 15, 10 and 14 mm, respectively. This important finding suggests that the nanoencapsulation may be a promising drug delivery system for TD extracts. Recently, Agboola et al. (2016) have reported that EO from flowers (40 mg/mL) showed promising growth inhibitory effect against *E. coli*, *Proteus mirabi*, *Bacillus megaterium*, *Klebsiella pneumonia*, *B. cereus*, and *S. pyrogens* (Agboola et al., 2016). Moreover, at a concentration of 72 mg/mL, this oil caused a complete growth inhibition of three fungal species including *Cochliobolus lunatus*, *Fusarium solani*, and *Fusarium lateritum*. Heleno et al. (2011) have also observed that this EO provided moderate activity (MIC = 250 µg/mL) against cariogenic bacteria such as *S. mitis*, *S. sanguinis*, *S. sobrinus* and *L. casei*. This observation suggests that TD may be used in preventing dental caries. Orsomando et al. (2016) have demonstrated that EO exhibited mild to moderate growth inhibitory activity against *E. faecalis* (ZI = 8 mm) and *E. coli* (ZI = 9 mm). Importantly, such an extract was strongly active against *S. aureus* with MIC value of 2 mg/mL and ZI of 14 mm. Authors have also found that this oil selectively inhibited (IC_{50} of ~60 μg/mL) NadD, an enzyme essential for the survival of most bacterial pathogens.

7.7. Antiviral effect

Ethanolic leaf extract of TD showed no antiviral effect (SI less than 1) (Cos et al., 2002). Subsequent fractionation of this extract by suspending consecutively in 60% methanol, petroleum ether and ethyl acetate led to an aqueous fraction with a pronounced antiHIV-1 activity (SI > 461). This finding has been substantiated by Maregesi et al. (2010) who reported that the methanolic and aqueous extracts from the leaves exhibited mild antiviral activity against HIV-1 and HIV-2. Moreover, the aqueous leaf extract was active against HSV-1 and HSV-2, with IC_{50} values less than 100 μg/mL (Chiang et al., 2004). Recently, the aqueous extract from the roots was found to suppress the replications of HSV-1 with CC_{50} value of 460 μg/mL (Radol et al., 2016). Together, all these findings provide supporting evidence for the use of decoction of TD in the treatment of viral diseases.

7.8. Antioxidant effect

Giacomo et al., (2015) have reported that the aqueous leaf extract of TD exhibited the most important free radical scavenging effect, followed by the methanolic and dichloromethane extracts, respectively. Importantly, the antioxidant capacity of aqueous leaf extract at a concentration of 0.044 μg/mL was comparable to that provided by 80 mU of superoxide dismutase. The equivalent ABTS-radical scavenging capacity of such an extract was estimated by Thongsom et al. (2013) to 93.09±37.91 μM TEAC per mg of dry extraction weight. In another study, such scavenging capacity was increased (241.04±11.93 mmol Trolox of dry extraction weight) probably because of the variation in the phenolic content of extracts from TD (Hiransai et al., 2016). On the other hand, the equivalent DPPH-radical scavenging capacities of the aqueous leaf extract amounted to 94.89±2.69 mmol Trolox and 20.99±2.79

mg NAC per gram of dry extraction weight. (Giacomo et al., 2015) have also found that the aqueous extract of TD decreased significantly and dose-dependently the lipid hydroperoxide formation in a cell-free system. This finding suggests that TD may prevent peroxidative damage of plasma lipids. Likewise, the ethanolic leaf extract showed noteworthy scavenging activity (IC_{50} = 0.93±0.20 μg/mL) against DPPH free radicals, and such an effect was higher than that produced by ascorbic acid (IC_{50} = 0.48±0.10 μg/mL) used as a positive control (Juang et al., 2014). Importantly, at the dose of 50 mg/kg, this extract exhibited moderate protective effect against oxidative stress induced by spinal cord injury in rats. In another study, EO showed significant scavenging activity against DPPH and ABTS radicals, with IC_{50} values of 108.8 and 41.7 μg/mL, respectively (Orsomando et al., 2016). Likewise, the ethanol extract from TD flowers provided a strong DPPH scavenging effect with IC_{50} value of 205.80 μg/mL (Gama et al., 2014). At the molecular level, the antioxidant capacity of TD may be ascribed to its phenolic content (Giacomo et al., 2015). In fact, Pantoja Pulido et al. (2017) have found that caffeic acid derivatives **55-58** produced consistent DPPH radical-scavenging effects

7.9. Antiproliferative effect

Lee et al. (2011) have found that methanolic leaf extract of TD (IC_{50} = 59.2±3.7 μg/mL) along with compound **2** (IC_{50} = 6.1±0.1 μg/mL) showed significant antiproliferative effect against glioblastoma U373 cells. Such an effect was dose-dependent and occurred at doses of 10 μg/mL methanolic extract and 4 μg/mL tagitinin C (Liao et al., 2011). Interestingly, Lee et al. (2011) have established that the antiglioblastoma effect of methanolic extract occurred independently of caspase activation. Additionally, **2** caused arrest of malignant glioblastoma cells in G2/M phase (Liao et al., 2011). Importantly, survivin, a critical factor of drug resistance in cancer chemotherapy was dose-dependently downmodulated in cells treated with either methanolic leaf extract (dose of 10 μg/mL) or compound **2** (dose range of 2.5-10 μg/mL) (Liao et al., 2011). Moreover, the methanolic leaf extract and **2** also showed cytotoxic effects on human hepatoma

Hep-G2 cells with IC_{50} values of 40.0±2.0 and 2.0±0.1 μg/mL, respectively (Liao et al., 2013). Specifically, **2** was found to induce a dose-dependent increase in tumour cell population in sub-G1 phase and their arrest in S phase. Such an effect was selective and occurred through caspase-dependent apoptosis. Importantly, the tumorigenicity of xenografts derived from Hep-G2 cells was significantly retarded in mice treated with 15 μg per day of **2**. In another reports, **2** also showed a growth inhibitory effect (IC_{50} = 0.706 mg/mL) against human colon carcinoma cell lines HTC-116 (Goffin et al., 2002). Likewise, the methanolic leaf extract exhibited moderate antiproliferative effect against renal (TK10), breast (MCF-7) and melanoma (UACC62) human cancer cell lines with GI_{50} values of 9.65, 12.70, and 7.86 μg/mL respectively (Fouche et al., 2008). Interestingly, 3β-acetoxy-8β-isobutyryloxyreynosin at concentration of 10 μg/mL, significantly caused 63.0% of inhibition of lesion formation in mouse mammary organ culture assay (Gu et al., 2002). Calderón et al. (2006) have found that the ethanolic leaf extract of TD also exhibited chemosuppressive effect against MCF-7 cells (GI_{50} = 35 μg/mL) and other human cancer cell lines including lung (H-460), and central nervous system (SF-268) with GI_{50} values of 33 and 53 μg/mL, respectively. Likewise, ethyl acetate extract from the aerial parts of TD caused significant cytotoxic effect (IC_{50} = 1.3 μg/mL) on human colon cancer (Col2) cells (Gu et al., 2002). In addition, such an extract at a concentration of 4 μg/mL induced HL-60 cellular differentiation. Further, compounds **2** and **14** were identified as the major responsible for the antiproliferative effect on Col2 cells ($IC_{50} \leq 5$ μg/mL). On the other hand, **36**, **37** and **39**, were found to induce HL-60 cell differentiation (differentiation rate > 30%) at a concentration of 4 μg/mL. In another set of experiments, the aqueous extract from the whole plant also exhibited mild antiproliferative effect (GI_{50} = 356.7±16.9 μg/mL) on human leukaemia cell lines P3HR1 (Chiang et al., 2004). Likewise, 80% EtOH extract from aerial parts of TD showed significant cytotoxic effect (IC_{50} = 4.10 μg/mL) against HL-60 cells (Kuroda et al., 2007). Such an effect was related mainly to sesquiterpenoids including **17**.

Specifically, cell lines including breast BSY-1, central nervous system (SF 539, SNB-78), lung (DMS273, DMS114), ovary (OVCAR-3, OVCAR-5, OVCAR-8 and SK-OV-3), stomach (MKN1, MKN28, MKN74), prostate (DU-145, PC-3) were relatively sensitive (average log GI_{50} = -5.17) to **17**. This finding is consistent with data indicating that the presence of two α,β-unsaturations in STLs improves cytotoxic activity of this family of compounds (Rocha et al., 2012).–On the other hand, a 72-hour exposure to EO produced a dose-dependent antiproliferative effect against malignant melanoma A375, breast adenocarcinoma MDA-MB231, human colon carcinoma HCT116 and human glioblastoma multiform T98 G, with IC_{50} values of 3.02, 3.79, 3.46 and 12.82 μg/mL, respectively. Interestingly, the cytotoxic effects of EO against A375, MDA-MB231, and HCT116 cells were comparable to that produced by cisplatin (Orsomando et al., 2016).

7.10. Hypolipidemic and antiobesity effects

At doses of 17.5 and 175 μg/mL, the aqueous leaf extract of TD significantly inhibited the adipogenic differentiation of human mesenchymal stem cells (hMSCs) (Giacomo et al., 2015). This finding suggests that TD aqueous extract is endowed with antiobesity effect. The authors also claimed that such an effect was owed to increased pAMPK expression in hMSCs upon exposure to TD and the free radical scavenging capacity of this plant. Importantly, at a concentration of 175 μg/mL, the previous extract significantly increased the expression of HO-1 in hMSCs. This observation indicates that the antioxidant effect of *Tithonia* is not merely due to a direct free-radical scavenging activity, but it is also mediated by activation of protective molecular systems such as HO-1. the aqueous leaf extract (400 mg/kg per day) provided a significant decrease in total serum cholesterol (from 159.62±2.6 to 127.43±0.7 mmol/L), and LDL (from 116.98±3.0 to 57.95±4.2 mmol/L) in diabetic rats after 21 days of treatment (Olukunle et al., 2014). However, the serum HDL was significantly increased (25.70±0.9 to 52.42±0.6 mmol/L). On the other hand, in normal rats, saponins isolated from TD leaves, reduced significantly the levels of triglyceride, total cholesterol and serum LDL at dose ranges of 60-100, 40-100, and 20-100 mg/kg, respectively (Ejelonu et al., 2017). However, at doses of 20-100 mg/kg, the serum HDL was also significantly reduced. Putting together, all these findings suggest that TD extracts, particularly the saponin-rich fraction, can be used to treat and prevent dyslipidaemia in both healthy and unhealthy subjects.

7.11. Anti-ulcer effect

TD showed promise as means of prevention from gastric ulcer. At doses of 10-100 mg/kg, DM leaf extract produced a 90% decrease in ethanol-induced gastric ulcer in rats (Sánchez-Mendoza et al., 2011). Such an effect was related to **2**. Indeed, doses of 1.3, 10 and 30 mg/kg of **2** reduced ulcerative lesions at 37.7%, 70.1% and 100%, respectively in rats. Importantly, the authors also reported that such ethanol-induced ulcerative lesions were not attenuated by NG-nitro-L-arginine methyl ester (70 mg/kg, i.p.), N-ethylmaleimide (10 mg/kg, s.c.), or indomethacin (10 mg/kg, s.c.). These observations suggest that the gastroprotective mediators such as nitric oxide, sulfhydryl groups or prostaglandins E_2 do not participate in the antiulcer activity of TD. In addition, extracts from the aerial parts of TD exhibited mild (methanolic extract) to moderate (aqueous extract) growth inhibitory effect against *Helicobacter pylori* with MIC values of 62.5 μg/mL (methanolic extract) and 500 μg/mL (aqueous extract) (Castillo-Juárez et al., 2009). This finding suggests that TD can be applied to eradication of *Helicobacter pylori* in the treatment of chronic gastritis.

7.12. Antiemetic effect

At oral dose of 150 mg/kg, methanolic leaf extract of TD produced a 39.51% reduction in copper sulphate-induced retches in male chicks (Ahmed and Onocha, 2013). This finding suggests that TD is endowed with antiemesis effect. Importantly, such an effect was higher than that of chlorpromazine, used as a positive control. Moreover, the authors claimed that the phenolic compounds occurring in the extract could be the main responsible for this effect. However, the underlying mechanism of action awaits further investigation.

7.13. Hepatoprotective effect

The crude aqueous extract from the aerial parts of *T. diversifolia* (0.1 g/mL/kg, i.p.) markedly reduced CCl_4 -induced liver damage in rats (Lin et al., 1993). Likewise, the GOT levels in TD-treated rats were significantly decreased, while GPT were increased. These findings suggest that the aerial parts of TD are endowed with hepatoprotective effects. Such effects were more marked than those provided by aqueous extract from the stem (0.1 g/mL/kg, i.p.), suggesting that further screening of the hepatoprotective effect of TD should focus on the aerial parts of this plant. In agreement with this finding, Giacomo et al., (2015) found that ethanol leaf extract (at doses ≤ 50 µg/mL) provided a protective effect against H_2O_2-induced damage in normal rat liver cells.

7.14. Antivenin effect

Miranda et al. (2016) have reported that EO from fresh TD leaves significantly reduced blood clotting induced by *Bothrops atrox* venom. Specifically, in the presence of 0.6 µL and 1.2 µL EO, the clotting time of citrated human plasma was extended to statistically significant values of 116.4±1.2 and 114.3±1.3, respectively. This observation indicates that EO from TD can be used as an adjuvant in the treatment of snakebites.

7.15. Antidiarrheal effect

Tona et al. (1999) have demonstrated that decoction of fresh TD leaves was active (MIC < 100 µg/mL) against *Escherichia coli*, *Escherichia paracoli*, *Citrobacter diversus*, *Klebsiella pneumoniae*, *Salmonella enteritidis*, *Shigella flexneri*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Secondly, Authors have also found that the extract was very active against *Entamoeba histolitica* (MAC = 62.5 µg/mL). This effect was substantiated by Tona et al. (1998) who claimed that aqueous leaf extract showed strong chemosuppressive activity (MIC = 62.5 µg/mL) against *E. histolitica*. Importantly, (Tona et al., 2000) reported that the polyphenol-rich fractions of TD extract might be responsible for the antiamoebic effect of this plant species (MAC = 1.3 µg/mL). These findings suggest that TD is endowed with antidiarrheal potential as diarrhoea can be caused by the aforementioned microorganisms. Besides, TD also showed a pronounced antispasmodic effect as reported by (Tona et al., 2000). Indeed, the leaf decoction (80 µg/mL) caused a 79.4±7.9 to 76.9±4.1% inhibition in ACh or KCl-induced ileum contractions.

Table 3. ***Tithonia diversifolia*****: alleged therapeutic effects and pharmacological evidence**

Pharmacological activities	Alleged therapeutic claims	Study extracts/compounds	Experimental design	Results	References
immunomodulatory effect	asthma, bronchitis, cystitis, inflammatory conditions, hematoma, lymphadenitis	aqueous leaf extract	PHA-induced proliferation of PBMCs;	it decreased dose-dependently at concentrations of 0.66-25.00 μg/mL (IC_{50} of 4.42 μg/mL)	(Hiransai et al., 2016)
			NO generation by LPS-activated RAW264.7	concentration-dependent inhibition at concentrations of 0.94-30μg/mL (IC_{50} of 11.63 μg/mL)	
		methanolic leaf extract	oedema induced by carrageenan and granuloma induced by	inhibition of oedema and granuloma	(Owoyele et al., 2004)

		cotton pellet in rats		
	methanolic leaf extract	carrageenan-induced oedema in mice	inhibition at doses of 150 and 300 mg/kg	(Sijuade et al., 2016)
	LRE, PE and infusion	paw oedema and croton oil ear oedema assays in mice.	inhibition by LRE and PE at doses of 10-150 mg/kg; at doses of 0.05 and 0.5 mg/ear, all these extracts produced significant inhibition of neutrophil recruitment in the inflamed ears of the mice	(Chagas-Paula et al., 2011)
	tagitinins A, C and F isolated from the leaves	LPS-induced IL-6, IL-8 and TNF-α production by activated human neutrophils	inhibition at dose of 100 μM; tagitinin A induced TNF-α secretion even in the absence of inflammatory stimuli	(Abe et al., 2015)

		apoptosis of neutrophils	increase in activated neutrophils; tagitinin C induced apoptosis even in the absence of inflammatory stimuli	
		myeloperoxidase generation by LPS-activated human neutrophils	it was decreased by tagitinin F at concentration of 100 μM	
	aqueous extract	carrageenan-induced oedema in rats	decreased oedema at dose of 10 mL/kg.	(Lin et al., 1993)
	tagitinins A and C isolated from the dried aerial parts	ear oedema induced by 12-O-tetrade-canoylphorbol	moderately decrease	(García et al., 2006)

		13-acetate in mice		
	sesquiterpene lactones	superoxide anion generation in PMA-stimulated human neutrophils	reduction by 1,3-dihydroxy-3,10-epoxy-8-(-2-methylpropanoyloxy)-germacra-11(13)-ene-6,12-olide, 1,3-dihydroxy-3,10-epoxy-8-(2-methylpropanoyloxy)-germacra-4,11(13)-diene-6,12-olide, 1,3-dimethoxy-3,10-epoxy-8-(2-methylpropanoyloxy)-germacra-4,11(13)-diene-6,12-olide and 1-hydroxy-3-methoxy-3,10-epoxy-8-(2-methylpropanoyloxy)-germacra-4,11(13)-diene-6,12-olide at concentrations of 0.6–10 μM	(Herrera et al., 2007)
	ethanolic leaf extract	*in vitro* activity of NF-κb	inhibition at a concentration of 50 μg/mL; diversifolin, diversifolin 3-O-methyl ether, and tirotundin act through alkylation of cysteine residues in the DNA binding loop of NF-kB	(Bork et al., 1997, 1996, Rüngeler et al., 1999, 1998)

	aqueous leaf extract.	PHA-induced lymphocyte proliferation	inhibition	(Lasure et al., 1995)
		PHA-induced (^{3}H)-thymidine uptake of the lymphocytes	dose-dependent inhibition	
	Ethanol leaf extract	high-performance LC coupled to high-resolution in reversed-phase chromatography and electrospray ionization	dual inhibition of COX-1 and 5-LOX	(Chagas-Paula et al., 2015)

		saponins afforded by the leaves	WBC count in normal rats	increase at doses of 40–80 mg/kg after 21 days of treatment	(Ejelonu et al., 2017)
analgesic effect	body pain; dysmenorrhea, stomach pain; musculoskeletal disorders; sore throat; lymphadenitis	methanol leaf extract	pains induced by hot plate and formalin in rats	decrease at doses of 50-200 mg/kg; at dose of 100 mg/kg, the painkilling effect was higher than that produced with indomethacin (5 mg/kg)	(Owoyele et al., 2004)
		methanol leaf extract	pains induced by heat and mechanical pressure in mice	reduction at doses of 150 and 300 mg/kg; the maximum anti-nociceptive activity was achieved at 300 mg/kg	(Sijuade et al., 2016)
antimalarial effect	malaria; febrile illnesses	aqueous, ether and methanol extracts from the aerial parts	growth inhibition of *Plasmodium falciparum*	ether and methanol showed moderate chemosuppressive activity against FCA and FCB1 strains; ether extracts exhibited the highest antiplasmodial effect with IC_{50} of 0.75 and 0.83 μg/mL for FCA and FCB1, respectively	(Goffin et al., 2002)

	aqueous extract	growth inhibition of *Plasmodium falciparum*	modest chemosuppressive effect against chloroquine-sensitive strains with IC_{50} of 15.6 and 24.5 µg/mL for the leaf and flower extracts respectively	(Muganga et al., 2010)
	tagitinin C	growth inhibition of *Plasmodium falciparum*	antiplasmodial effect against chloroquine-sensitive (IC_{50} = 0.33 µg/mL) and chloroquine-resistant strains FCB1 (IC_{50}=0.24 µg/mL) and W2 (IC_{50} = 0.25 µg/mL)	(Goffin et al., 2002)
	80% methanol leaf extract	growth inhibition of *P. falciparum*	moderate chemosuppressive effect against the chloroquine-sensitive strains, with IC_{50} of 31.25 to 62.5 µg/mL	(Maregesi et al., 2007)
	methanol and dichloromethane extracts	growth inhibition of *P. falciparum*	strong antiplasmodial action against chloroquine-sensitive and resistant strains (IC_{50} < 2.0 µg/mL); the highest effect was achieved with dichloromethane leaf and flower extracts with IC_{50} < 1.5 µg/Ml	(Muganga et al., 2010)
	petroleum ether fraction	parasitaemia of mice infected	moderate reduction at doses of 500 and 1000 mg/kg	(Elufioye and

		with *P. falciparum*		Agbedahunsi, 2004)
	ethanol leaf extract	parasitaemia of mice infected with *P. falciparum*	strong reduction at doses of 200-600 mg/kg; the highest antiplasmodial effect was achieved at a dose of 600 mg/kg	(Dada and Oloruntola, 2016)
	combination of TD with *Lawsonia inermis*	growth inhibition of *P. falciparum*	synergic chemosuppressive effect against both the Chloroquine sensitive (IC_{50} = 0.43±0.02 µg/mL) and Chloroquine-resistant (IC_{50} = 2.55±0.19 µg/mL) strains of *P. falciparum*	(Afolayan et al., 2016)
		parasitaemia of mice infected with *P. falciparum*	83.6% reduction of parasitaemia in mice	

	mixture of TD, *C. odorata* and *L. inermis*	growth inhibition of *P. falciparum*	no effect	(Afolayan et al., 2016)
		parasitaemia of mice infected with *P. falciparum*	moderate reduction	
	petroleum ether extracts from the aerial parts	growth inhibition of blood and liver stage *P. falciparum*	growth inhibition of the blood ($IC_{50} < 10$ µg/mL) and liver ($IC_{50} = 18$ µg/mL) developmental stages of the parasites	(de Madureira et al., 2002)
	ethanol extract from the aerial parts	parasitaemia of mice during the early, repository and established stage of infection	dose-dependent reduction at doses of 50–400 mg/kg per day	(de Madureira et al., 2002)

			with *P. falciparum*		
			parasitaemia of mice infected with *P. falciparum*	reduction at the dose of 200 mg/kg	
antidiabetic effect	diabetes	80% ethanolic extract	blood glucose and plasma insulin in TD-treated KK-Ay mice and normal mice	decrease glucose level at single doses of 500 and 1500 mg/kg; decrease insulin after 3-weeks of treatment with a dose of 500 mg/kg; the insulin tolerance test showed a significant decrease in blood glucose in TD-treated diabetic mice and no consistent change in normal mice	(Miura et al., 2005)
		aqueous leaf extract	blood glucose in alloxan-induced	at dose of 400 mg/kg, 36% and 82.3% reduction of glycaemia respectively at days 1 and 21 of treatment	(Olukunle et al., 2014)

	diabetic rats treated with TD		
aqueous leaf extract	blood glucose in alloxan-induced diabetic rats and normal rats	at the dose of 500 mg/kg, dose-dependent reduction of blood glucose in alloxan-induced diabetic and non-diabetic mice after 30 days of treatment; malondialdehyde, was dose-dependently reduced in TD-treated diabetic mice	(Thongsom et al., 2013)
Tirotundin and tagitinin A, isolated from the crude oil	transient transfection reporter and mammalian one-hybrid assays	at 10 μM, activation of LXR/FXR pathways	(Lin, 2013)
sesquiterpenes	glucose uptake by differentiated 3T3-L1 adipocytes	increase at a dose of 10 μg/mL by tagitinin G, tagitinin I, 1β-hydroxydiversifolin-3-O-methyl ether and 1β-hydroxytirotundin-3-O-methyl ether	(Zhao et al., 2012)

		ethanolic extract	in vitro dipeptidyl peptidase IV inhibitory activity	decrease with IC_{50} value of 15,385.27 mg/mL	(Purnomo et al., 2014)
		tagitinin A and tirotundin	transient transfection reporter assay	activation of PPARγ	(Lin, 2012)
antimicrobial effect	bacterial infections; ringworm; cholera; typhoid fever; athlete's foot;	ethanolic leaf extract	growth inhibition of *S. aureus*	increase at concentration of 10 µg/spot	(Bork et al., 1996)
		aqueous leaf extract and TD-based silver nanoparticles	growth inhibition of *M. foliorum, B. subtilis, R. equi* and *P. aeruginosa*	induction by the TD silver nanoparticles while the leaf extract showed no effect	(Tran et al., 2013)

fungal, ear and nose diseases	n-hexane and methanolic leaf extracts	growth inhibition activity	hexane extract showed moderate activity against *Candida albicans* (MIC = 1000 µg/mL) while methanolic extract showed antibacterial effect against *B. cereus* and *S. aureus* with MIC values of 500 and 1000 µg/mL respectively	(Maregesi et al., 2008)
	dichloromethane, ethyl acetate and methanol leaf extracts	*in vitro* using micro-broth diffusion method	*S. aureus* (ZI = 18 mm) and *Pseudomonas aeruginosa* (ZI = 14 mm) were inhibited by dichloromethane leaf extract at a concentration of 25 mg/mL; *E. coli* was less sensitive to different plants extracts	(Douglas and Jeruto, 2016)
	TD soap	growth inhibition activity of *E. coli* and *Candida albicans*	induction at doses ≥ 9.0% w/w	(Kareru et al., 2010)
	ethyl acetate, hexane and	growth inhibitory effect of bacteria and *Candida*	ethyl acetate extract was the most active, followed by the hexane and methanol extracts. All the extracts were active against *Candida albicans*	(Obafemi et al., 2006)

	methanolic leaf extracts	*albicans* using agar-well diffusion method		
	methanol leaf extract	growth inhibitory activity of *E. coli, B. subtilis, P. vulgaris* and *S. aureus*	at a dose of 80 mg/mL, it was very active against *E. coli* and *B. subtilis*, with average diameter of ZI of 20.33 mm and 23 mm respectively; it was also active against *P. vulgaris* and *S. aureus*	(Gutierrez et al., 2013)
	flower essential oil	growth inhibition of bacteria and fungi	at dose of 40 mg/mL, it was active against *E. coli*, *Proteus mirabi*, *Bacillus megaterium*, *Klebsiella pneumonia*, *Bacillus cereus*, and *Streptococcus pyrogens*; at dose of 72 mg/mL, it completely inhibited the growth of *Cochliobolus lunatus*, *Fusarium solani*, *Fusarium lateritum*	(Agboola et al., 2016)

		essential oil	growth inhibition	moderate to low antibacterial activity against *E. faecalis* (ZI=8 mm) and *E. coli* (ZI=9 mm), while it was highly active against *S. aureus* with MIC=2 mg/mL and ZI=14 mm	(Orsomando et al., 2016)
			activity of NadD from *S. aureus*	inhibition (IC_{50} of ~60 µg/mL)	
antioxidant effect	anti-poison; improve liver functions	aqueous and dichloromethane extracts	free radical scavenging effect	Induction	(Giacomo et al., 2015)
			plasma lipid hydroperoxide formation in a cell-free system	inhibition by aqueous extract	
		aqueous extract	ABTS-radical scavenging assay	the equivalent ABTS-radical scavenging potential was 93.09 ± 37.91 µM TEAC per mg of dry extraction weight	(Thongsom et al., 2013)

	aqueous extract	DPPH-radical scavenging assay	the equivalent DPPH-radical scavenging potential was 94.89 ± 2.69 mmol Trolox and 20.99 ± 2.79 mg NAC per gram of dry extraction weight	(Hiransai et al., 2016)
	essential oil	ABTS and DPPH-radical scavenging effects	induction with IC_{50} values of 108.8 and 41.7 μg/mL, for DPPH and ABTS respectively	(Orsomando et al., 2016)
	caffeic acid derivatives	DPPH radical-scavenging effect	induction by (1S,3S,5S)-dicaffeoylquinic acid, (1S,3R,4S)-dicaffeoylquinic acid, (1S,3S,4S)-dicaffeoylquinic acid and (1R,3S,5S)-dicaffeoylquinic acid	(Pantoja Pulido et al., 2017)
	ethanolic leaf extract	DPPH radical-scavenging assay; H_2O_2-induced damage in rat liver cells;	it showed noteworthy scavenging effect against DPPH free radicals (IC_{50}=0.93 ± 0.20 μg/mL); at concentrations ≤ 50 μg/mL, it provided hepatoprotective effect; at oral dose of 50 mg/kg, it provided modest protective effect against oxidative stress in rats	(Juang et al., 2014)

			oxidative stress induced by spinal cord injury in rats		
anticancer effects	unstated	methanolic leaf extract and tagitinin C	cytotoxicity using MTT reduction assay	reduction of the proliferation of U373 cells with IC_{50} of 59.2±3.7 µg/mL and 6.1±0.1 µg/mL; induction of autophagy; cell death occurred independently of caspase activation	(Lee et al., 2011)
		methanolic leaf extract and tagitinin C	cytotoxicity using MTT reduction assay	concentration-dependent anti-glioblastoma effect at doses of 10 and 4 µg/mL; tagitinin C caused arrest of malignant glioblastoma cells in G2/M; survivin was significantly and dose-dependently underexpressed in presence of methanolic extract (10 µg/mL) and tagitinin C (2.5, 5 and 10 µg/mL)	(Liao et al., 2011)

	methanolic leaf extract and tagitinin C	cytotoxicity using MTT reduction assay; tumorigenicity of xenografts	cytotoxic effects on Hep-G2 cells with IC_{50} of 40.0 ± 2.0 and 2.0 ± 0.1 μg/mL; increase in tumour cell population in sub-G1 phase and arrest in S phase; the antiproliferative effect of tagitinin C on Hep-G2 was selective, and occurred via caspase-dependent apoptosis; at dose of 15 μg per day, tagitinin C retarded significantly the tumorigenicity of xenografts derived from Hep-G2 cells	(Liao et al., 2013)
	sesquiterpene lactones	cytotoxicity using MTT reduction assay; mouse mammary organ culture assay	inhibitory effect on HTC-116 (IC_{50} = 0.706 mg/mL); Col2 cells (IC_{50} ≤ 5 μg/mL) by tagitinin C; 1β,2α-epoxytagitinin C; tithofolinolide; 3β-acetoxy-8β-isobutyryloxyreynosin; 4α,10α-dihydroxy-3-oxo-8β-isobutyryloxyguaia-11(13)-en-12,6α-olide; induction of HL-60 cell differentiation (> 30%) at 4 μg/mL; 63.0% inhibition of lesion formation in mouse mammary organ culture assay at 10 μg/mL	(Goffin et al., 2002)

ethyl acetate extract from the aerial parts	cytotoxicity using MTT reduction assay	cytotoxic effect on Col2 cells (IC_{50} = 1.3 µg/mL) and induction of 70% HL-60 cell differentiation at 4 µg/mL	(Gu et al., 2002)
aqueous extract from the whole plant	cytotoxicity using MTT reduction assay	cytotoxic effect against P3HR1 with GI_{50} = 356.7 ± 16.9 µg/mL	(Chiang et al., 2004)
80% EtOH extract from aerial parts	cytotoxicity using MTT reduction assay	cytotoxic effect on HL-60 cells (IC_{50} = 4.10 µg/mL); cell lines including breast BSY-1, central nervous system (SF 539, SNB-78), lung (DMS273, DMS114), ovary (OVCAR-3, OVCAR-5, OVCAR-8 and SK-OV-3), stomach (MKN1, MKN28, MKN74), prostate (DU-145, PC-3) were relatively sensitive to 1β–methoxydiversifolin 3-*O*-methyl ether (GI_{50} = -5.17)	(Kuroda et al., 2007)
essential oil	cytotoxicity using MTT assay	concentration-dependent cytotoxic effect against A375, MDA-MB231, HCT116 and T98 G after 72 h of tumour cell exposition	(Orsomando et al., 2016)

				to EO. IC_{50} values were 3.02, 3.79, 3.46 µg/mL and 12.82 µg/mL for A375, MDA-MB231, HCT116 cells and T98 G, respectively.	
		methanol leaf extract	cytotoxicity using MTT assay	moderate antiproliferative effect against TK10, MCF-7 and UACC62 with GI_{50} of 9.65, 12.70, and 7.86 respectively	(Fouche et al., 2008)
		ethanolic leaf extract	cytotoxicity using MTT assay	cytotoxic effect against MCF-7, H-460, and SF-268 with GI_{50} values of 35, 33, 53 µg/mL respectively	(Calderón et al., 2006)
anti-obesity effect	unstated	aqueous leaf extract	in vitro studies on hMSC; the antiadipogenic effect was assessed using Oil-Red O staining	inhibition of adipocyte differentiation at doses of 17.5 µg/mL and 175 µg/mL, increase in pAMPK expression and free radical scavenging effects. 72h-exposure of hMSCs at doses of 17.5 µg/mL or 175 µg/mL, provided a significant decrease in ROS levels; at dose of 175 µg/mL, increased expression of HO-1 in hMSCs	(Giacomo et al., 2015)

hypolipidemic effect	hypertension	aqueous leaf extract	lipid status of alloxan-induced diabetic rats	at dose of 400 mg/kg, decrease in total cholesterol (from 159.62±2.6 to 127.43±0.7mmol/L), and LDL (from 116.98±3.0 to 57.95±4.2 mmol/L) as well as increase of HDL (from 25.70±0.9 to 52.42±0.6 mmol/L).	(Olukunle et al., 2014)
		saponins isolated from the leaves	lipid status of normal rats	reduction of triglyceride, total cholesterol and LDL at doses of 60–100 mg/kg, 40–100 mg/kg, and 20–100 mg/kg, respectively and increase of HDL at doses of 20 to 100 mg/kg	(Ejelonu et al., 2017)
gastroprotective effect	stomach pains; gastrointestinal complaints	dichloromethane leaf extract, tagitinin C	gastric ulcer induced by ethanol in rats	at doses of 10-100 mg/kg, 90% decrease of gastric ulcer; prevention of ulcerative lesions by percentages of 37.7%, 70.1% and 100% by tagitinin C at doses of 1.3, 10 and 30 mg/kg, respectively	(Sánchez-Mendoza et al., 2011)
		aqueous and methanolic extract from aerial parts	growth inhibition	cytotoxic effect against *H. pylori* with MIC values of 62.5 and 500 µg/mL respectively for methanolic and aqueous extracts	(Castillo-Juárez et al., 2009)

antiemetic effect	indigestion	methanol leaf extract	retches induced by copper sulphate in male chicks	at oral dose of 150 mg/kg, 39.51% reduction of retches	(Ahmed and Onocha, 2013)
antidiarrheal effect	diarrhoea; typhoid fever; indigestion; constipation; gastrointestinal disorders	aqueous leaf extract	growth inhibition	strong cytotoxic activity against *E. histolitica* (MIC = 62.5 µg/mL)	(Tona et al., 1998)
		decoction of fresh leaves	growth inhibition assay, ileum contraction assay	cytotoxic effect against E. coli, E. paracoli, C. diversus, K. pneumoniae, *S. enteritidis*, *S. flexneri*, *S. aureus*, *P. aeruginosa* (MIC<100 µg/mL) and *E. histolitica* (MAC = 62.5 µg/mL); at a concentration of 80 µg/mL, 79.4 ±7.9 or 76.9 ± 4.1 % inhibition of ACh or KCI-induced contractions.	(Tona et al., 1999)
		polyphenol-rich fraction of aqueous leaf extract	growth inhibition assay	growth inhibitory effect against *E. histolitica* (MAC=1.3 µg/mL)	(Tona et al., 2000)

antileishmanial effect	unstated	dichloromethane leaf rinse extract	*in vitro* antileishmanial assay	at 10 μg/mL, leishmanicidal effect against promastigote forms of *Leishmania braziliensis* with LD_{50}=1.5 ± 0.50 μg/mL; tagitinin C, 1β,2α-epoxytagitinin C, tirotundin, tirotundin 3-O-methylether, tagitinin F, 4β,10α-dihydroxy-3-oxo-8β-isobutyryloxyguai-11(13)-en-6α,12-olide and tagitinin A exhibited antileishmanicidal effect with LD_{50} ranging from 6.0 ± 2.5 to 37.4 ± 7.1 μM; tagitinin C was found to cause significant cytotoxic effect against macrophages (SI=1.4); tirotundin 3-O-methyl ether, tagitinin F, and 4β,10α-dihydroxy-3-oxo-8β-isobutyryloxyguai-11(13)-en-6α,12-olide reduced the internalization of parasites	(De Toledo et al., 2014)
antitrypanosomal effect	unstated	aqueous leaf extract	parasitaemia of rats infected with *Trypanosoma brucei brucei*	decrease to 2.60±1.1 at 3rd day post infection at the dose of 400 mg/kg per day	(Olukunle et al., 2010)

antivenin effect	snake antivenin	EO from fresh leaves	blood clotting time of citrated plasma exposed to snake venom	inhibition of blood clotting induced by *Bothrops atrox* venom. The clotting time of citrated human plasma for *Bothrops atrox* venom was 116.4±1.2 and 114.3±1.3 for 0.6 μL and 1.2 μL of EO, respectively	(Miranda et al., 2016)
hepatoprotective effect	hepatitis; improve liver functions; jaundice	aqueous extract from aerial parts and stem	CCl_4 induced liver damage in rats	prevention of liver damage induced at the dose of 0.1 g/mL/kg, i.p., decreased levels of GOT and GPT	(Lin et al., 1993)
		ethanol leaf extract	H_2O_2-induced liver damage in normal rat liver cells	at doses ≤ 50 μg/mL, protective effect against H_2O_2-induced damage	(Giacomo et al., 2015)
antiviral effect	AIDS; hepatitis; herpes zoster;	80% ethanolic extract	growth inhibition of viruses	no effect against viruses (SI < 1). Subsequent fractionation of this extract by suspending consecutively in 60% methanol, petroleum ether and ethyl acetate led to an aqueous fraction with a pronounced antiHIV-1 activity (SI > 461)	(Cos et al., 2002)

	smallpox; measles	aqueous root extract	cytotoxicity through MTT of Vero cells	anti-HSV1 with MNC of 20 μg/mL and CC_{50} of 460 μg/mL	(Radol et al., 2016)
		80% methanol and water leaf extracts	In vitro growth inhibition of viruses	they were active against HIV-1 and HIV-2	(Maregesi et al., 2010)
		aqueous leaf extract	In vitro growth inhibition of viruses	inhibition of HSV-1 and HSV-2 replications (IC_{50}<100 μg/mL)	(Chiang et al., 2004)
repellent activity	Unstated	essential oil	arm-in-cage test	increase in the protection time against bites of *Anopheles gambiae*, *Aedes aegypti*, and *Culex quinquefasciatus*; the highest repellent effect was achieved against *A. gambiae*	(Oyewole et al., 2008)
		methanol leaf extract	feeding assay	significant decrease (LC_{50} = 1.52 mg/mL) in the survival of *A. gambiae* after 7 days of feeding; mild larvicidal activity	(Wachira et al., 2014)

8. Safety

Very few studies have looked at adverse effects associated with exposure to TD extracts and focused mainly on ethanolic and aqueous extracts of this plant. Dada and Oloruntola (2016) reported that a single oral administration of 1600 mg/kg of ethanolic leaf extract is well tolerated in mice. In contrast, the ethanolic extract from aerial parts, at doses lesser than 1600 mg/kg, caused dose- and time-dependent alterations in kidney and liver functions and changes in haematological parameters in rats (Elufioye et al., 2009). However, no detectable histological lesions were recorded in the heart, spleen and brain. Interestingly, the kidney and liver damage induced by TD extract seemed to be reversible.

TD-induced alteration in haematological parameters was also supported by Hiransai et al. (2016). They found that the aqueous leaf extract of TD showed significant cytotoxic effect against PBMCs and RAW264.7 with CC_{50} values of 145.87 µg/mL and 73.67 µg/mL, respectively. Likewise, long-term use of this extract (50-140 mg/kg per day) was found to cause altered haematological parameters in rats including decreased PCV, low WBC counts and increased serum GPT (Oyewole et al., 2007). Liver damage and loss of body weight were reported as well. Specifically, weight loss was time-dependent and averaged 6 g (5.9%) and 10 g (9.8%) on days 7 and 14 of treatment, respectively. The maximum tolerated dosage (MTD) and LD50 of the aqueous leaf extract were also determined and amounted to 100 and 120 mg/kg, respectively. At doses higher than MTD (100-400 mg/kg), this extract showed significant changes in haematological, biochemical and histopathological parameters in rats after 14 days of treatment (Fankule and Abatan, 2007). In contrast, TD was well tolerated in rats following 7 days of treatment with 100 mg/kg of such an extract (Adebayo et al., 2009). However, at the dose of 200 mg/kg, Adebayo et al. (2009) have reported the occurrence of liver and heart damage as evidenced by increased levels of alkaline phosphatase in such tissues.

Additionally, a 90-days repeated administration of 10 mg/kg of the extract, caused decreased WBC counts and increased alkaline phosphatase levels in rats (Passoni et al., 2013).

Putting together, these findings suggest that TD is relatively well-tolerated in animals, when administered orally at lower doses (< 100 mg/kg) and for a short-term period (less than 7 days). Taken in high doses than necessary, it can cause serious side effects including anaemia, dyspnoea, asthenia, immunosuppression, hepatic dysfunctions and kidney damage. There is also some alleged risk of contact dermatitis associated with its content in STLs (Mark et al., 1999). Usually, such adverse effects are moderate and surmountable, but they can become pronounced and irreversible at higher doses or with chronic exposure to the plant.

In an effort to unravel the main components governing the toxicity, rats were treated orally for 90 days with various doses of two chemically different extracts of TD including LRE and PE (Passoni et al., 2013). At doses of 10 and 100 mg/kg, PE caused changes in the levels of several haematological and biochemical parameters, including the erythrocytes number, AST, ALT, alkaline phosphatase, albumin, total proteins, and creatinine. Histological analysis revealed hepatic steatosis especially at doses higher than 100 mg/kg, and no evident alterations in the kidneys. Likewise, the haematological and biochemical parameters were altered in rats treated with LRE (doses of 10 and 50 mg/kg). Histologically, at the dose of 50 mg/kg, serious kidney damage including destruction of glomeruli and distal tubules, was also recorded. In addition, a 21-day treatment with saponins isolated from the leaves caused at doses of 20-100 mg/kg, a moderate to strong increase in the activity of ALT, AST, ALP, GGT as well as high levels of creatinine in normal rats (Ejelonu et al., 2017). Together, these findings suggest that the toxic effects of TD extracts may be ascribed to CAs, STLs and saponins.

9. Clinical application and future outlooks

Tithonia diversifolia has a long tradition of use by local people for treating and preventing ailments and diseases. Few studies provided supporting evidence for most of the ethnomedicinal claims stated for TD extracts and focused mainly on the anti-inflammatory, antimalarial, antidiabetic, antioxidant, and anticancer effects (Table 4). However, there is little or dearth of information about the analgesic, antivenom, repellent, antiviral, antiemetic, antitrypanosomial, and leishmanicidal effects. This may be worth deserving much more interest. Moreover, many other ethnomedicinal uses of TD including for/as deworming, haemorrhoids, diuretic, urethral stones, cough, appetite stimulant, boosting energy, "folk illnesses" and nicotine addiction are still missing supporting evidence. This could be due to the lack of established experimental design in which these claims can be validated. Likewise, literature on the pharmacotoxicology of EO is very poor if considering that volatiles are endowed with interesting biological effects.

As discussed above, TD is a reservoir of bioactive principles with great therapeutic potentials. In some cases, particularly as anti-inflammatory, analgesic, antidiabetic, antioxidant, antimalarial and antiproliferative drug candidate, the bioactivity of TD was better and safer than that produced by conventional remedies. Therefore, it may represent a valid therapeutic avenue, mostly for diseases with unmet needs. Table 4 summarized the documented pharmacological activities of TD and provided hypotheses for their corresponding indications in clinical practice. As far as we know, there is only one study conducted to use TD for future antimicrobial medicine by synthesizing silver nanoparticles using aqueous leaf extract of this plant (Tran et al., 2013). TD is already commercially available in Taiwan, but there are still many walls to fall before it is used in clinical practice. As current research on TD has been conducted in a preclinical setting, there is no documented dose prescribed for humans. So, the effective doses retrieved from preclinical studies should be translated into realistic human-equivalent doses. In this regard, the use of the allometric scale is the best indication such an estimation (Wojcikowski and Gobe, 2014). However, in some cases, the preclinical effective doses are too high to be replicable in clinical practice. For instance, doses applied to treat malaria and diabetes in rats are higher than the MTD. To overcome this important issue, future research should focus on identifying the toxic components of TD extracts and developing extraction techniques to reduce them.

In addition, the complex nature and inherent variability of constituents of TD raise concerns over the clinical effectiveness and reproducibility of the effects of various extracts of this plant. The standardization of extracts through a modern analytical technique can represent an important tool to circumvent this issue. Fingerprinting analyses of markers occurring in TD should represent a core topic of future investigation on this plant. In the meantime, tagitinin C can serve as a fingerprinting marker for quality control purpose, as it has emerged as one of the major constituents responsible for the biological effects of TD.

Pharmacological studies have provided supporting evidence for a therapeutic potential of TD however, clinical trials are warranted to assess the efficacy of TD for any disorder. But above all, future studies have to be done especially around pharmacokinetics, phytochemistry and toxicology. Concerning the toxicology, we should extend research to herb-herb and herb-drug interactions, considering that TD can be used in association with other plants or prescribed medicines. This could either cancel or exacerbate the effects of the plant, or even lead to severe side effects. Thus, future pharmacotoxicological studies should be dedicated to clarifying the potential benefits and/or risks associated with these interactions. Other routes of administration should be also considered in future toxicity studies. Moreover, beyond STLs, CAs, saponins and flavonoids, many other compounds —not yet characterized— may be responsible for the effects of TD. Future investigation on this plant should focus on their identification, isolation, purification, pharmacotoxicological characterization. Researchers are strongly encouraged to fill the gap in this field as the need for new drugs is more relevant than ever and TD can afford untapped bioactive compounds.

Developing TD-based medicines or TD-derived products also requires implementation of programs to ensure that adequate quantities of raw materials are available for succeeding generations. Currently, the supply resource of this plant is not a concern, however, we should keep in mind the sustainability issue if considering the possibility to produce drugs from TD. This goal can be achieved through a better management/conservation of the genetic resources of the plant, protection of its natural habitat and mutual agreement with native people whose traditional knowledge has contributed to making such a drug possible.

Overall, TD is source of compounds with significant therapeutic implications and favourable safety profile. Their clinical application could be decisive in reducing high costs and side effects associated with modern medicines. However, there is no clinical evidence for their therapeutic effect. Therefore, it seems premature to draw firm conclusions about the alleged therapeutic effects of this plant. More rigorously designed investigations are needed in view of recommending the whole plant or its bioactive components for the treatment and prevention of a broad range of diseases. This systematic review provides the tradition and science behind the use of TD for an informed decision for future investigation on this plant. This research is of particular importance in that it links the pharmacology to ethnomedicine and phytochemistry and provides insight into the potential clinical application of this plant species.

Table 4. *Tithonia diversifolia*: pharmacological activities and their corresponding indications in clinical practice.

Pharmacological activities	Effective doses	Possible clinical indications	References
Immunomodulatory effect	150 mg/kg	immunodeficiency; pains; eczema; dysmenorrhea;	(Sijuade et al., 2016)
Analgesic effect	200 mg/kg	wound healing; pains; dysmenorrhea; abscesses; musculoskeletal disorders; throat diseases	(Sijuade et al., 2016)
Antimalarial effect	200-600 mg/kg	Malaria	(Dada and Oloruntola, 2016)
Antidiabetic effect	500 mg/kg	type I and type II diabetes	(Miura et al., 2005)
Antimicrobial effect	72 mg/mL	wounds; candidiasis; amoebic dysentery; dermatological diseases; *Klebsiella* infections; pneumonia; gastroenteritis; typhoid fever; skin eruptions; cholera; trypanosomiasis	(Agboola et al., 2016)
Antioxidant effect	50 mg/kg	improve liver function; antiaging care	(Juang et al., 2014)
Anticancer effect	10 µg/mL	breast cancer; leukaemia; glioblastoma; colon cancer; hepatocellular carcinoma; lung cancer; ovary cancer; prostate cancer; stomach cancer	(Liao et al., 2011)
Anti-obesity effect	175 µg/mL	Obesity	(Giacomo et al., 2015)

Hypolipidemic effect	60–100 mg/kg	dyslipidaemia; prevention of cardiovascular events	(Olukunle et al., 2014)
Gastroprotective effect	10-100 mg/kg	stomach pains; gastritis; eradication of *H. pylori*	(Sánchez-Mendoza et al., 2011)
Antiemetic effect	150 mg/kg	indigestion; motion sickness	(Ahmed and Onocha, 2013)
Antidiarrheal effect	80 µg/mL	diarrhoea; indigestion; gastrointestinal disorders	(Tona et al., 1999)
Antileishmanial effect	10 µg/mL	Leishmaniosis	(De Toledo et al., 2014)
Antiviral effect	460 µg/mL	hepatitis; measles; herpes simplex infection; AIDS; Dengue fever; Zika	(Radol et al., 2016)

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CHAPTER 2: Determinants, Prevalence and Trend of Use of Medicinal Plants among People Living with HIV: a Cross-Sectional Survey in Dschang, Cameroon

(This work is currently under review)

Abstract

People living with HIV (PLHIV) in Cameroon often seek help from traditional health practitioners (THPs) and use medicinal plants (MP). Most MP, however, are still lacking supporting evidence for their efficacy and safety, and their use, often undisclosed to the caring physicians and scarcely investigated, may jeopardize the effectiveness and tolerability of standard therapies. Therefore, we conducted a six-month questionnaire-based survey of 247 consecutive PLHIV and 16 THPs to explore the extent of MP use in Dschang (Cameroon). As a result, 54.9% of PLHIV reported using a total of 70 plant species, 91.3% of users were satisfied with MP and unwanted effects were reported only in 2 cases. Importantly, MP users were less educated than nonusers, had longer disease duration and were more often unemployed. On the other hand, only 3 THPs acknowledged the use of MP, although most of them had insufficient knowledge and serious misconceptions about HIV/AIDS.

Keywords: medicinal plants; survey; prevalence; HIV/AIDS; Dschang Cameroon.

Introduction

With circa 36.7 million people living with HIV (PLHIV) worldwide and 1 million AIDS-related deaths in 2016, HIV/AIDS remains a major global concern (UNAIDS, 2018). Although no region of the globe has been spared from this pandemic, sub-Saharan Africa, which accounts for more than 70% of the total number of PLHIV, stands out as the epicentre of this epidemic (2). Despite efforts to combat this disease, a definitive cure or protective vaccine has not yet been discovered (3). The advent of combination antiretroviral therapy (cART) allowed HIV/AIDS to shift from an inevitably fatal condition to a manageable chronic disease (4). This therapeutic option which consists of combining three or more antiretroviral drugs from a minimum of two different pharmacological classes, has remarkably improved the life quality and longevity of PLHIV (5). Moreover, cART has decreased the global incidence of HIV-related opportunistic infections. However, access to cART is still limited in some parts of the world, especially in resource-poor settings (6). On the other hand, the chronicity of the infection and long-term exposure to antiretroviral drugs have created new health challenges including cardiovascular, neurologic and metabolic disorders in HIV-infected population (7,8). Overall, antiretroviral therapy still suffers from several largely unmet medical needs, which could induce PLHIV to seek help from complementary and/or alternative medicines such as medicinal plants (9,10).

Use of medicinal plants (MP) is deeply rooted in history, and traditional health practitioners (THPs) represent the custodians of indigenous knowledge about MP (11,12). Most MP, however, are still lacking supporting evidence for their efficacy, tolerability and safety, and their use, often undisclosed to the referring physicians, may raise concerns about possible interference with standard therapies (13,14). Importantly, knowledge of MP is fading because it is transmitted over generations most often without any written record (15). Globally, actions are needed not only to preserve such an ancestral knowledge resource, but also to ascertain the pharmacotoxicological activities of MP, and to ensure that adequate quantities are available for posterity.

The current study is part of an ongoing research project aiming at identifying plants with promising prospect for the development of novel lead compounds to control HIV in the absence of a cure or vaccine. In the present study, we surveyed knowledge and attitudes towards MP in PLHIV, exploring their use prevalence, the reasons for their use, the modality of their use along with their perceived value. In addition, we sought to investigate potential factors that could determine or prone MP consumption in PLHIV. On the other hand, a sample of THPs was surveyed about MP use and HIV-related knowledge.

Methods

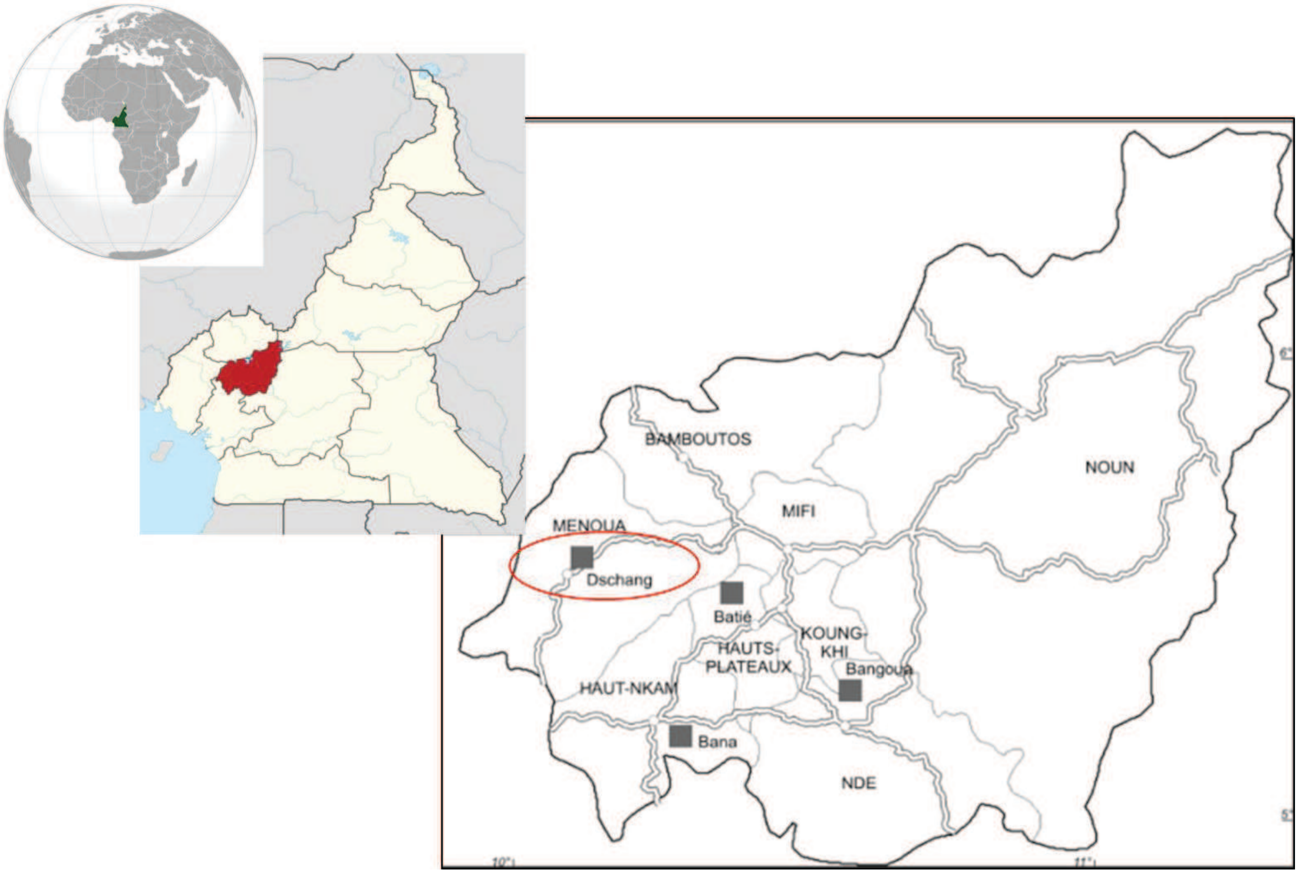
Study design and administrative procedure

This study was a cross-sectional questionnaire survey carried out between January and June 2017 among PLHIV and THPs in the city of Dschang. Permission was previously granted by the head of Dschang health district (N° 192/AR/MINSANTE/DRSPO/DSD) and the director of the Dschang District hospital (Supporting information SI. 5), respectively. Ethics approval was also obtained from the Cameroon National Ethics Committee (Supporting information SI. 6).

Study area

Dschang is the capital city of the Ménoua division, in the West Region of Cameroon (Fig. 1). Since 2015, it is home to 221,031 inhabitants and the Bamiléké are the predominant ethnic group. The city of Dschang is best known for its university (University of Dschang). The Dschang Health District is divided into 22 health areas, with a total of 66 health facilities including 1 district hospital. With a capacity of 300 beds, 10 care units and 95 medical and paramedical staff, the Dschang District Hospital is the reference health facility in the Dschang Health District. It has a care unit specially dedicated to PLHIV (UPEC, Unité de Prise en Charge), although screening, prevention and care activities are integrated into the various care services throughout the hospital. All patients attending the national HIV treatment program are required to report each month to the pharmacy for a refill of their prescriptions and at least once every three months to their referring physicians for their medical follow-up. The psychosocial support of PLHIV is mainly provided by trained health care auxiliaries (psychosocial counsellors) and to some extent the members of local HIV/AIDS associations, community relay workers and social workers.

Figure 1: Map of study area



Participants

Eligible PLHIV had to be over 18 years old, enrolled in the national HIV treatment program and consented to participate in the survey. PLHIV who met the above inclusion criteria were enrolled consecutively - without financial incentive - during routine visits to the Dschang District Hospital (UPEC).

THPs, including healers and diviners, were enrolled in this study as well. They were identified by the chairperson of Société Coopérative des Producteurs des Plantes Médicinales de l'Ouest - Cameroun (SOCOPMO, Cooperative Society of Producers of Medicinal Plants of West - Cameroon) based on their good reputation in traditional healing practice.

Interviews

A one-to-one interview of PLHIV was conducted by a trained and experienced psychosocial counsellor in their preferred language (English or French) using a questionnaire. Likewise, THPs were interviewed by Maurice Kenzo, chairperson of SOCOPMO. In all cases, the interview did not last more than 30 minutes.

The questionnaire addressed to PLHIV was designed following Loraschi et al. (2016) with adaptations and was pretested before being used for the interview. It was a 15-item mixed questionnaire structured into three main sections (Supporting information SI. 7, Annex 1). The first section was dedicated to the socio-demographic characteristics of the respondents including gender, education level, home location, ethnicity and profession. The second part focused on the duration since HIV diagnosis, the current cART regimen and associated benefits/side effects. In the last section, study participants were asked about their MP use experience for HIV symptoms management and/or related conditions. MP users were invited to provide information about the names of plants, the reasons for MP use, the modality of their use as well as ratings of desired and unwanted effects.

The HIV Knowledge Questionnaire (HIV-KQ-18) designed by Carey and Schroder (2002) was used with slight adaptations as research instrument to assess the HIV-related knowledge of THPs (Supporting information SI. 7, Annex 2). For each knowledge item, THPs were invited to select one answer from a defined list of three choices including “true”, “false” and “don’t know”. The correct answers were scored as “1” and the incorrect/“don’t know” responses as “0”. The percentage of correct answers were determined for each HIV knowledge item following Saddki et al. (2016). Further, THPs who reported to use MP in treating HIV/AIDS and related symptoms, were interviewed about attitudes towards MP.

The questionnaires were anonymous to ensure confidentiality, to avoid possible reticence of PLHIV to unveil the use of MP to their healthcare givers, as well as to overcome any potential ethical and/or legal issues related to the use of illegal or endangered MP by PLHIV or by THPs.

Botanical identification of MP

MP users were invited to provide photographs and/or specimens of plants. If needed, field trips were made, and plants were sampled and deposited at the University of Dschang (Laboratory of Phytochemistry, Department of Biochemistry). At the end of the survey, a complete set of plant specimens, together with the collection notes (plant parts, photographs, vernacular/local name, habitat, and traditional use) were sent to the National Herbarium of Cameroon for botanical identification (Supporting information SI. 8).

Statistical analysis

Data were processed in MS Excel and subsequently validated following the International Quality Standard ISO 28590:2017 guidelines. Outcome variables were presented as counts or percentage where appropriate. The chi-square analysis was performed at the 5% level of significance using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com).

Results

Demographics of the study participants

A total of 247 PLHIV were enrolled in this survey. Descriptive baseline characteristics of HIV-infected participants are illustrated in Table 1. Participants were more than 70% females, about 82% with primary or secondary education but only less than 9% with university degree, 1 out of three with disease duration ≤2 years and 1 out of five >9 years, more than 60% with work (although usually precarious, data not shown), almost all on cART and more than 90% satisfied with it.

On the other hand, 16 THPs were surveyed, including 13 traditional healers and 3 diviners. Most of them were males (n = 13, 81.3%) and had at least 10 years of seniority in traditional health practice (n = 10, 62.5%).

Table 1: Demographics of HIV-infected participants and association with MP use. N: sample size; AZT: zidovudine; 3TC: lamivudine; NVP: nevirapine; TDF: tenofovir; EFV: efavirenz

Features	**N (%)**	**MP nonusers (%)**	**MP users (%)**	**p-value**
Gender				0.5637
Female	176 (71.3)	73 (68.9)	94 (72.9)	
Male	71 (28.7)	33 (31.1)	35 (27.1)	
Level of education				0.0304
Primary	129 (52.2)	51 (48.1)	75 (58.1)	
Secondary	75 (30.4)	34 (32.1)	35 (27.1)	
University	19 (7.7)	12 (11.3)	6 (4.7)	
Unschooled	4 (1.6)	0 (0.0)	0 (0.0)	
Missing	20 (8.1)	9 (8.5)	13 (10.1)	

Duration of illness in years (Years since HIV diagnosis)				0.0019
≤2 years	83 (33.6)	46 (43.4)	34 (26.4)	
3-5 years	60 (24.3)	24 (22.6)	35 (27.1)	
6-9 years	47 (19.0)	19 (17.9)	25 (19.4)	
>9 years	49 (19.8)	16 (15.1)	29 (22.5)	
Missing	8 (3.2)	1 (0.9)	6 (4.7)	
Current cART regimen				0.1386
TDF/3TC+EFV	219 (88.7)	97 (91.5)	112 (45.3)	
TDF/3TC+NVP	6 (2.4)	0 (0.0)	4 (1.6)	
AZT/3TC/NVP	19 (7.7)	7 (6.6)	12 (4.9)	
No treatment	1 (0.4)	0 (0.0)	1 (0.4)	
Missing	2 (0.8)	2 (1.887)	0 (0.0)	
Occupational status				0.0004
Yes	152 (61.5)	60 (56.6)	43 (33.3)	
No	93 (37.7)	45 (42.5)	85 (65.9)	
Missing	2 (0.8)	1 (0.9)	1 (0.8)	
Satisfaction with cART regimen				0.5537
No	14 (5.7)	4 (3.8)	8 (6.2)	
Yes	228 (92.3)	101 (95.3)	117 (90.7)	
Missing	5 (2.0)	1 (0.9)	4 (3.1)	

Prevalence of MP Use among PLHIV

A total of 235 HIV positive participants have answered the question about the use of MP (response rate = 95.1%). Respondents reporting the use of MP but not answering open-ended questions about MP and vice-versa were considered MP users. Overall, 129 PLHIV (54.9% of the respondents) declared having used MP to manage HIV symptoms and related conditions (Table 1). MP users were mainly females (n = 94, 72.9% of MP users) and came from the West region of Cameroon (n = 123, 95.3%). Most had 3-5 years of illness (n = 35, 27.1%) and primary school education level (n = 75, 58.1%). Nearly all MP users were currently receiving the first-line cART (n = 128, 99.2%) and most of them were satisfied with their therapy (n = 117, 90.7%). In comparison to nonusers, MP users were less educated, had longer HIV diagnosis duration and were more often unemployed (Table 1). However, there was no difference between MP users and nonusers regarding gender, current cART regimen and satisfaction with cART regimen. Dissatisfaction with cART was reported only by few subjects (n = 8, 6.2%, 5 MP users and 3 nonusers) and the most frequent reason was side effects including abdominal pain (n = 1), abdominal pain and diarrhoea (n = 1), dizziness (n = 1), heart palpitation and burning feet (n = 1), unspecified (n = 1). Reasons for dissatisfaction are listed in Table 2.

Table 2: Reasons for dissatisfaction with current cART regimen. n: number of citations.

Reported reasons for dissatisfaction with cART	**N**	**MP users (*n*)**	**MP nonusers (*n*)**
side effects	5	2	3
“decline in CD4 count”	2	2	0

"I stopped cART therapy a year ago because I did not perceive any improvement in my HIV serological status"	1	1	0
"my work does not allow me to take my pills"	1	1	0
"no change in serological status"	1	1	0

Reasons and determinants of MP use

MP were used with conventional drugs to manage non-HIV-related diseases (n = 94, 72.9% of MP users), to recover from HIV-related conditions (n = 83, 64.3%), or to cure HIV/AIDS (n = 12, 9.3%). Commonly reported pathological conditions treated with MP included malaria, cough and abdominal pain. The complete list is shown in Table 3. Interestingly, MP users claimed moderate (n = 60, 57.7%) to complete (n = 35, 33.7%) relief of these diseases/conditions. Only 8 subjects (7.7% of MP users) admitted that MP had not been beneficial to them. Of 6 individuals who answered the questions regarding the unwanted effects associated with MP use, only 2 subjects (33.3 % of respondents) denounced fatigue and weight loss.

Table 3: Frequently reported diseases and conditions (n > 5) treated with MP

Diseases/conditions	**Occurrence (*n*)**	**% of total reports**
Malaria	27	18.4
Cough	20	13.6
abdominal pain	16	10.9
typhoid fever	13	8.8
dysentery	11	7.5

Fever	10	6.8
weakness/fatigue	10	6.8
diarrhoea	9	6.1
headache	9	6.1
anaemia	7	4.8
Bile	5	3.4
skin problems	5	3.4
stomach hurts	5	3.4

Reported MP and modality of use

Of the 70 MP mentioned by 106 informants (82.2% of total MP users, mean±SEM: 2.2±0.2 MP/subject, min 1, max 11), 49 plant species have been botanically identified, among which the most popular (in at least 4 users) are listed in Table 4. MP were mainly herbs and were usually used after PLHIV started the antiretroviral treatment (n = 46, 63.0% of respondents). The plant parts most commonly collected for the preparation of herbal remedies included leaves (n = 129, frequency of citations: 68.3%), bark (n = 17, 9.0%) and fruits (n = 16, 8.5%). Other ingredients including palm oil, palm wine, and palm kernel oil, were often added. Most frequently, MP were administered orally in the form of decoction and without any specific dosage or mode of conservation. They were collected and/or purchased from fields (n = 79, frequency of citations: 66.4%), market (n = 26, 21.8%), traditional healers (n = 13, 10.9%) and others (n = 1, 0.8%). MP were used as self-medications (n = 63, frequency of citations: 55.8%) or upon the recommendation of THPs (n = 42, 37.2%), acquaintance and relatives (n = 6, 5.3%), nurse (n = 1, 0.9%) and media (n = 1, 0.9%).

Table 4: List of the most popular (in at least 4 users) MP.

Users (n)	Scientific names	Vernacular/common names	Part used	Reasons for use												
				abdominal pain	anaemia	Bile	cold	cough	diarrhoea/dysentery	fever	stomach hurts	headache	malaria	typhoid fever	weakness	other
20	*Cymbopogon citratus (DC.) Stapf*	Fipagrassi; Fhou Ngouoya/ Citronelle	L				x	X		X		x	x	x		x[1]
16	*Psidium guajava L.*	Goyavier	B; L; R	x		X		X	x	X		x	x	x	x	x[2]
15	*Carica papaya L.*	Papayer	F; L; R	x				X	x				x	x		x[3]

[1] Oedema, hypertension, itching, diabetes
[2] indigestion
[3] "poison de nuit": it refers to the afflictions suffered after a meal ingested during a dream

12	*Ageratum conyzoides (L.) L.*	Tchouamo'o/ roi des herbes	L	x		X		X		X	x	x	x	x		x[4]
12	*Mangifera indica L.*	Manguier	B; L; R	x					x			x	x	x	x	
11	*Citrus limon (L.) Osbeck*	Citron	F	x				X	x			x	x	x	x	x[5]
9	*Aloe barbadensis Mill.*	Aloe	L	x		X			x	X	x	x	x			
7	*Ananas comosus (L.) Merr.*	Ananas	F										x	x		
7	*Manihot esculenta Crantz*	Pkwem/ Cassava	L		x										X	x[6]
6	*Eucalyptus globulus Labill.*	Ecalyptus	L					X		X						

[4] Chest pain, nightmare
[5] Tuberculosis
[6] Foot cramps

6	*Ocimum gratissimum L.*	Massep; basilic sauvage; Kotmajo	L				x						x			x[7]
5	*Persea americana Mill.*	Pia’a/avocat	L										x	x		
4	*Eremomastax speciosa (Hochst.) Cufod.*	Houeou;Wouomekwa; Panzem ze mo’/ rouge un côté	L	x	x											x[8]
4	*Kalanchoe crenata (Andrews) Haw.*	Djoudjou; Ntonkenou’	L			X		X								x[9]
4	*Vernonia amygdalina Delile*	bitter leaf/ Ndolè	L	x						X	x		x	x		
4	Unidentified	Tseutseuneck/ épingle noir											x	x		

[7] Nausea, constipation
[8] Nappy rash
[9] Otitis

HIV-related knowledge of THPs and their attitudes

Few TPHs interviewed (n = 3, 18.8%) acknowledged the use of MP to manage diseases in PLHIV. Specifically, the leaves of *Aloe vera* (n = 3), *Moringa oleifera* (n = 1) and "Mbeuheu Ser" (n = 1), were administered orally in the form of infusion or decoction with the purpose to treat symptomatic conditions such as diarrhoea (n = 1). Other desired goals include strengthening immune function (n = 1) and increasing CD4+ T cell count (n = 1). Beyond the bitter taste of MP extracts, HIV-infected clients claimed no unwanted effects.

The HIV-related knowledge of THPs as assessed by the frequency of correct answers to the adapted version of HIV-KQ-18, was relatively low (48.0% of correct answers). In particular, all THPs believed that HIV and AIDS are the same, that there is a cure for AIDS and that pulling out the penis before a man climaxes/cum keeps a woman from getting HIV during sex, and only 1 out of 16 knew that there is no vaccine so far for HIV. Moreover, less than 2 out of 3 THPs provided correct answers to most of the remaining items (Table 5). Interestingly, all THPs knew about the possibility to spread HIV from mother to newborn or through just only one sexual intercourse, and that having sex with many partners increases the chance to be infected with HIV, and most of them knew about female condoms (14 out of 16), and that HIV is not spread by mosquitoes (12 out of 16). THPs using MP provided a higher proportion of correct answers in comparison to nonusers, but the difference was not statistically significant (58.3% of correct answers vs 45.7% for the nonusers, P = 0.149).

Table 5: Frequency of correct answers for each HIV-related knowledge item. Correct answers appear in parentheses (T = true; F = false).

HIV knowledge item	N (%)	Users (%)	Nonusers (%)
A pregnant woman with HIV can give the virus to her unborn baby (T)	16 (100)	3 (100)	13 (100)
A person can get HIV even if she or he has sex with another person only one time (T)	16 (100)	3 (100)	13 (100)
Having sex with more than one partner can increase a person's chance of being infected with HIV (T)	16 (100)	3 (100)	13 (100)
There is a female condom that can help decrease a woman's chance of getting HIV (T)	14 (87.5)	3 (100)	11 (84.6)
HIV can be spread by mosquitoes (F)	12 (75.0)	3 (100)	9 (69.2)
It is possible to get HIV when a person gets a tattoo (T)	10 (62.5)	3 (100)	7 (53.8)
A person can get HIV from a toilet seat (F)	9 (56.3)	3 (100)	6 (46.2)
A person can get HIV through contact with saliva, tears, sweat, or urine (F)	9 (56.3)	2 (66.7)	7 (53.8)
A person with HIV can look and feel healthy (T)	7 (43.8)	2 (66.7)	5 (38.5)
Coughing and sneezing DO NOT spread HIV (T)	5 (31.3)	0 (0.0)	5 (38.5)
Taking a test for HIV one week after having sex will tell a person if she or he has HIV (T)	5 (31.3)	3 (100)	2 (15.4)

Showering, or washing one's genitals/private parts, after sex keeps a person from getting HIV (F)	3 (18.8)	0 (0.0)	3 (23.1)
There is a vaccine that can stop adults from getting HIV (F)	1 (6.3)	0 (0.0)	1 (7.7)
HIV and AIDS are the same thing (F)	0 (0.0)	0 (0.0)	0 (0.0)
There is a cure for AIDS (F)	0 (0.0)	0 (0.0)	0 (0.0)
Pulling out the penis before a man climaxes/cums keeps a woman from getting HIV during sex (F)	0 (0.0)	0 (0.0)	0 (0.0)
Total	123 (48.0)	28 (58.3)	95 (45.7)

Discussion

The present study sought to gain a cross-sectional picture of the overall use of MP in HIV-infected population. The city of Dschang was purposefully selected as a pilot site due to its rich biodiversity, long tradition of MP use and multi-ethnic population (19). Moreover, Dschang can be regarded as "Cameroon in miniature" because it exhibits both the rural and urban landscapes of the country and traditional health beliefs are widespread in the countryside (20).

Out of 247 PLHIV consecutively enrolled in this study, 54.9% reported the use of at least 1 MP. This finding is not so surprising as it reflects the status of the use of MP, estimated to nearly 80% in the general population in Cameroon (21,22). However, our prevalence estimate is marginally higher than the 33.7% use prevalence in Uganda and clearly lower than the 97.3% use prevalence among PLHIV in Trinidad (Bahall, 2017; Namuddu et al., 2011). This discrepancy could stem from the diverse sociodemographic features of the informants, and from the heterogeneity in the design of the different surveys.

Typical MP users were less educated than nonusers, unlike previous research (9,23,26–29). This can be explained by the fact that PLHIV with a low education level would be more likely to be influenced in their decision to use MP. In line with prior studies (9,29,30), PLHIV with longer disease duration were more inclined to use MP than nonusers. One possible explanation could be that the long-term toxicity of cART, as well as its lack of effectiveness over time, may lead PLHIV to seek alternative and/or complementary medicines such as MP. In the same vein, MP users were more likely to be unemployed, which may financially handicap their access to conventional therapies. This observation, however, was not in keeping with previous research in which MP use was associated with greater financial resources (27,31).

Use of MP is considered a proxy for the tolerability and efficacy of conventional therapies (16). In the current study, however, almost all MP users were satisfied with their cART regimen. Notwithstanding, they used MP, not in lieu of standard therapies, but rather as an adjunct to manage conditions that may be related to HIV or cART side effects. This finding, somewhat surprising, should be perceived in a positive tone since the use of herbal remedies did not compromise adherence to antiretroviral therapy which is a pressing concern among MP users (32,33). Moreover, it indicates that besides being a therapeutic option, MP are an integral part of the lifestyle of PLHIV who wish, through their use, to align with their health beliefs. Additionally, in resource-poor settings where MP use is culturally rooted in mentalities, and where access to health care is challenging, PLHIV may be more likely to use MP.

Most HIV-infected participants reported to retrieve benefits from the use of MP. Anecdotally, in the present survey, two MP users have claimed *Combretum micranthum G.Don* was able to clear HIV. Whether these claims correspond to a real clinical benefit awaits future research.

Interestingly, unpleasant side effects related to MP use were denounced only in few cases and included fatigue and weight loss. Some caution is however required in interpreting this finding since users are less likely to report MP-related side effects (34). All in all, these findings suggest that MP are perceived as a safer complementary mean to manage HIV symptoms and related conditions.

MP were usually harvested from wild and this open access modality may account for their high use prevalence in HIV-infected population. Most frequently, the leaves were used, which is less detrimental to the plants. One of the reported MP, namely *Garcinia kola Heckel* is listed as endangered species in Cameroon since 2004 (35). The most commonly mentioned MP including *Aloe barbadensis Mill.*, *Ageratum conyzoides (L.) L.*, *Mangifera indica L., Cymbopogon citratus (DC.) Stapf*, *Eucalyptus globulus Labill.*, *Ocimum gratissimum L.*, *Carica papaya L.*, *Vernonia amygdalina Delile*, *Persea americana Mill.*, and *Psidium guajava L.*, have also been reported in other surveys investigating MP use by PLHIV in different countries such as Uganda (36,37), Gabon (38), South Africa (39) and Nigeria (40), indicating convergent local ethnobotanical and ethnomedical traditions, possibly suggesting shared ethnobotanical knowledge due to geographical proximity and migratory processes. Among these plants, at least the leaves of Aloe species and *Persea americana Mill.* are documented for their anti-HIV1 activity (41,42). However, little is known about the efficacy and tolerability of these MP when taken concomitantly with conventional HIV medications (43,44). MP use is therefore a matter of concern for HIV care providers given the possibility of unanticipated MP-related side effects or herb-drug interactions which may jeopardize the efficacy and/or safety of standard therapies. Nevertheless, the risk of adverse drug reactions can be minimized dramatically if patients disclose their use of MP to their caring physicians (45).

In most cases, MP were self-prescribed without appropriate medical supervision, raising concern about potential interference with concurrent conventional therapies. Prior evidence suggests that PLHIV are less likely to discuss the use of MP with their caring physicians (23). The underlying motives for non-disclosure include the physicians' reluctance about MP use and patients' perceptions of MP as "natural" and, therefore, effective or at least risk-free (46–48). Additionally, MP use is not asked during consultation and patients most often are unaware of the name of herbal remedies (46,47). Doctors' reluctance to MP may come from not feeling adequately educated on MP, and the lack of supporting evidence for their efficacy and safety in humans (49). Therefore, future directions for national AIDS programmes should focus on facilitating more informed patient-physician communication about MP use. In this regard, patients' descriptive characteristics including the occupational status, educational level and disease duration, may be applied by physicians in detecting the most likely MP users and, therefore, in addressing relevant and personalized educational interventions.

THPs were the main advisors of PLHIV on the use of MP, presumably because traditional medicine is more affordable, accessible and socio-culturally acceptable than allopathic medicine (50,51). Thus, in the rest of our study, the knowledge and attitudes of THPs towards HIV/AIDS were surveyed. THPs were drawn from members of SOCOPMO, a cooperative society of producers of MP, encompassing about forty THPs from all over the western region of Cameroon. Three out of the 16 THPs surveyed claimed the use of MP to manage conditions related to HIV/AIDS. Amongst the cited MP, *Moringa oleifera Lam.* and *Aloe vera (L.) Burm.f.* have also been reported elsewhere in similar studies (52–55). Though these plants are widely valued by PLHIV, there is some evidence to suggest they may compromise the efficacy of antiretroviral drugs (41,56,57). The results of our survey raise additional concerns, since THPs had insufficient knowledge and/or serious misconceptions about PLHIV, HIV transmission, the curability of HIV/AIDS and the existence of anti-HIV vaccines. Given the shortage of health staff and the burden of HIV/AIDS in this part of the globe, facilitating a collaboration between allopathic and traditional medicine is highly needed.

As a caveat, PLHIV were enrolled from attendees of the national HIV treatment program, who generally receive cART and other conventional cares (58), missing, however, the experiences of patients who were not engaged in that program. This may generate a selection bias that would make our sample not fully representative of the entire HIV-infected population. Another limitation of this research includes the fact that the informants could be exposed to recall bias. The face-to-face interview was purposely used as recommended by Hunt et al. (2010) to improve informants' recall and assist them in their answers. In this specific regard, as far as we are concerned, patients' interviews were conducted in a private room by a psychosocial counsellor to avoid their reticence to disclose the use of MP to their caring physicians and to discuss their condition freely. On the other hand, THPs' interviews were run by the chairperson of SOCOPMO to avoid them being reluctant to discuss with people uninitiated in traditional beliefs. Additionally, we relied solely on self-reported MP use to categorize study participants into users and nonusers, and this approach can sometimes be inaccurate. In prospect, it would be worth considering the possibility to assess the plasma concentrations of the reported MP or their metabolites in all study participants, as well as to collect data from the hospital records of PLHIV, to objectively assess their clinical conditions. The lack of statistically significant differences between MP users and nonusers among THPs may be due to the small sample size. Therefore, future research is required in a larger sample. This study may however lay the groundwork for designing proper data collection instruments for a countrywide survey.

Conclusions

To the best of our knowledge, this exploratory study is the first one that showcases the overall use of MP among PLHIV in Cameroon. Overall, MP use is popular within HIV-infected population, making it crucial to scrutinize meticulously the risks and benefits associated with this practice. Given the possibility that MP may jeopardize the efficacy and tolerability of conventional therapies, physicians need to be educated on the use of MP. Likewise, they should systematically assess the use of MP in their patients during routine visits to provide them with appropriate advice. Traditional knowledge of MP should be examined on purpose to make the most of the use of plants. Moreover, THPs need to be educated on HIV/AIDS, as part of a program aiming to integrate traditional medicine in a global response to HIV/AIDS epidemic.

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Author Contributions

Conception and design of the survey: FM MC AMT PCBN ATT GP. Data collection: ATT PCBN AKEE GP MANN. Data analysis: MC AMT FM. Interpretation of results: MC FM AMT ATT MANN. Drafting of the manuscript: MC AMT FM. All authors were involved in revising it critically for important intellectual content, and all authors approved the final version to be published. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved and declare to have confidence in the integrity of the contributions of their co-authors.

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CHAPTER 3: Comparison of a *Cannabis sativa L.* extract and its Derivative Cannabidiol on Human Polymorphonuclear Leukocyte Function

(This work is currently under review)

Abstract

Cannabis has long history of use for medical purposes. Several reports support that cannabis and its derivative cannabidiol (CBD) offer significant therapeutic benefits for a wide scope of pathological conditions. Among them, the clinical issues rooted in inflammation do stand out, nonetheless the underlying mechanisms are not yet plainly understood. Circumstantial evidence points to polymorphonuclear neutrophil leukocytes (PMN) are targets for the anti-inflammatory effects of cannabis. Therefore, we conducted this study to assess the effects of a cannabis oil extract standardized in 5% CBD (CM5) on human PMN functions, including cell migration, oxidative metabolism and production of proinflammatory cytokines. We then sought to investigate whether such effects could be ascribed to its content in CBD. As a result, we found that CM5 0.05-50 μg/mL and CBD 10^{-8}-10^{-5} M inhibit PMN functions to a comparable extent, indicating that CBD may be the main responsible of the anti-inflammatory effects of cannabis. The effects of CBD and CM5 show however remarkable differences, suggesting that beyond CBD, other components of cannabis may contribute to its biological effects. As a whole, such results support the use of cannabis and CBD to stem inflammation, however also warrant in-depth investigation of the underlying cellular and molecular mechanisms to better exploit their therapeutic potential.

Introduction

Cannabis (*Cannabis sativa L.*, fam. Cannabaceae) has long been accredited with medicinal properties (Zlas et al., 1993). Currently, several reports support that it offers significant therapeutic benefits for a wide scope of pathological conditions, most of which are rooted in inflammation (recently reviewed by (Abrams, 2018).

Medical cannabis is now available in many countries either on recommendation by doctors for their patients or even on prescription. However, a world of controversy related to the addictive potential of this plant species surrounds its use in clinical practice. Having been able to largely identify the compounds responsible for the unwanted psychotropic activity of cannabis, many states have passed legislation limiting its content of Δ^9-tetrahydrocannabinol (Δ^9-THC), known as the major psychoactive component of cannabis (Mechoulam and Gaoni, 1967), to the benefit of cannabidiol (CBD).

Indeed, CBD occurs naturally in appreciable amounts in the leaves, seeds, stalk and flowers of cannabis plants (Andre et al., 2016). Currently, CBD is generating a huge therapeutic interest because it is devoid of any drug abuse liability (Babalonis et al., 2017). In addition, CBD carries no meaningful side effects across a wide dose range (up to 1500 mg/day p.o.) in humans (Bergamaschi et al., 2011; Iffland and Grotenhermen, 2017). More importantly, it provides a large variety of therapeutic potentials (reviewed by (Pisanti et al., 2017). Among them, its ability to stem inflammation do stand out (Burstein, 2015), nonetheless, the underlying mechanisms are not yet plainly understood. Circumstantial evidence suggests that polymorphonuclear neutrophil leukocytes (PMN) may be involved in the anti-inflammatory effects of CBD (Krohn et al., 2016; McHugh et al., 2007; Wang et al., 2017).

Based on this background, we conducted this study to assess the effects of a cannabis oil extract standardized in 5% CBD (CM5) on human PMN functions, including cell migration, oxidative metabolism and production of proinflammatory cytokines. We then sought to investigate whether such effects could be ascribed to its content in CBD.

Materials and Methods

Test substances: Cannabis oil extract containing 5% CBD (dark green viscous liquid, batch n° 74717009) and pure CBD (white/off-white or slightly yellow powder, batch n° P54/29/046) were kindly provided by LINNEA SA. Certificates of analysis of both reagents are provided as supporting information. Solutions were prepared in dimethylsulfoxide (DMSO, Sigma) and further diluted in either Hanks' Balanced Salt Solution (HBSS) modified with 10 mM HEPES or RPMI medium to obtain final concentrations (CM5 0.05-50 μg/mL and CBD 10^{-8}-10^{-5} M).

Isolation of human PMN: Human PMN were obtained from buffy coats of blood donations from consenting healthy donors through the courtesy of the local blood bank (Ospedale di Circolo, Fondazione Macchi, Varese, Italy). In brief, PMN were isolated by Dextran sedimentation followed by Ficoll-Paque Plus density-gradient centrifugation (GE Healthcare, Milan, Italy), as described previously (Scanzano et al., 2015). Contaminating erythrocytes and platelets were eliminated by 5-min hypotonic lysis in distilled water with added NH4Cl 8.3 g/L, KHCO3 1.0 g/L, and ethylenediamine tetraacetic acid 37 mg/L. Cells were then washed twice in NaCl 0.15 M. Experiments were performed only when the purity and viability of isolated PMN as assessed by light microscopy, were over 95%.

Cytotoxicity assays: Cytotoxicity of test substance was assessed on PMN by means of the MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2Htetrazolium bromide] reduction method as previously described by (Mosmann, 1983). In short, freshly isolated PMN were resuspended at 1 × 10^6 cells/ml in RPMI 1640 medium supplemented with 10% FBS and 1% penicillin/streptomycin. Cells were then seeded in duplicate in a 96-well round bottom plate (250 μl of suspension per well) and cultured for 24 h in the presence or absence of test substance at 37 °C in 5% CO_2. The absorbance (OD) was measured using a microplate spectrophotometer with a 570 nm test wavelength and a 690 nm reference wavelength. Results were expressed as mean absorbance value of duplicates.

Reactive oxygen species (ROS) production assay: Intracellular ROS production was assayed by use of the redox-sensitive dye C-DCFH-DA (Molecular probes, Eugene, Oregon, USA) as previously described by (Cosentino et al., 2008). Fluorescence was measured by means of spectrofluorimeter (PerkinElmer LS-50B, PerkinElmer Instruments, Bridgeport, CT, USA) set at 488 nm excitation wavelength and 525 nm fluorescence emission. In each experiment, the test substance was added to the cells after a 60-s resting period, either alone (resting conditions), together with (coincubation) or 1 h before (preincubation) 0.1 μM N-formyl-Met-Leu-Phe (fMLP; Sigma–Aldrich). ROS changes, expressed as fluorescence intensity in arbitrary units (AU), were calculated as the difference (Δ) between resting levels and peak levels induced by the treatment.

Cell migration assay: PMN migration was investigated by the modified Boyden chamber assay according to our previous study (Marino et al., 2018). Briefly, after instrument assembly, the test substance was added in the upper chamber to PMN alone and in the presence of 10 ng/mL interleukin-8 (IL-8, Sigma–Aldrich) or 0.1 μM fMLP in the lower chamber. In some experiments, a spontaneous migration was run with no stimulus in the lower chamber. Both chambers were separated by a 3 μm pore-sized filter. After a 90-min incubation at 37 °C, the filter was harvested, dehydrated, fixed, and finally stained with haematoxylin. PMN migration was then quantified by light microscopy measuring the distance (in μm) from the surface of the filter to the leading front of cells.

Real-time PCR of IL-8, IL-6, TNF-α mRNA: Freshly isolated PMN were resuspended in RPMI 1640 medium. The test substance was then added to PMN alone, or in the presence of 0.1 μM fMLP. Following 3 h incubation at 37 °C in 5% CO_2, cells were harvested, and total

RNA was extracted by PerfectPure RNA Cell Kit™ (5 Prime). The amount of extracted RNA was estimated by spectrophotometry at λ= 260 nm. Total mRNA obtained from PMN was reverse-transcribed using a random primer, high-capacity cDNA RT kit (Applied Biosystems©). The amount of obtained cDNA was estimated by spectrophotometry at λ = 260 nm, in order to start Real-Time PCR reaction with a cDNA concentration of 1 μM. cDNA was amplified with SsoAdvanced™ Universal Probes Supermix (BIORAD) for analysis of IL-8, IL-6, TNF-α gene expression (Supporting information SI. 9). Linearity of real-time PCR assays were tested by constructing standard curves by use of serial 10-fold dilutions of a standard calibrator cDNA for each gene and regression coefficients (r^2) were always >0.999. Gene expression level in a given sample was represented as $2^{-\Delta Ct}$ where ΔCt = [Ct (sample) - Ct (housekeeping gene)]. Relative expression was determined by normalization to 18S cDNA (analysed by StepOne software™ 2.2.2 - Applied Biosystems).

Statistical analysis: Data are presented as mean±SD. Differences between groups were assessed by Student's *t*-test using Microsoft Excel 2016. $P<0.05$ was considered statistically significant.

Results

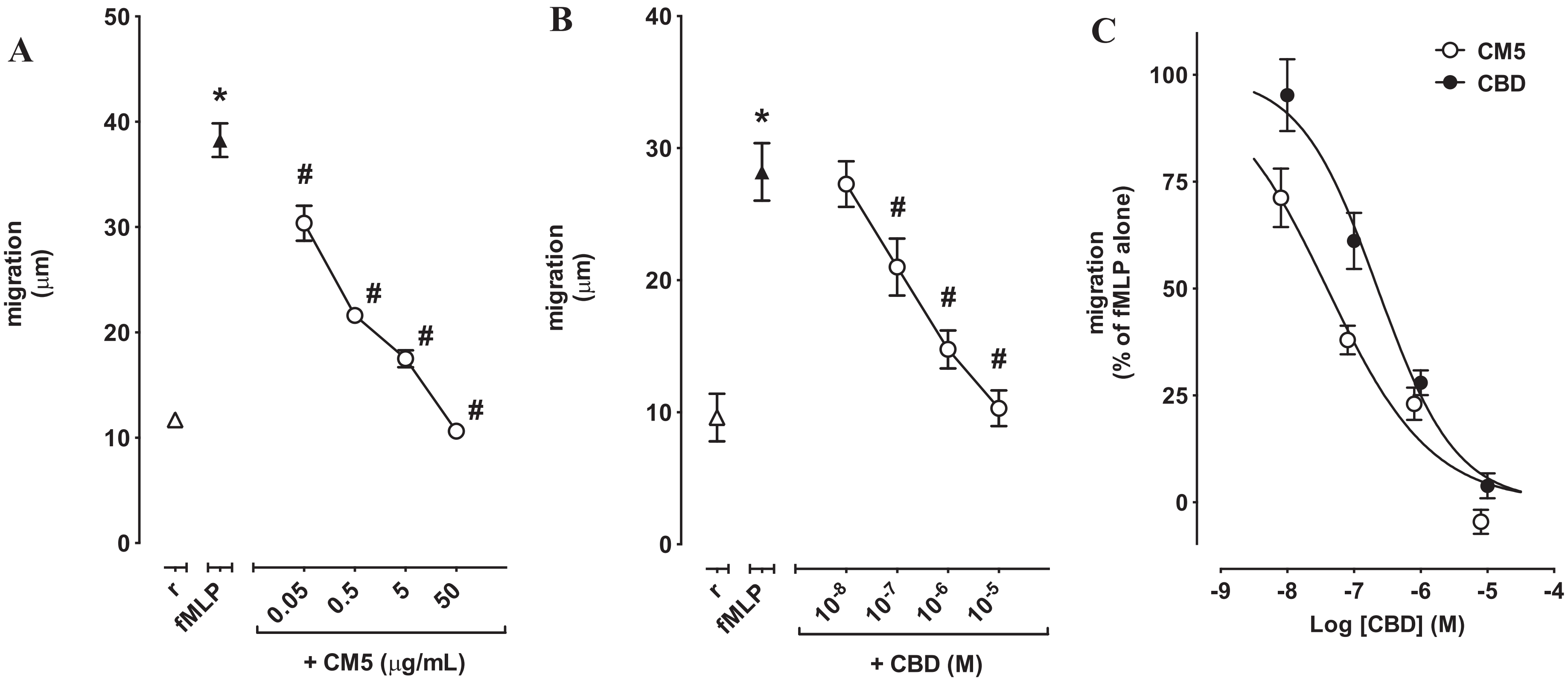
Cytotoxicity of CM5 and CBD on PMN

CM5 0.05-50 μg/mL and CBD 10^{-8}-10^{-5} M carried no meaningful effect on PMN viability in comparison to the control groups (data not shown).

Effects of CM5 and CBD on migration of fMLP-stimulated PMN

PMN migration was increased by fMLP [from 11.6±1.6 μm in resting cells up to 37.0±4.7 μm, $P<0.0001$], and this effect was concentration-dependently reverted by CM5 0.05-50 μg/mL down to 10.6±1.6 μm (*$P<0.0001$* vs fMLP, n = 8-11, Fig. 1A). Likewise, CBD at about equimolar concentrations (10^{-8}-10^{-5} M) decreased fMLP-induced migration in a concentration-dependent way [from 30.2±5.0 μm down to 10.3±3.0 μm, n = 5-8, *$P<0.0001$* vs fMLP, Fig. 1B]. Interestingly, CBD was less active on migration inhibition of fMLP-stimulated PMN [IC_{50} = 2.3*10^{-7} M, 95% confidence interval (CI) 1.4*10^{-7} - 3.8*10^{-7} M] than CM5 [IC_{50} = 4*10^{-8} M (expressed in CBD equivalent), 95% CI 2.4*10^{-8} - 6.7*10^{-8} M] (Fig. 1C). In resting conditions, however, neither CBD nor CM5 did not affect PMN migration (data not shown)

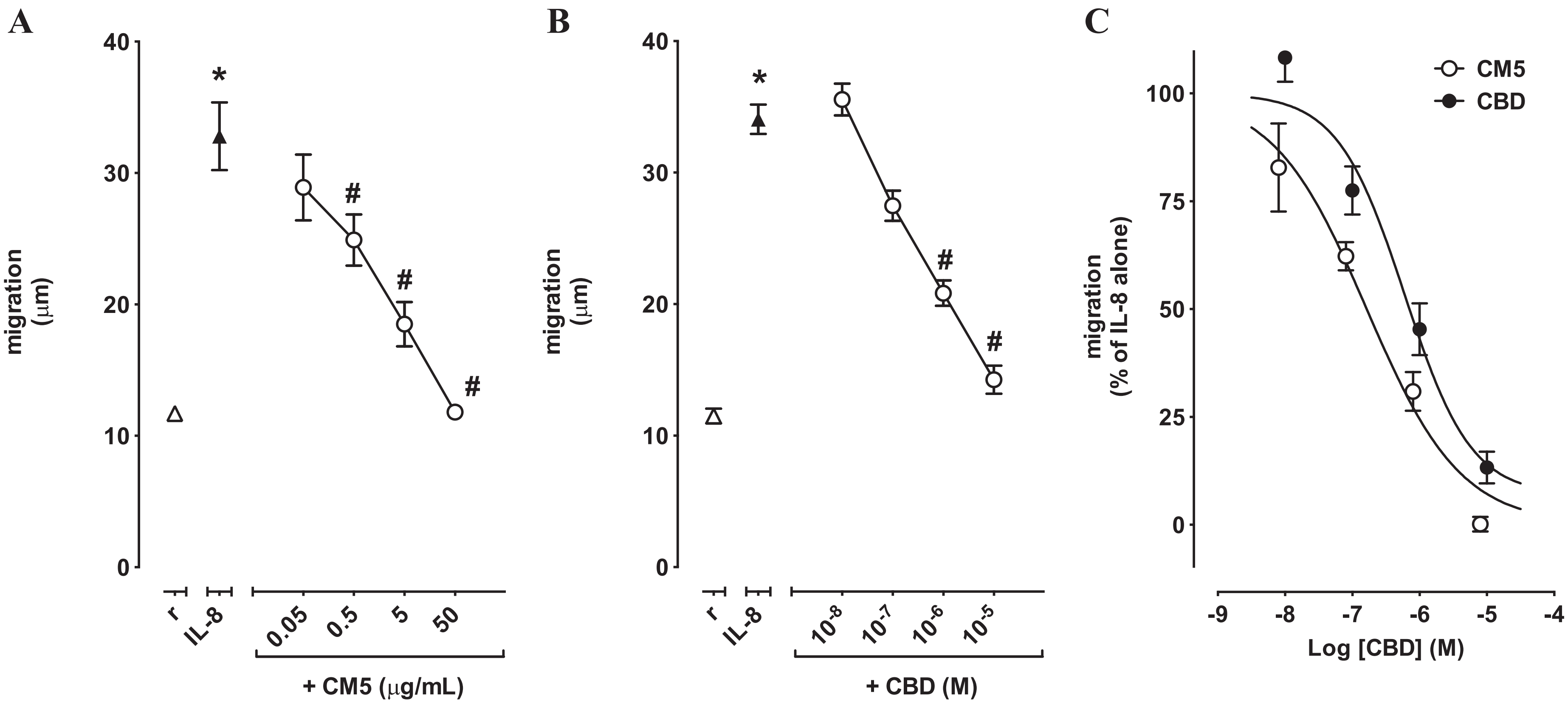
Figure 1. Effects of CBD and CM5 on fMLP-induced PMN migration. Panel A and B: Values are mean±SD (n = 5-11). * = *P<0.0001* vs resting and # = *P<0.001* vs fMLP alone. Panel C: Values represent the percent of inhibition of migration of fMLP–stimulated PMN.



Effects of CM5 and CBD on IL-8-induced migration of PMN

IL-8 increased PMN migration [from 11.7±0.8 μm in resting cells up to 32.8±5.8 μm, *P<0.001*], and such an effect was concentration-dependently reverted by CM5 0.05-50 μg/mL down to 11.8±1.1 μm (n = 5, *P<0.0001* vs fMLP, Fig. 2A). Similarly, CBD at about equimolar concentrations (10^{-8}-10^{-5} M) reversed IL-8-induced PMN migration [from 34.1±3.3 μm down to 14.3±2.6 μm, n = 7-13, *P<0.0001*, Fig. 2B]. However, the inhibitory effect of CBD on cell migration [$IC_{50} = 6.0*10^{-7}$ M, 95% CI $2.1*10^{-7}$ - $1.7*10^{-6}$ M] was less pronounced than that of CM5 [$IC_{50} = 1.6*10^{-7}$ M (expressed in CBD equivalent), 95% CI $9.0*10^{-8}$ - $3.0*10^{-7}$ M] (Fig. 2C). Likewise, neither CBD nor CM5 did not affect PMN migration in resting conditions (data not shown)

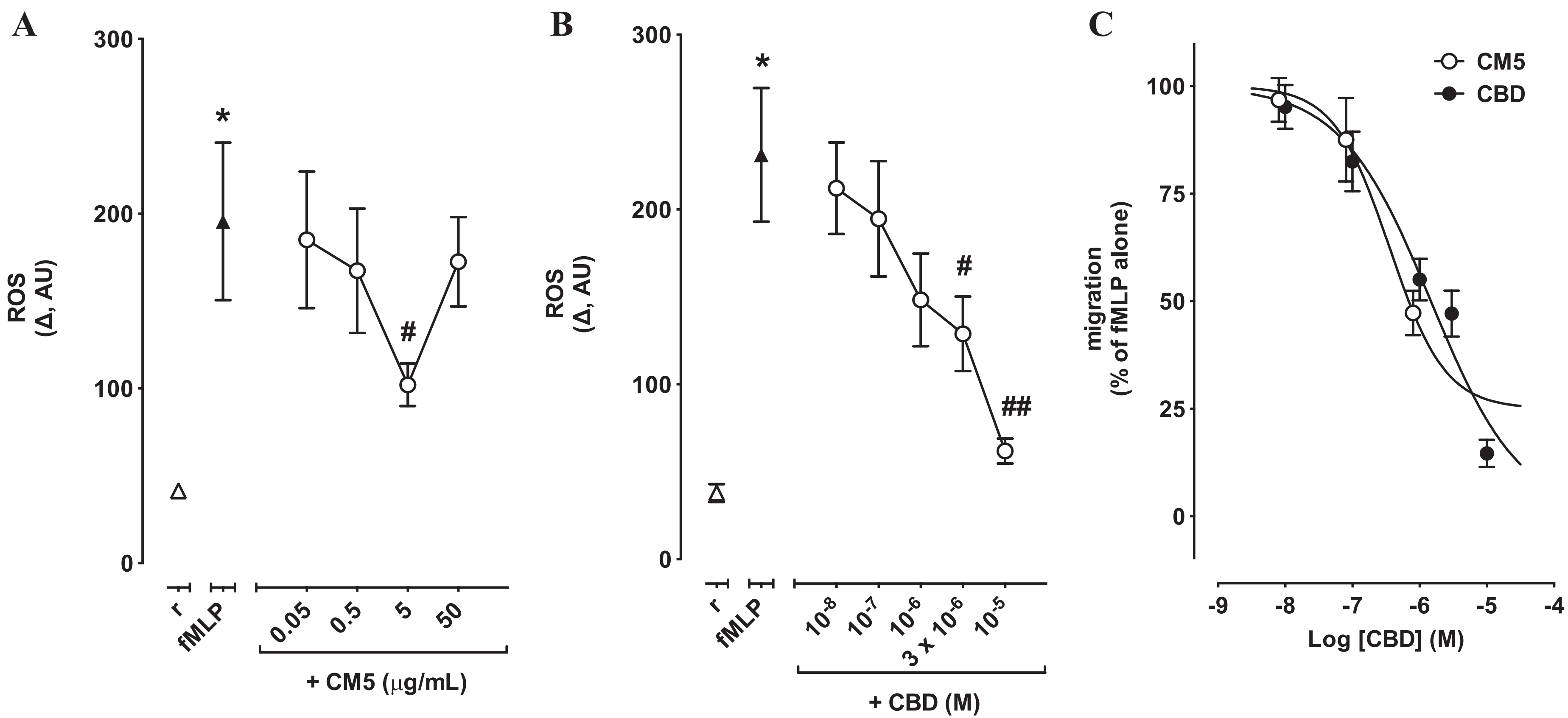
Figure 2. Effects of CBD and CM5 on IL-8-induced PMN migration. Panel A and B: Values are mean±SD (n = 5-13). * = *P<0.001* vs resting and # = *P<0.001* vs IL-8 alone. Panel C: Values represent the percent of inhibition of migration of IL-8–stimulated PMN.



Effects of CM5 and CBD on ROS levels in PMN

ROS production was increased by fMLP [from 41.3±10.8 AU in resting cells to 195.6±127.6 AU, *P<0.01*, Fig. 3A]. PMN preincubation with CM5 for 1 h concentration-dependently attenuated fMLP-induced ROS production reaching the maximum effect at 5 µg/mL (down to 102.1±34.4, n = 8, *P<0.05* vs fMLP alone, Fig. 3A). Surprisingly, CM5 50 µg/mL did not affect fMLP-induced ROS production and even increased ROS levels in resting PMN to a similar extent as fMLP (Supporting information, Fig. S4). In contrast, pretreatment of PMN for 1 h with CBD at about equimolar concentrations (10^{-8}-10^{-5} M) attenuated fMLP-induced ROS production in a concentration-dependent way [from 231.2±114.6 AU down to 61.9±21.4 AU at 10^{-5} M, n = 9, *P<0.01* vs fMLP alone, Fig. 3B). By comparison, CM5 [$IC_{50} = 3.6*10^{-7}$ M (expressed in CBD equivalent), 95% CI $1.8*10^{-7}$ - $7.2*10^{-7}$ M] was more active on ROS inhibition than CBD ($IC_{50} = 1.5*10^{-6}$ M, 95% CI $9.7*10^{-7}$ - $2.2*10^{-6}$ M) (Fig. 3C). Importantly, coincubation with either CBD or CM5 did not affect fMLP-induced ROS production in PMN (data not shown)

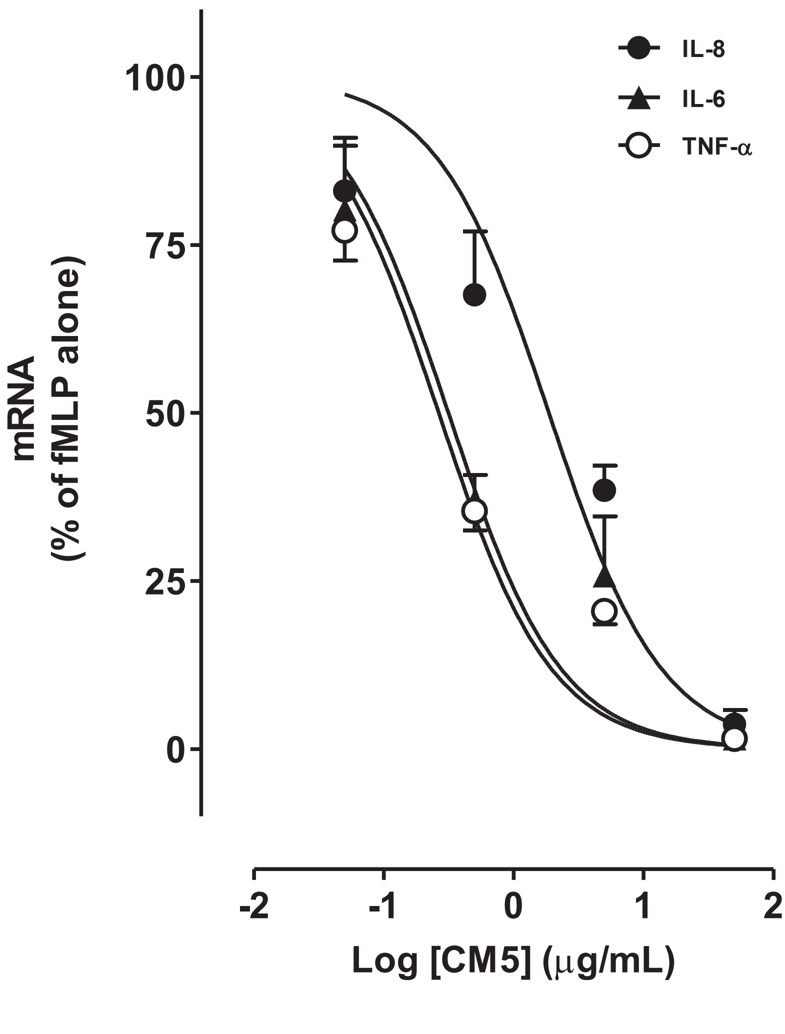
Figure 3. Effect of CBD and CM5 on fMLP-induced ROS production in PMN. Values are mean±SEM (n = 8-9). ROS changes were calculated over 30 min as the difference (Δ) between resting levels and peak levels induced by fMLP. * = *P<0.001* vs resting, # = *P<0.05* and ## = *P<0.001* vs fMLP alone.



Effects of CM5 and CBD on mRNA expression of IL-8, IL-6 and TNF-α.

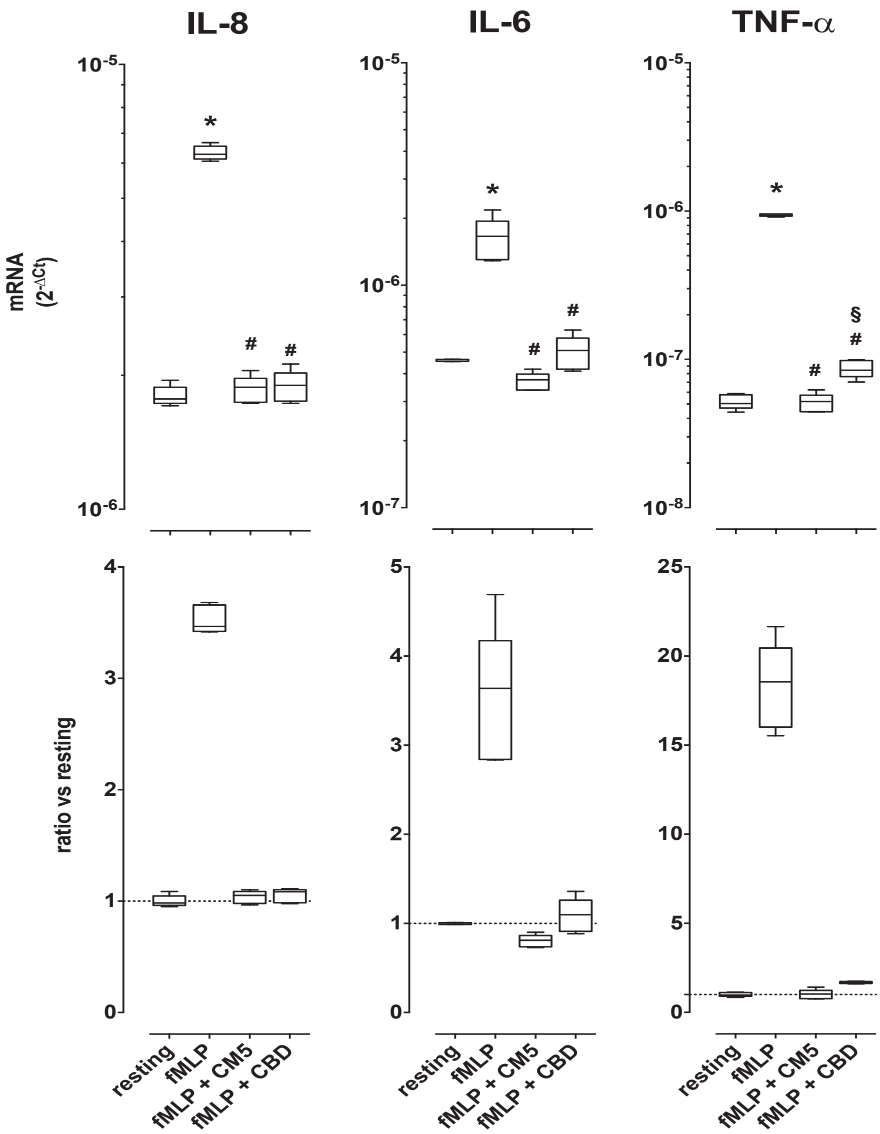
mRNA expression of IL-8, IL-6 and TNF-α was upregulated in fMLP-activated PMN and this was concentration-dependently restrained by CM5 0.05-50 μg/mL, reaching the maximum effect at 50 μg/mL (Fig. 4). Noteworthily, CM5 was less active on IL-8 mRNA (IC_{50} = 1.9 μg/mL, 95% CI 1.0 - 3.4 μg/mL) than on IL-6 mRNA (IC_{50} = 0.3 μg/mL, 95% CI 0.2 - 0.6 μg/mL) and on TNF-α mRNA (IC_{50} = 0.3 μg/mL, 95% CI 0.2 - 0.4 μg/mL). In contrast, CM5 did not affect mRNA expression of all these genes in resting PMN (data not shown).

Figure 4. Effect of CM5 on IL-8, TNF-α and IL-6 mRNA levels in PMN. Values are mean±SEM (n = 5) and are shown as percent of inhibition in fMLP-activated PMN.



Further, we decided to focus on the assessment of the effects of CM5 50 μg/mL on mRNA expression of IL-8, IL-6 and TNF-α, in comparison with CBD effects at the equimolar concentrations (10^{-5} M). As a result, both CM5 50 μg/mL and CBD 10^{-5} M reversed fMLP-induced mRNA expression of IL-8, IL-6 and TNF-α in a comparable extent (Fig. 5). Interestingly, the CM5-induced inhibitory effect on TNF-α mRNA expression was more pronounced than that of CBD 10^{-5} M ($P<0.05$ vs CBD, n = 5).

Figure 5. Effects of CM5 50 μg/mL and CBD 10^{-5} M on IL-8, TNF-α and IL-6 mRNA levels in PMN. Values are mean±SEM (n = 5) and are shown as absolute values. * = $P<0.001$ vs resting, # = $P<0.01$ and ## = $P<0.001$ vs fMLP alone.



Discussion

PMN lead the first wave of host defence against a wide range of infectious pathogens (Kolaczkowska and Kubes, 2013). They exert their role in defence through migration and homing into inflamed tissues, secretion of cytokines, proteases, reactive oxygen species (ROS) generation and neutrophil extracellular trap (NET) formation (Kumar and Sharma, 2010). However, aberration in the neutrophil response may contribute to ongoing inflammation in a plethora of diseases (Angulo et al., 2017; Cantin et al., 2015; Tsukamoto et al., 2010; Wright et al., 2014).

The present results provide compelling evidence that CM5 and CBD inhibit PMN effector functions including cell migration, oxidative metabolism and production of proinflammatory cytokines. Were in keeping with prior research the effects of CBD on migration of fMLP-stimulated PMN (McHugh et al., 2007) and on ROS production (Wang et al., 2017), the other findings being provided for the first time.

CBD affected PMN effector functions in a comparable extent to CM5, suggesting that CBD may be the main responsible of the effects of cannabis on PMN. However, CM5 was more active than CBD at about equimolar concentrations, suggesting that beyond CBD, other components present in cannabis may contribute to its biological effects. Thus, the observed effects of the cannabis extract on PMN may be the result of synergistic or additive interactions of its several components. These other ingredients of cannabis – not yet characterized – may be said to exhibit the entourage effect to enhance the activity on PMN functions of cannabis compared to CBD. Future investigation on cannabis should focus on their identification, isolation and purification and pharmacotoxicological characterization.

The inhibitory effects on ROS production in fMLP-activated PMN only occurred when cells were pre-treated for 1 h with the test substance. In contrast, when CBD or CM5 was added together with fMLP, there was no decrease of ROS production in PMN. This finding was in line with the work of (Wang et al., 2017) and it suggests that the ROS attenuation effect of cannabis and its derivatives is not merely ascribed to a competitive antagonism at fMLP receptors. Future studies are therefore required to decipher the mechanistic understanding of such an effect.

CM5 50 µg/mL did not disrupt fMLP-induced ROS production and even increased ROS levels in resting PMN to a similar extent as fMLP. In contrast, CBD at equimolar concentration (10^{-5} M) exhibited the maximum inhibitory effect of ROS production in fMLP-activated PMN, indicating that the surprising increase of ROS levels by CM5 50 µg/mL should not be ascribed to its CBD content, but rather to the entourage effect. Future direction on cannabis research should include the identification and isolation of the components that are mainly responsible for ROS levels increase in PMN.

Our study also revealed that cannabis and its derivatives downmodulate the gene expression of three proinflammatory cytokines including TNF-α, IL-8 and IL-6 in a concentration-dependent manner. Whether this decrease in mRNA expression correlates with the relevant protein levels deserves further investigation. Measurements of these cytokines in culture supernatants should therefore help to give insight. Interestingly, CM5 was more active on mRNA expression of TNF-α and IL-6 than on IL-8, suggesting it might possibly have higher therapeutic efficacy for diseases in which TNF-α and IL-6 are involved.

Globally, the inhibition of PMN functions induced by CBD or CM5 carried no meaningful effect on cell viability, suggesting that it probably results from pharmacological mechanisms rather than from any kind of cytotoxic activity. Importantly, the observed effects on PMN were concentration-dependent, suggesting they may involve receptors expressed on PMN. Further research is needed to unravel the molecular underpinnings of the actions of CBD or CM5 on PMN.

As a whole, such results support the use of cannabis and CBD as anti-inflammatory agents, however also warrant in-depth investigation of the underlying cellular and molecular mechanisms to better exploit their therapeutic potential.

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Author Contributions

Conception and design of the study: FM MC AMT BP. Cell migration assay: AMT ML. ROS production assay: AMT AL. RT PCR: AMT ML. Data analysis: MC AMT FM. Interpretation of results: MC FM AMT BP. Drafting of the manuscript: MC AMT FM. BP All authors were involved in revising it critically for important intellectual content, and all authors approved the final version to be published. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved and declare to have confidence in the integrity of the contributions of their co-authors.

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GENERAL DISCUSSION AND CONCLUDING REMARKS

Botanical resources are still representing an important pool for the identification of novel therapeutics (Kinghorn et al., 2011). Indeed, the plant kingdom comprises a large variety of species producing a wide diversity of biologically relevant secondary metabolites which are still far from being exhaustively investigated (Cragg and Newman, 2013; Fabricant and Farnsworth, 2001; Verpoorte, 2000, 1998).

One major asset of drug discovery involving vegetal resources is the existence of ethnomedical background providing clues to plant-derived compounds therapeutically relevant to humans (Corson and Crews, 2007; Dajas et al., 2016; Heinrich, 2010a, 2010b; Heinrich and Gibbons, 2001; Kinghorn et al., 2011; Sakurada et al., 2016). Ethnomedical knowledge of plants can be gained easily from obvious sources such as books, journal articles, notes placed on voucher herbarium specimens by the botanist at the time of collection, reports and computer databases. However, this information may often be inaccurate, hence the need to turn to the holders of ancestral knowledge such as indigenous people and traditional health practitioners (Loraschi and Cosentino, 2016). However, ethnomedical knowledge of plants is sometimes kept secret by custodians of traditional knowledge and access is restricted to traditional insiders. Therefore, when deciding to conduct an ethnobotanical survey, it is appropriate to involve community-based facilitators, such as community or association leaders, in the survey team, in order to avoid any reluctance from the holders of ethnomedical information to share their knowledge.

Following ethnobotanical survey, plants selected as candidate for drug discovery campaign or their derivatives require detailed knowledge about their botany, pharmacotoxicology, safety, habitat, abundance, botanical authentication, whether they are threatened, or endangered, and which permits are necessary in order to collect and investigate them (David et al., 2015; Fabricant and Farnsworth, 2001). However, extant knowledge about a medicinal plant may sometimes be conflicting, hence the need to literature mining in order to compile it into a usable form. In this regard, the literature review will, therefore, consist of a systematic search and critical appraisal of extant knowledge on the selected plants, with the scope of documenting the state of the art, pinpointing controversies and gaps in current knowledge, and building a strong foundation for future research. This knowledge can be retrieved not only from easily accessible and obvious sources such as international databases (Google Scholar, SciFinder, Web of Science, PubMed etc) and other electronic resources, but also from the very large regional (or maybe even global) body of unpublished resources or resources which are not covered in the main databases (e.g. books, newspapers, magazines, PhD and MSc dissertations, national and local government reports). The keyword mechanisms and other systems of indexing or document classification, as well as straightforward text search are frequently used for the search and retrieval of sets of articles (generally abstracts and citations), followed by subsequent refinements through Boolean combinations of search terms, iterative refinement of searches, and so forth.

Resulting from literature mining and ethnomedical claims is the adoption of a relevant pharmacological testing system. In any case, the testing systems should represent the biological activities that best match the ethnomedical uses of the selected plant species. It is important to bear in mind that plant extracts are complex mixtures containing various components and, therefore, their overall activity results from interactions between their naturally occurring ingredients (Heinrich, 2010b). Thus, a high bioactivity may result from synergic and/or additive interactions between plant components (Junio et al., 2011; Wagner and Ulrich-Merzenich, 2009; Zimmermann et al., 2007). In such a scenario, isolation of a pure ingredient from the plant extract will disrupt these interactions, thereby resulting in a decrease of the activity. In the worst scenario, a promising bioactivity can be missed if the concentration of the bioactive compounds present in the crude extract are too low, or they are poorly soluble or instable. Likewise, the presence of fluorescent or coloured contaminants such as chlorophylls may interfere with biological assays, generating false positive or false negative results (Li and Vederas, 2009).

Upon developing a drug discovery program involving plants, it would make sense to immediately search for raw materials sources from various geographic areas. Cultivation programs should also be envisioned to achieve the goal of adequate and continuous supply of plant-based products. However, sometimes, resupply from the original plant species may be insufficient to meet market demands (Miralpeix et al., 2013; Staniek et al., 2014), and alternative resupply approaches should be developed that may rely on use of plant cultures or chemical synthesis of plant-based bioactive ingredients.

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Supporting Information

SI 1. Records after duplicates removed

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SI 5. Authorization granted by the director of DDH

MINISTERE DE LA SANTE PUBLIQUE
******
DELEGATION REGIONALE DE L'OUEST
******
DISTRICT SANTE DE DSCHANG
******
HOPITAL DE DISTRICT DE DSCHANG
******

MINISTRY OF PUBLIC HEALTH
****
WEST REGION DELEGATION
****
DSCHANG HEALTH DISTRICT
****
DSCHANG DISTRICT HOSPITAL
****

Réf 091 /AR/MSP/DRO/DSD/HDD.-

AUTORISATION DE RECHERCHE

****

Je soussigné, **Dr BOUTING MAYAKA Georges** Directeur de l'Hôpital de District de Dschang, autorise l'étudiant **EDINGUE Anick** de master I en Épidémiologie et santé publique de la filière science Biomédicale de l'Université de Dschang d'effectuer une recherche sur le thème : « **Ethnobotanical survey of medicinal plants used in the treatment of HIV-AIDS in Dschang** »

En foi de quoi la présente autorisation de recherche lui est délivrée pour servir et valoir ce que de droit

Dschang le 16 JAN 2017

Le Directeur,

Dr. BOUTING MAYAKA GEORGES
DIRECTEUR
HOPITAL DE DISTRICT DE DSCHANG

SI 6. Authorization granted by the Cameroon National Ethics Committee

REPUBLIQUE DU CAMEROUN
Paix – Travail - Patrie

MINSTERE DE LA SANTE PUBLIQUE

SECRETARIAT GENERAL

COMITE REGIONAL D'ETHIQUE DE LA RECHERCHE POUR LA SANTE HUMAINE DU CENTRE

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REPUBLIC OF CAMEROON
Peace – Work – Fatherland

MINISTRY OF PUBLIC HEALTH

SECRETARIAT GENERAL

CENTRE REGIONALETHICS COMMITTEE FOR HUMAN HEALTH RESEARCH

CE N° 00172 /CRERSHC/2017

Yaoundé, le 20 FEV 2017

CLAIRANCE ETHIQUE

Le Comité Régional d'Ethique de la Recherche pour la Santé Humaine du Centre **(CRERSH/C)** a reçu la demande de clairance éthique pour le projet de recherche intitulé : **« Ethnobotanical survey of the indigenous knowledge of medicinal plants used to manage HIV/AIDS in Dschang, Cameroon »** soumis par Monsieur **Franklin CHU BUH.**

Après son évaluation, il ressort que le sujet est digne d'intérêt, les objectifs sont bien définis et la procédure de recherche ne comporte pas de méthodes invasives préjudiciables aux participants. Par ailleurs, le formulaire de consentement éclairé destiné aux participants est acceptable.

Pour ces raisons, le Comité Régional d'éthique approuve pour une période de six (06) mois, la mise en œuvre de la présente version du protocole.

L'intéressé est responsable du respect scrupuleux du protocole et ne devra y apporter aucun amendement aussi mineur soit-il sans l'avis favorable du Comité Régional d'Ethique. En outre, il est tenu de:

- collaborer pour toute descente du Comité Régional d'éthique pour le suivi de la mise en œuvre du protocole approuvé ;
- et soumettre le rapport final de l'étude au Comité Régional d'éthique et aux autorités compétentes concernées par l'étude.

La présente clairance peut être retirée en cas de non-respect de la réglementation en vigueur et des directives sus mentionnées.

En foi de quoi la présente Clairance Ethique est délivrée pour servir et valoir ce que de droit.

REPUBLIQUE DU CAMEROUN
Comité régional d'Ethique de la Recherche pour la Santé Humaine du Centre
CRERSH/C
CENTRE
MINISTERE DE LA SANTE PUBLIQUE

LE PRESIDENT
[illegible]OBO BETE Casimir
Pharmacien.

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SI 7. Questionnaires

UNIVERSITA' DEGLI STUDI DELL'INSUBRIA
Centro di Ricerca in Farmacologia Medica
Segreteria: Via Ottorino Rossi n. 9, 21100 Varese VA
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RESEARCH PROJECT: ETHNOBOTANICAL SURVEY OF THE INDIGENOUS KNOWLEDGE OF MEDICINAL PLANTS USED TO MANAGE HIV/AIDS IN DSCHANG/CAMEROON

INTERVIEWERS TO COMPLETE TABLE

Respondents ID	
Interviewers Name	
Supervisors Name	
Interview Date	

Annex 1: QUESTIONNAIRE ADRESSED TO HIV PATIENT

I. Identification

1.1. Gender: Male □ Female □

1.2. Education level: Elementary □ Secondary □ University □

1.3.

Profession:…………………………………………………………………………………………………
……

1.4. Home

location:……………………………………………………………………………….........................

1.5. Ethnic

group:…………………………………………………………………………………………….

II. HIV status

2.1. Date of announcement of the HIV

seropositivity:…………………………………………………………

2.2. Current

treatment:…………………………………………………………………………………………

2.3. Satisfaction: No □ Yes □

2.4. If not satisfied of HIV treatment,

why?...

III. Plants used for HIV Control:

3.1. Have you ever used plants to manage HIV symptoms? No □ Yes □

3.2. If yes, when did you start with the herbal medications:

Pre-ARV medication □ After ARV medication □

3.3. From whom did you find out about plants?

□ Traditional healers

□ Herbalists (city vendors)

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□ Others (name them):……………………………………………………………………………………..

3.4. For which reasons do you use plants?

□ Relieve symptoms

□ Achieve cure

□ Others (name them):……………………………………………………………………………………..

3.5. For which symptoms do you use plants?

□ Oral candidiasis □ Malaria □ Weakness □ Fever □ Cough

□ Vaginal candidiasis □ Abdominal pains □ Lethargy □ Headache □ Herpes zoster

□ Skin infection □ Diarrhea □ STDs[10] □ Tuberculosis

[10] Sexual Transmitted Diseases

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3.6. List the name of plants you used for HIV medications indicating the precise reasons for their use, the part used, methods of preparation, mode of administration, adverse effects and perceived effectiveness of herbal medication (fill the following table):

N°	Common name	Procurement method	Part used	Reasons for plant use	Methods of preparation/con-servation	Mode of administration	Satisfaction[11]	adverse effects
1								

[11] Satisfaction status:
□ no help
□ moderate relieve of symptoms
□ completely relieved symptoms
□ cures HIV completely

2								
3								
4								

5								
6								

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7								
8								

9								

Name of the investigator

Signature of the investigator

Date

Annex 2: QUESTIONNAIRE ADRESSED TO TRADITIONAL PRACTIONNER

I. Identification

Gender: Male □ Female □

Education level: Elementary □ Secondary □ University□

Profession:…….

Home

location:………………………………………………………………………………………………..

Seniority in traditional medicinal

sector:………………………..

Ethnic

group:……………………………………………………………………………………………….

II. Knowledge about HIV/Aids Invalid source specified.[12]

1. Have you ever heard about Aids.

Yes □ No □

[12] Carey, M. P. (2002). Development and psychometric evaluation of the brief HIV knowledge questionnaire (HIV-KQ-18). AIDS Education and Prevention, 14, 174-184.

2. HIV and AIDS are the same thing.

True □ False □ Don't know □

3. There is a cure for AIDS.

True □ False □ Don't know □

4. A person can get HIV from a toilet seat.

True □ False □ Don't know □

5. Coughing and sneezing DO NOT spread HIV.

True □ False □ Don't know □

6. HIV can be spread by mosquitoes.

True □ False □ Don't know □

7. It is possible to get HIV when a person gets a tattoo.

True □ False □ Don't know □

8. A pregnant woman with HIV can give the virus to her unborn baby.

True □ False □ Don't know □

9. Showering, or washing one's genitals/private parts, after sex keeps a person from getting HIV.

True □ False □ Don't know □

10. A person with HIV can look and feel healthy. True □ False □ Don't know □

11. There is a vaccine that can stop adults from getting HIV. True □ False □ Don't know □

12. A person can get HIV even if she or he has sex with another person only one time.

True □ False □ Don't know □

13. A person can get HIV through contact with saliva, tears, sweat, or urine.

True □ False □ Don't know □

14. Pulling out the penis before a man climaxes/cums keeps a woman from getting HIV during sex.

True □ False □ Don't know □

15. There is a female condom that can help decrease a woman's chance of getting HIV.

True □ False □ Don't know □

16. Having sex with more than one partner can increase a person's chance of being infected with HIV.

True □ False □ Don't know □

17. Taking a test for HIV one week after having sex will tell a person if she or he has HIV.

True □ False □ Don't know □

III. Plants used for HIV Control:

1. Do you use plants for HIV medications? No □ Yes □

2. If yes, List the name of such plants indicating the precise reasons for their use, the part used, methods of preparation, mode of administration, method of procurement, place of collection, and the adverse effects of herbal medication (fill the following table):

N°	Vernacular name of plants	Place of collection/threat	Date/season of collection	Parts used	Reasons of plant use	Methods of preparation/con-servation	Mode of administration	Adverse effects
1								
2								

3								
4								

5								
6								

7								

Name of the investigator

Signature of the investigator

Date

SI 8. Botanical authentication Certificate

REPUBLIQUE DU CAMEROUN
Paix - Travail - Patrie

INSTITUT DE RECHERCHE AGRICOLE POUR LE DEVELOPPEMENT

CENTRE REGIONAL DE RECHERCHE DE NKOLBISSON

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Yaoundé, le 19 JUIN 2017

N/Réf. 158 / IRAD/DG/CRRA-NK /SSRB-HN/07/2017

ATTESTATION D'IDENTIFICATION D'ECHANTILLONS BOTANIQUES

Le Chef de l'Herbier National soussigné atteste que les échantillons botaniques de monsieur **MABOU Alex** étudiant de Biochimie à la Faculté des Sciences de l'Université de Douala ont été identifiés à l'Herbier National par monsieur TADJOUTEU Fulbert (botaniste) comme l'indique le tableau ci-dessous.

Noms communs	Noms scientifiques	Réf. HNC	Noms communs	Noms scientifiques	Réf. HNC
Fleur jalousie	Tithonia diversifolia	48790/HNC	Basilic	Ocimum basilicum	42782/HNC
Rouge un coté	Eremomastax speciosa	16371/SRFC	Biter kola	Garcinia kola	65745/HNC
Aloe	Aloe barbadensis	/	Aubergine	Solanum melongena	43055/HNC
Foléré	Hibiscus sabdariffa	42810/HNC	Quinqueliba	Cinchona succiruba	25850/HNC
Manguier	Mangifera indica	32875/HNC	Quinine sauvage	/	/
Roi des herbes	Ageratum conizoides	9504/SRFC	Cotmajo	Ocimum gratissimum	44996/HNC
Ngui d'afrique	Viscum sp.	/	Citron	Citrus medica	65106/HNC
Avocatier	Persea americana	33945/HNC	Djinja	Zingiber officinale	43143/HNC
Colatier	Cola acuminata	48653/HNC	Herbe du lapin	Emilia coccinea	61778/HNC
Goyavier	Psidium guayava L.	45028/HNC	Fruit noir	Canarium schwenfurthii Engl.	59834/HNC
Ecalyptus	Eucalyptus globulus	4077/SRFC	Essok	Garcinia lucida	57192/HNC
Citronelle	Cymbopogon citratus	18628/SRFC	/	Mondia whitei	48774/HNC
Papayer	Carica papaya	18647/SRFC	/	/	/

En foi de quoi la présente attestation est délivrée pour servir et valoir ce que de droit.

LE CHEF DE L'HERBIER NATIONAL

REPUBLIQUE DU CAMEROUN Republic of Cameroon IRAD / CRRA-NK NATIONAL HERBARIUM HERBIER NATIONAL CENTRE REGIONAL DE RECHERCHE AGRICOLE DE NKOLBISSON Institute of Agricultural Research for Development Institut de Recherche Agricole pour le Développement

Dr. Ngo Ngwé Marie Florence Sandrine

SI 9. Real-Time PCR probes for gene expression (BIO-RAD)

Gene Symbol	UniGene ID	Interrogated Sequence *RefSeq/GenBank mRNA*	Detected Coding Transcripts	Amplicon Context Sequence	Chromosome Location	Amplicon Lenght	Annealing temperature (°C)	Efficiency (%)

TNF	Hs.241570	NC_000006.11, NG_007462.1, NG_012010.1, NT_007592.15, NT_113891.2, NT_167244.1, NT_167245.1, NT_167246.1, NT_167247.1, NT_167248.1, NT_167249.1	ENST00000328965, ENST00000445232, ENST00000594551, ENST00000443707, ENST00000412275, ENST00000449264, ENST00000577810, ENST00000326294, ENST00000448781, ENST00000420425, ENST00000394126, ENST00000356271, ENST00000394128, ENST00000394127, ENST00000422942, ENST00000501516, ENST00000536318, ENST00000431269, ENST00000376122, ENST00000383496, ENST00000264203,	GGGGTCTTCCAGCTGGAGAAGGGTGACCGACTCAGCGCTGAGATCAATCGGCCCGACTATCTCGACTTTGCCGAGTCTGGGCAGGTCTACTTTGGGATCATTGCCCTGTGAGGAGGACGAACATC	6:31545204-31545328	95	60	99

			ENST00000375144, ENST00000375142, ENST00000401084, ENST00000439554					
IL6	Hs.654458	NC_000007.13, NG_011640.1, NT_007819.17	ENST00000404625, ENST00000426291, ENST00000401651, ENST00000407492, ENST00000401630, ENST00000406575, ENST00000258743, ENST00000420258	GTATACCTAGAGTACCTCCAGAACAGATTTGAGAGTAGTGAGGAACAAGCCAGACTGTGCAGATGAGTACAAAAGTCCTGATCCAGTTCCTGCAGAAA	7:22769178-22769276	69	60	98
IL8	Hs.624	NC_000004.11, NT_022778.16	ENST00000307407, ENST00000401931	GCAGAGCACACAAGCTTCTAGGACAAGAGCCAGGAAGAAACCACCGGAAGGAACCATCTCACTGTGTGTAAACATGACTTCCAAGCTGGCCGTGGCTCTCTTG	4:74606303-74606405	73	60	99