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An aqueous *Citrullus colocynthis* peel extract inhibits neutrophil reactive oxygen species production and attenuates lung inflammation in mice

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Received 12 January, 2015; Accepted 12 August, 2015

*Citrullus colocynthis* peel aqueous extract (CCPAE) is widely used to treat disorders such as inflammation, ulcers and infections, but its pharmacological target is not known. The objectives of this work were to study the effect of *C. colocynthis* peel aqueous extract, on human neutrophil reactive oxygen species (ROS) production *in vitro*, and to evaluate its protective effect on lipopolysaccharide (LPS)-induced lung inflammation *in vivo* in mice. Neutrophils were isolated from blood of healthy volunteers. ROS generation was measured by luminol-amplified chemiluminescence. Superoxide anion generation was detected by the cytochrome c reduction assay. H₂O₂ was detected by horseradish peroxidase (HRP)-amplified chemiluminescence assay. Myeloperoxidase (MPO) activity was measured by the tetramethylbenzidine oxidation method. Lung inflammation was induced in mice by LPS instillation. CCPAE inhibited luminol-amplified chemiluminescence of resting neutrophils and N-formylmethionyl-leucyl-phenylalanine (fMLP)- or phorbolmyristate acetate (PMA)-stimulated neutrophils, in a concentration-dependent manner. CCPAE also inhibited superoxide anion generation; and did not scavenge H₂O₂ and superoxide anions nor inhibited MPO activity *in vitro* suggesting that it inhibits nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activation. *In vivo* studies showed that CCPAE attenuated LPS-induced lung inflammation in mice. This study shows that CCPAE inhibits neutrophil ROS production and attenuates LPS-induced lung inflammation in mice. Inhibition of NADPH oxidase activation by CCPAE could explain its anti-inflammatory action.

**Key words:** *Citrullus colocynthis*, colocynth, inflammation, neutrophils, reactive oxygen species (ROS), nicotinamide adenine dinucleotide phosphate (NADPH) oxidase.

INTRODUCTION

*Citrullus colocynthis* (L.) Schrad. (Cucurbitaceae), commonly known as “bitter apple” is a plant that grows abundantly in Tunisia (Pottier-Alapetite, 1981), and widely in other parts of the world (Abbes el al., 2006). In the Tunisian traditional medicine, this plant has been used to treat various diseases including, hypertension and rheumatism (Le Flock, 1983; Boukef et al., 1982), while in other countries it is used to treat constipation,
oedema bacterial infections, cancer and diabetes, and as
an abortifacient (Abbes et al., 2006). The ethnoveterinary
uses of this plant include its use as cathartic, purgative
and vermifuge, and for the treatment of fever, cancer,
amenorrhea, jaundice, leukemia, rheumatism and tumour
(Abbes et al., 2006). The ethnoveterinary use efficiency of
this plant was consolidated by a number of studies which
demonstrated that C. colocynthis Schrad has a potent
anti-tumour (Tannin-Spitz et al., 2007), anti-microbial
(Marzouk et al., 2009, 2010a) and antioxidant activity
(Marzouk et al., 2010b). Many secondary metabolites
from C. colocynthis, including cucurbitacins, flavonoids,
caffeic acid derivatives and terpenoids, have been
previously reported (Yankov and Hussein, 1975; Hatam
et al., 1989; Maatooq et al., 1997) and could explain the
biological activity of this plant.

Inflammatory disorders are due to excessive production
of pro-inflammatory mediators, such as tumor necrosis
factor (TNFα), granulocyte-macrophage colony-
stimulating factor (GM-CSF), interleukin (IL)-1, IL-6, IL-8,
leukotriene B4 and platelet-activating factor (PAF), to the
activity of inflammatory cells such as neutrophils,
monocytes and macrophages, and to the excessive
production of reactive oxygen species (ROS) (Ley, 2002;
Nathan, 2006). Polymorphonuclear neutrophils play a key
role in host defenses against invading microorganisms
(Hampton et al., 1998), but excessive neutrophil
activation participates in tissue damage associated with
inflammatory disorders (Babior, 2000). In response to
a variety of agents neutrophils migrate to inflammatory
sites, where they release proteases, bactericidal
peptides, and large quantities of ROS, in a process
known as the respiratory burst (Babior, 1984). Oxygen
reduction by neutrophil NADPH oxidase, a
multicomponent enzyme system, yields superoxide anion
(O2−) (El-Benna et al., 2005), while myeloperoxidase
(MPO) produces hypochloric acid from hydrogen
peroxide (Klebanoff, 2005).

This study was undertaken to analyze the effect of C.
 colocynthis peel aqueous extract (CCPAE) on ROS
production by human neutrophils; and to evaluate the
effect of this product on intratracheal lipopolysaccharide
(LPS)-induced lung inflammation in mice.

MATERIALS AND METHODS

Chemicals and reagents

Luminol, cytochrome c, IMLF, PMA, zymosan, superoxide
dismutase (SOD), catalase and HRPO Escherichia coli (O55:B5)
lipopolysaccharide (LPS) were from Sigma-Aldrich (Saint-Quentin
Fallavier, France). Ficoll and Dextran T500 were from GE
Healthcare, phosphate buffered saline (PBS), Hank’s balanced salt
solution (HBSS), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic
acid (HEPES) and glucose were from Gibco. 2,7-
dichlorofluorescein-diacetate (DCFH-DA) was from Acros Fine
Chemicals. Stock solutions of fMLF (10μmol/L) and PMA (1 mg/ml)
were prepared in dimethyl sulfoxide (DMSO) and stored at -20°C.
The different solutions were diluted in PBS immediately before use.

Preparation of CCPAE

In this study, three batch of Tunisian C. colocynthis, collected from
the island of Jerba in South of Tunisia were used. The colocynth
peels were, dried at 37°C, blended and suspended in sterile 0.9%
NaCl, then centrifuged at 2000 rpm for 3 min. From each batch, the
supernatants of different preparations of CCPAE were used for the
experiments. The results obtained with different preparations from
different batches are reproducible and the same dose effect
responses were found.

Isolation of human neutrophils

Venous blood was collected from healthy adult volunteers and
neutrophils were isolated by Dextran sedimentation and density
gradient centrifugation as previously described (El Benna and
Dang, 2007). Erythrocytes were removed by hypotonic lysis.
Following isolation, the cells were resuspended in appropriate
medium, such as HBSS. The cells were counted and their viability
was determined with the Trypan Blue exclusion method.

Measurement of ROS production by chemiluminescence

Isolated neutrophils were resuspended in HBSS at a concentration
of 1 million per ml. Cell suspensions (5 x 10⁵) in 0.5 ml of HBSS
containing 10 μM luminal in the presence or absence of CCPAE
were preheated to 37°C in the thermostatted chamber of a
luminometer (Berthold-Biolumat LB937) and allowed to stabilize.
After a baseline reading, cells were stimulated with 10⁻⁶ M IMLF or
100 ng/ml PMA. Changes in chemiluminescence were measured
over a 30-min period.

Measurement of superoxide production

Isolated cells were resuspended in HBSS at a concentration of 1
million/ml. Cell suspensions in 1 ml of HBSS containing 1 mg/ml
cytochrome c in the presence or absence of CCPAE were
preincubated to 37°C in the thermostatted chamber of a
spectrophotometer (Uvikon) and allowed to stabilize. After a
baseline reading, cells were stimulated with 10⁻⁶ M IMLF or 100
ng/ml PMA. Changes in absorbance were measured at 550 nm
over a 15-min period.

Detection of H₂O₂

In order to investigate whether CCPAE reacts directly with H₂O₂
CCPAE was incubated in PBS with H₂O₂ (80 μM) for 15 min in the
presence of luminol (10 μM) and the reaction was initiated by
adding HRPO (5U). Changes in chemiluminescence were

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measured over a 15 min period.

Preparation of azurophilic granules and measurement of MPO activity

Neutrophils were lysed by nitrogen cavitation and the granule fraction was purified by Percoll gradient centrifugation (Udby and Borregaard, 1989). The granules were sonicated in 0.2 cetyltrimethylammonium bromide (CTAB) and MPO activity was assessed using the H$_2$O$_2$-dependent tetramethylbenzidine (TMB) oxidation assay at 650 nm.

LPS-induced lung inflammation in mice

These experiments were approved by our Institutional Committee on Animal Care and Use and the experimental protocol complied with Tunisian legal requirements for animal studies. Male Balb C mice aged 7 weeks and weighing 22 to 26 g were purchased from SIFAT (Société des industries pharmaceutique de Tunisie) and housed in standard wire-topped cages and the temperature-controlled units. Food and water were supplied ad libitum. The mice received an intraperitoneal injection of 200 mg/kg CCPAE on day 0 (D0), followed by 200 mg/kg on day 1 (D1). Three hours after the second injection, the mice received a cocktail of anesthetics [75 mg/kg ketamin (Virbac Santé Animale) plus 1 mg/kg medetomidine (Pfizer)], before intratracheal LPS instillation (5 µg/mouse). The mice were aroused by an intraperitoneal injection of 1 mg/kg atipamezol (Pfizer), a medetomidine antagonist, and were killed 24 h later.

Bronchoalveolar lavage (BAL) and lung sampling

The mice were anesthetized by an intraperitoneal injection of 50 mg of urethane (Sigma) and killed by exsanguination. The lungs were lavaged twice with 1 ml of physiological saline, removed from the chest cavity, and immediately placed at -80 °C until use. The lavage fluid (1.8 ml) was immediately placed on ice. Free alveolar cells were recovered from the lavage fluid by centrifugation at 400 g for 15 min at 4 °C. The total protein concentration in the supernatant was measured with the Quick-Start Bradford assay (Bio-Rad, Marnes-la-Coquette, France). The cell pellet was suspended in 150 µl of physiological saline and an aliquot was used to determine the total white cell count with a hemocytometer. For differential counts, the cell suspension was cytospun (Cytospin-2, Shandon Products Ltd.), fixed in methanol, and stained with Diff Quick solution (Medion Diagnostics, Plaisir, France). One hundred cells were counted with an oil immersion lens (1000x).

Statistical analysis

Data were reported as mean ± standard error. The Newman-Keuls multiple comparisons test was used, and P values <0.05 were considered to denote significant differences.

RESULTS

CCPAE inhibits luminol-amplified chemiluminescence in human neutrophils, independently of the stimulus

To investigate the effect of CCPAE on neutrophil ROS production, human neutrophils were incubated with different CCPAE concentrations and ROS were detected by luminol-amplified chemiluminescence. Results show that CCPAE inhibited luminol-amplified chemiluminescence in resting neutrophils (Figure 1A) and in neutrophils stimulated with fMLF (Figure 1B) or PMA (Figure 1C). The effect of CCPAE shows an inhibitory effect starting at a concentration between 20 and 40 µg/ml. As fMLF and PMA activate neutrophils through different transduction pathways, these results suggested that CCPAE decreases neutrophil ROS production by either inhibiting a final common target, such as the NADPH oxidase or MPO, or by scavenging ROS.

CCPAE inhibits fMLF- and PMA-stimulated superoxide anions production by human neutrophils

Luminol-amplified chemiluminescence technique used above allows the detection of several ROS molecules (such as superoxide anions, H$_2$O$_2$) and several neutrophil functions (such as NADPH oxidase activation, degranulation and MPO activity). To investigate precisely the effect of CCPAE on neutrophil NADPH oxidase activation, superoxide anion production was measured using cytochrome c reduction assay. Since superoxide production is not detectable in resting neutrophils, the effect of CCPAE was tested only on PMA- and fMLF-stimulated cells. Results show that CCPAE inhibited superoxide anion production by neutrophils stimulated with PMA or fMLF (Figure 2). The effect of CCPAE shows an inhibitory effect starting at a concentration as low as 20 µg/ml. As fMLF and PMA activate the NADPH oxidase through different transduction pathways, these results suggested that CCPAE directly inhibits a final common target, such as the NADPH oxidase, or scavenges superoxide anions.

CCPAE does scavenge superoxide anions nor H$_2$O$_2$ nor inhibit MPO activity

Firstly, to investigate whether CCPAE scavenges superoxide anions, xanthine/xanthine oxidase was used to produce superoxide anions, which were then detected by the cytochrome c reduction assay. Results show (Figure 3A) that CCPAE had no effect on superoxide anions production by this system. Secondly, to investigate whether CCPAE scavenges H$_2$O$_2$, commercial H$_2$O$_2$ which was detected by the luminol-amplified chemiluminescence assay was used. Results show (Figure 3B) that CCPAE had no effect on H$_2$O$_2$. Thirdly, to investigate the effect of CCPAE on MPO activity, azurophilic granules extracts were incubated with different concentrations of CCPAE, and MPO activity was measured using H$_2$O$_2$-TMB oxidation assay. Results
Figure 1. Effect of CCPAE on ROS production by human neutrophils. Human neutrophils (5 × 10⁵) were incubated in the presence or absence of different CCPAE concentrations in resting conditions (A), and stimulated with fMLF (10⁻⁶ M) (B), or PMA (100 ng/ml) (C). Luminol-amplified chemiluminescence was measured for 30 min (mean ± SEM of 5 experiments, *p<0.05).

Figure 2. Effect of CCPAE on superoxide anion production by neutrophils. Human neutrophils (1 × 10⁶) were incubated in the presence or absence of CCPAE, and stimulated with fMLF (10⁻⁶ M) or PMA (100 ng/ml). Cytochrome c reduction was measured at 550 nm in a spectrophotometer for 10 min (mean +/- SEM of 5 experiments, *p<0.05).
Figure 3. Effect of CCPAE on superoxide anions and H$_2$O$_2$ in vitro. (A) Xanthine oxidase was incubated in the presence or absence of CCPAE, xanthine was added and superoxide anions was detected by the cytochrome c reduction assay. (B) H$_2$O$_2$ was incubated in the presence or absence of CCPAE and detected using HRP-amplified chemiluminescence (mean +/- SEM of 3 experiments, *p<0.05).

show (Figure 4) that CCPAE had no effect on MPO activity.

**Effect of CCPAE on BAL fluid protein content and cellularity in LPS-treated mice**

Intratracheal administration of 5 µg of LPS to mice induced a significant increase in BAL protein content after 24 h, compared with animals treated with either the vehicle or CCPAE alone (Figure 5). Interestingly, the BALF protein content after intratracheal LPS challenge was significantly lower when animals were pretreated with 200 mg/kg CCPAE (Figure 5).

Moreover, intratracheal LPS administration induced a significant increase in both the BALF total cell count (p < 0.05 vs. vehicle or CCPAE alone, Figure 6A and B) and the BALF neutrophil count after 24 h (p < 0.05 vs. vehicle or CCPAE alone, Figure 6A and C). Neither the vehicle nor CCPAE at 200 mg/kg modified the BALF cell count. However, intraperitoneal CCPAE injection at 200 mg/kg significantly reduced both the BALF total cell count after intratracheal administration of LPS (p < 0.05 vs. LPS alone, Figure 6A and B), and the neutrophil recruitment when compared with LPS alone (p < 0.05 vs. LPS, Figure 6A and C).

**DISCUSSION**

In this study, it was shown that CCPAE inhibited neutrophil ROS production, as measured by luminol-amplified chemiluminescence in both resting and stimulated neutrophils. However, CCPAE did not scavenge superoxide anions or H$_2$O$_2$, but markedly inhibited superoxide anions production by neutrophils. In addition, CCPAE attenuated LPS-induced lung inflammation in mice.

CCPAE inhibited luminol-amplified chemiluminescence in human neutrophils stimulated with the chemotactic peptide fMLF or the protein kinase C activator PMA. As fMLF and PMA induce NADPH oxidase activation through different transduction pathways, these results suggest that CCPAE does not affect a specific transduction pathway, but directly inhibits a final common biochemical target, such as the NADPH oxidase or MPO, or that it scavenges reactive oxygen species. Besides, it was found out that CCPAE had an inhibitory effect on cytochrome c reduction, a specific technique for superoxide anion detection, suggesting that CCPAE could react either by inhibiting NADPH oxidase activity or by scavenging superoxide anions.

Luminol-amplified chemiluminescence can be used to assay both intracellular and extracellular ROS production by neutrophils, as luminol is a membrane-permeable molecule. Luminol-amplified chemiluminescence is dependent on superoxide anions, H$_2$O$_2$, and on peroxidases, such as cytosolic peroxidases and MPO (Dahlgren and Karlsson, 1999). To determine whether CCPAE reacted with H$_2$O$_2$, a more specific technique was used to detect H$_2$O$_2$ in vitro. CCPAE did not affect
Figure 4. Effect of CCPAE on MPO activity. MPO was incubated with or without CCPAE and its activity was measured in terms of tetra-methylbenzidine oxidation at 655 nm (mean ± SEM of 5 experiments, *p<0.05).

Figure 5. Effect of CCPAE on the protein concentration of mouse bronchoalveolar lavage fluid (BALF). Total content of protein was measured in BALF 24 h after intratracheal instillation of lipopolysaccharide (LPS 5 µg/mouse) or in controls. LPS induced a massive increase in the protein content, which was significantly attenuated by CCPAE (200 mg/kg) (n=8, mean ± SEM, *p<0.05).
Figure 6. Effect of CCPAE on BALF cell content. BALB/c mice were treated with vehicle, CCPAE (200 mg/kg), LPS (5 µg/mouse) or CCPAE (200 mg/kg) plus LPS (5 µg/mouse), and cells were counted in BALF. A, Total cell counts; B and C, Neutrophil counts (n=8, mean ± SEM *p<0.05).
the amount of H$_2$O$_2$, suggesting that CCPAE does not react with H$_2$O$_2$. However, CCPAE inhibited NADPH oxidase activity in vitro and exerted a potent anti-inflammatory effect in the lungs of mice exposed to LPS, reducing the BALF protein content, total cell number and neutrophil count. The inhibitory effect of CCPAE on NADPH oxidase activity could thus explain its anti-inflammatory action in vivo.

Persistent asthma, chronic obstructive pulmonary disease (COPD) and emphysema are chronic inflammatory lung diseases (Lemanske and Busse, 1997; Ward, 1997). COPD and asthma involve several types of inflammatory cells and soluble mediators (Barnes et al., 2003). COPD is associated with destruction of the alveolar epithelium and flooding of the alveolar spaces with proteinaceous exudates containing abundant neutrophils (Di Stefano et al., 1998). In our study, BALF from mice exposed to LPS contained a large amount of protein, reflecting high-permeability pulmonary oedema. The BALF protein concentration was significantly reduced by CCPAE treatment, suggesting that CCPAE reduces lung vascular permeability and oedema, and might therefore protect the integrity of the alveolocapillary membrane. The reduction in neutrophil infiltration could explain these beneficial effects, as neutrophils are considered a primary cellular effector of alveolocapillary damage in COPD and asthma (Pesci et al., 1998; Fabbri et al., 2003).

It was found out that CCPAE inhibited NADPH oxidase activity in vitro, an effect possibly explaining the anti-inflammatory action observed in vivo. NADPH oxidase stimulation triggers murine macrophages to produce ROS (Lincoln et al., 1995; Gelderman et al., 1998). Secreted ROS enhances the secretion of TNFα, IL-8 and other proinflammatory cytokines (Nelson et al., 1998). In particular, alveolar macrophage-derived TNFα and IL-8 recruit neutrophils to sites of inflammation (Gibson et al., 2001). NADPH oxidase inhibition by CCPAE could attenuate these inflammatory reactions.

Fruit and vegetables are important sources of antioxidants, including ascorbic acid, carotenoids, flavonoids and hydrolysable tannins. Epidemiological studies indicate that populations that consume foods rich in specific polyphenols have a lower incidence of inflammatory disorders, such as cardiovascular and cerebrovascular disease, as well as certain cancers (Huxley and Neil, 2003; Temple and Gladwin, 2003). Several studies have demonstrated the high antioxidants activity of coloynith. This activity is attributed especially to a number of plant secondary metabolites including cucurbitacin glycosides, flavonoids, caffeeic acid derivatives and terpenoids (Flavone C-glycosides) and cucurbitacin glycosides from C. coloynithis. (Delazar et al., 2006). In carrageenan-induced rat paw edema model, Marzouk et al. (2013) showed that C. coloynithis also has a potent anti-inflammatory action. The intraperitoneal administration of aqueous extracts of seeds and fruits of C. coloynithis, significantly reduced the paw edema induced by the noxious agent (Marzouk et al., 2013).

ROS are important contributors to tissue injury, inflammation, cancer and many other diseases. The antioxidant properties of flavonoids especially the flavonones glucoside (isosaponarin, isovitexin and isoorientin 3'-O-methyl ether) and the cucurbitacin glucosides (2-O-β-D-glucopyranosylcucurbitacin and 2-O-β-D-glucopyranosyl cucurbitacin L), probably contribute, at least to some extent, to the pharmacological and traditional medicinal uses of the C. coloynithis (Abbas et al., 2009). These compounds scavenge free radicals and inhibit lipid oxidation in vitro (Gil et al., 2000; Noda et al., 2002). Further studies are needed to identify the precise phenolic compounds responsible for the NADPH oxidase inhibition observed in this study.

Conclusion

As concluded from this study, CCPAE inhibited neutrophil luminol-amplified chemiluminescence in vitro, by inhibiting NADPH oxidase. CCPAE also attenuated inflammation induced by intratracheal endotoxin instillation in mice, leading to a decrease in the BALF protein concentration, total cellularity and neutrophil content.

Conflict of interest

The authors declare that they have no conflict of interest.

Abbreviations

fMLF, N-formyl-methionyl-Leucyl-phenylalanine; MPO, myeloperoxidase; PMA, phorbolmyristate acetate; ROS, reactive oxygen species.

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Review

A review on antimicrobial potential of species of the genus Vernonia (Asteraceae)

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Received 13 June, 2015; Accepted 4 August, 2015

Natural products are sources of various biologically active chemicals. Therefore, ethnopharmacological and ethnobotanical studies are essential to discover new substances for the treatment of diseases. In this context, many studies have been conducted of the Asteraceae family demonstrating medicinal properties of its representatives, such as species of the genus Vernonia, which are rich in bioactive substances like sesquiterpene lactones, flavonoids, tannins and steroids. This review presents an overview of Vernonia species with antimicrobial potential, their main phytochemical characteristics and ethnomedicinal uses.

Key words: Compositae, Vernonieae, phytochemistry, biological activity, antimicrobial, antibacterial, antifungal.

INTRODUCTION

Humans have always used plants for therapeutic purposes to control microbial infections and other medical conditions (Rangel et al., 2001). The increase of bacterial and fungal infections and the development of microbial resistance to synthetic drugs have led to renewed interest in recent years to investigate plants as natural sources of substances for therapy against microorganisms of medical and veterinary importance (Denning, 2002; Rocha et al., 2011; Nascimento et al., 2000; Bautista-Baños et al., 2003). Numerous plants have been used for prophylactic purposes and to cure infections. In this context, many studies have been conducted to find plant species with antimicrobial potential, such as assays of essential oils with antimicrobial properties against a variety of microorganisms (Silva et al., 2012).

The Asteraceae family (Compositae) has about 24,000 described species, belonging to 1,600 to 1700 genera distributed in 17 tribes and three sub-families (Funk et al., 2009; Petacci et al., 2012). They have cosmopolitan distribution and are widely found in the tropics, subtropics and temperate regions (Teles and Bautista, 2006; Hattori and Nakajima, 2008). Representing to the largest family of the eudicotyledons, Asteraceae is relevant for its cosmetic, aromatic and therapeutic properties (Nakajima and Semir, 2001; Hattori and Nakajima,
2008). Together with Calyceraceae, Campanulaceae, Menyanthaceae and Goodeniaceae, it forms a clade, the Asterales order (Pozner et al., 2012). The main genera of Asteraceae are Aster L., Inula L., Xanthium L., Eupatorium L., Carpesium L., Saussurea DC., Vernonia Schreb. and Taraxacum Zinn. (Wu et al., 2006).

Of the tribe Vernonieae, the genus Vernonia is one of the largest, predominantly in Africa and South America (Costa et al., 2008). Its species have wide ethnopharmacological use for treatment of several diseases as snakebite antidote, and as food in some African regions (Toyang and Verpoorte, 2013). Numerous phytochemical studies of the genus Vernonia have revealed the presence of diterpenes (Kos et al., 2006), triterpenes (Liang and Min, 2003), steroids (Tchinda et al., 2003), and the most frequently isolated chemical constituents are flavonoids (Carvalho et al., 1999) and sesquiterpene lactones (Buskuhl et al., 2010). This article presents a review of the antimicrobial potential, focusing on the antibacterial and antifungal activities of the species of the genus Vernonia, highlighting the biological, phytochemical and ethnopharmacological properties of the genus.

**METHODOLOGY**

The current review was achieved using an organized search of the scientific data published about antimicrobial activity, focusing on the antibacterial and antifungal activities of the species of the genus Vernonia. The search was conducted between November 1 and December 28, 2014, using the keyword search term “Vernonia antimicrobial activity”. The searches were carried out using various databases, including PubMed (http://www.ncbi.nlm.nih.gov/pubmed), Science Direct (http://www.sciencedirect.com/), Scopus (http://www.scopus.com/), Scielo (http://www.scielo.org/) and Google Scholar (http://www.scholar.google.com/).

**Antimicrobial potential of the genus Vernonia**

Vernonia is one of the largest genera of plants belonging to the tribe Vernonieae (Cichorioideae), Asteraceae family, with about 1,500 described species, being the largest genus of the tribe (Silva et al., 2013). Vernonia species are found in temperate, tropical and sub-tropical areas, especially in South America, Asia, Africa (Costa et al., 2008; Dematteis and Pire, 2006) and North America (Redonda-Martinez et al., 2012). More than 500 of these Vernonia plants are distributed in Africa and Asia and approximately 300 species distributed in tropical areas, from Mexico to Argentina (Yeap et al., 2010). This genus is named in honour of the English botanist William Vernon, who first identified this genus in the region that is now Maryland in the USA in the late 1600s (Toyang and Verpoorte, 2013).

In Brazil, country with the highest genetic diversity of the world (Lewinsohn and Prado, 2005), there are over 200 species of Vernonia, in the form of herbs, shrubs or trees with foliage in various habitats. As for the morpho-anatomical features, they present solitary capitulums that are racemose, panículated or scorpion with flowers of various colors. The fruits are cipselas and can be angled or costadas, glabrous or sericeous. They nearly always have a double pappus formed by an outer row of short bristles and an inner row of feathery, barbeladas or scabrous bristles, persistent or not (Galastris et al., 2010).

In traditional medicine, many Vernonia species are employed to treat various diseases. From the pharmacological point of view, species have been investigated revealing many properties, such as antiplasmodial (Stangeland et al., 2011), analgesic (Frutusso et al., 1994), anti-inflammatory (Malafronte et al., 2009), antimicrobial (Ogundare et al., 2006), antidiabetes (Akinola et al., 2010), antioxidant (Owolabi et al., 2011) and antitumor (Sangeetha and Venkataramanakumar, 2011).

Phytochemical studies have indicated that the main constituents of the genus are sesquiterpene lactones, triterpenes, steroids, carotenoids, flavonoids, lignoids, alkaloids and tannins (Toigo et al., 2004). The most common constituents are flavonoids, which often have antioxidant activity (Salawu et al., 2011), and sesquiterpene lactones, the latter being considered the chemotaxonomic markers in the genus (Albuquerque et al., 2007a). Several sesquiterpene lactones have been isolated from species of the genus with different types of bioactivity, such as molluscicidal, antifungal, antitumor, cytotoxic and insecticidal against herbivores insects (Wedge et al., 2000; Freire et al., 1996c; Lopes et al., 1991). In V. amygdalina the sesquiterpene lactones vernolide and vernodalol (Figure 1) were isolated, with potential antibacterial and antifungal activity (Erasto et al., 2006). In an investigation of the chemical composition of hexane and ethanol extracts from aerial parts of V. chalybae Mart by spectroscopic methods, terpenoids, flavonoids, alcohols and aliphatic ethers were isolated for the first time in the genus (Costa et al., 2008).

There have been numerous studies involving extracts, fixed oils and essential oils of Vernonia species (Table 1) with potential antimicrobial activity against strains of bacteria, protozoa and fungi that are pathogens to animals and plants, using extracts, fixed oils, and essential oils. In particular, phytochemical screening is promising to identify bioactive compounds with antimicrobial activity (Magadula and Erasto, 2009; Hamill et al., 2000).

In Ethiopia, a study of nine plants with the ethnomedicinal use indicated that Vernonia species are used for the treatment of eye infections, for wound healing and the treatment of bone related problems such as fractures. They are used by decoction of fresh leaves and subsequent application on the lesions or intake.
Table 1. Species of the genus *Vernonia* with antimicrobial activity.

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Part used</th>
<th>Biological preparation</th>
<th>Bioactive substances</th>
<th>Biological activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Vernonia adoensis</em></td>
<td>L</td>
<td>PE</td>
<td>Terpenoids</td>
<td>Antibacterial activity: Gram-positive and gram-negative bacteria</td>
<td>Kisangau et al. (2007)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Antifungal activity: <em>Candida albicans</em> yeast</td>
<td></td>
</tr>
<tr>
<td><em>Vernonia ambiguа</em></td>
<td>AP</td>
<td>CE/EE</td>
<td>Alkaloids, flavonoids, saponins, tannins, glycosides, cardiac glycosides, steroids and triterpenes</td>
<td>Antibacterial activity: Gram-positive and gram-negative bacteria</td>
<td>Aliyu et al. (2011)</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>EAE</td>
<td>Sesquiterpene lactones</td>
<td>Antibacterial activity: Gram-positive and gram-negative bacteria</td>
<td>Jisaka et al. (1993)</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>ME</td>
<td>Saponins, tannins and flavonoids</td>
<td>Antibacterial activity: Gram-positive and gram-negative bacteria</td>
<td>Akinpelu (1999)</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>ME/DEE/AqE</td>
<td>Sesquiterpene lactones and steroid glucosides</td>
<td>Antibacterial activity: Gram-positive and gram-negative bacteria</td>
<td>Otshudi et al. (1999)</td>
</tr>
<tr>
<td><em>Vernonia amygdalina</em></td>
<td>AP</td>
<td>ME/AE</td>
<td>-</td>
<td>Antibacterial activity: Gram-positive and gram-negative bacteria</td>
<td>Kambizi and Afolayan (2001)</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>EE</td>
<td>Sesquiterpene lactones</td>
<td>Antibacterial activity: Gram-positive and gram-negative bacteria</td>
<td>Erasto et al. (2006)</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>AqE</td>
<td>-</td>
<td>Antifungal activity: <em>Fusarium</em> sp filamentous fungi</td>
<td>Suleiman et al. (2008)</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>ME</td>
<td>Sesquiterpene lactones and steroid glucosides</td>
<td>Antibacterial activity: Gram-positive and gram-negative bacteria</td>
<td>Cheruiyot et al. (2009)</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>AE</td>
<td>-</td>
<td>Antibacterial activity: Gram-positive <em>Mycoplasma mycoides</em> subsp. mycoides</td>
<td>Muraina et al. (2010)</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>EE/AqE</td>
<td>Phenolic compounds</td>
<td>Antibacterial activity: Gram-positive and gram-negative bacteria</td>
<td>Salawu et al. (2011)</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>EE/AqE</td>
<td>Sesquiterpene lactones</td>
<td>Antibacterial activity: Gram-positive and gram-negative bacteria</td>
<td>Adetutu et al. (2011)</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>ME</td>
<td>Alkaloids, phenols, tannins, phytosterols, flavonoids and saponins</td>
<td>Antibacterial activity: Gram-negative bacteria</td>
<td>Noumedem et al. (2013)</td>
</tr>
<tr>
<td><em>Vernonia anthelmintica</em></td>
<td>S</td>
<td>ME</td>
<td>-</td>
<td>Antibacterial activity: Gram-positive and gram-negative bacteria</td>
<td>Jahan et al. (2010)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Antifungal activity: yeast and filamentous fungi</td>
<td></td>
</tr>
<tr>
<td><em>Vernonia auriculifera</em></td>
<td>L/St/R</td>
<td>HE/DE/DE/AE/ME</td>
<td>Triterpenes and sesquiterpenes</td>
<td>Antibacterial activity: Gram-positive and gram-negative bacteria</td>
<td>Kiplimo et al. (2011)</td>
</tr>
<tr>
<td></td>
<td>AP</td>
<td>CE/EE</td>
<td>-</td>
<td>Antibacterial activity: Gram-positive and gram-negative bacteria</td>
<td>Aliyu et al. (2011)</td>
</tr>
<tr>
<td><em>Vernonia brasiliiana</em></td>
<td>L</td>
<td>EO</td>
<td>Sesquiterpenes</td>
<td>Antibacterial activity: Gram-positive and gram-negative bacteria</td>
<td>Maia et al. (2010)</td>
</tr>
<tr>
<td><em>Vernonia cinerea</em></td>
<td>L/St/R</td>
<td>ME/EAE</td>
<td>-</td>
<td>Antibacterial activity: Gram-positive and gram-negative bacteria</td>
<td>Kelman et al. (2000)</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>HE/AE</td>
<td>Sesquiterpene lactones</td>
<td>Antibacterial activity: Gram-positive and gram-negative bacteria</td>
<td>Rabe et al. (2002)</td>
</tr>
</tbody>
</table>
Table 1. cont’d

<table>
<thead>
<tr>
<th>Vernonia galamensis</th>
<th>S</th>
<th>FO</th>
<th>Amino compounds</th>
<th>Antibacterial activity: Gram-positive and gram-negative bacteria</th>
<th>Antifungal activity: Yeast and filamentous fungi</th>
<th>Mbugua et al. (2007)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vernonia guineenses</td>
<td>R</td>
<td>DE/ME/AqE</td>
<td>-</td>
<td>Antibacterial activity: Gram-positive and gram-negative bacteria</td>
<td>Antifungal activity: Yeast and filamentous fungi</td>
<td>Toyang et al. (2012)</td>
</tr>
<tr>
<td>Vernonia hymenolepis</td>
<td>L</td>
<td>ME</td>
<td>Alkaloids, phenols and flavonoids</td>
<td>Antibacterial activity: Gram-negative bacteria</td>
<td></td>
<td>Nouredem et al. (2013)</td>
</tr>
<tr>
<td>Vernonia lasiopus</td>
<td>L/St</td>
<td>AqE</td>
<td>-</td>
<td>Antibacterial activity: Gram-positive and gram-negative bacteria</td>
<td></td>
<td>Kareru et al. (2008)</td>
</tr>
<tr>
<td>Vernonia oocaphala</td>
<td>AP</td>
<td>CE/EE</td>
<td>alkaloids, flavonoids, saponins, tannins, glycosides, cardiac glycosides, steroids and triterpenes</td>
<td>Antibacterial activity: Gram-positive and gram-negative bacteria</td>
<td></td>
<td>Aliyu et al. (2011)</td>
</tr>
<tr>
<td>Vernonia polyanthes</td>
<td>L</td>
<td>ME</td>
<td>Alkaloids, triterpenoids, coumarins and flavonoids</td>
<td></td>
<td></td>
<td>Braga et al. (2007)</td>
</tr>
<tr>
<td>Vernonia remotiflora</td>
<td>L</td>
<td>EO</td>
<td>Sesquiterpenes</td>
<td>Antibacterial activity: Gram-positive and gram-negative bacteria</td>
<td></td>
<td>Freire et al. (1996a)</td>
</tr>
<tr>
<td></td>
<td>St</td>
<td>HE/CE</td>
<td>Sesquiterpene lactones</td>
<td></td>
<td></td>
<td>Freire et al. (1996b)</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>HE/CE</td>
<td>Sesquiterpene lactones</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vernonia scorpioides</td>
<td>AP</td>
<td>EO</td>
<td>Terpenoids</td>
<td>Antibacterial activity: Gram-positive and gram-negative bacteria</td>
<td></td>
<td>Toigo et al. (2004)</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>EAE</td>
<td>-</td>
<td>Antibacterial activity: Gram-positive bacteria</td>
<td></td>
<td>Kreuger et al. (2012)</td>
</tr>
<tr>
<td>Vernonia smithiana</td>
<td>AP</td>
<td>EO</td>
<td>Terpenoids</td>
<td>Antibacterial activity: Gram-positive and gram-negative bacteria</td>
<td></td>
<td>Vagionas et al. (2007)</td>
</tr>
<tr>
<td>Vernonia species</td>
<td>L</td>
<td>ME</td>
<td>-</td>
<td>Antibacterial activity: Gram-positive and gram-negative bacteria</td>
<td></td>
<td>Kalayou et al. (2012)</td>
</tr>
<tr>
<td>Vernonia tenoreana</td>
<td>L/St</td>
<td>ME/EAE/AE/HE</td>
<td>Alkaloids, tannins (including phlobaphenic tannins), cardiac glycosides and anthraquinones</td>
<td>Antibacterial activity: Gram-positive and gram-negative bacteria</td>
<td></td>
<td>Ogundare et al. (2006)</td>
</tr>
</tbody>
</table>

AP - Aereal part; L - Leaves; R - Root; S - Seed; St – Stems; AE - Acetone extract; AqE - Aqueous extract; CE - Chloroform extract; DE - Dichloromethane extract; DEE - Diethyl ether extract; EAE - Ethyl acetate extract; EE - ethanol extract; EO - Essential oil; FO - Fixed oil; HE - Hexane extract; ME - Methanol extract; PE - Petroleum ether extract.

(Kalayou et al., 2012). This article presents an overview of ethnopharmacological uses, local prevalence and antimicrobial potential of species of the genus *Vernonia*, with corresponding information in Table 1.

**Vernonia adoensis Sch. Bip. ex Walp.**

In Africa, this species is used in folk medicine for the treatment of various diseases, especially infectious diseases such as HIV/AIDS (Lamorde et al., 2010), tuberculosis and gonorrhea (Kisangau et al., 2007) and malaria (Ragunathan and Solomon, 2009). In Tanzania the petroleum ether extract of the leaves against the bacteria *Staphylococcus aureus*, *Bacillus subtilis*,
The authors used the disk diffusion and agar diffusion methods. The extracts only showed bioactivity against the strain of *E. coli* (Kisangau et al., 2007).

**Vernonia ambigua** Kotschy and Peyr (Asteraceae)

In Africa, particularly in Nigeria, Cameroon and Tanzania, this species is used in folk medicine to treat urinary tract infections, cough and colds (Burkill, 1985) as well as malaria (Builders et al., 2011).

In Northern Nigeria, it is widely used in traditional medicine for the treatment of various infectious diseases (Aliyu et al., 2011). *V. ambigua* is an annual shrub, in “Yoruba” is known as “Orungo” and in “Hausa” as “Tabtaba or Tattaba” (Builders et al., 2011). A phytochemical screening revealed the presence of secondary metabolites alkaloids, flavonoids, saponins, tannins, glycosides, cardiac glycosides, steroids and triterpenes (Aliyu et al., 2011).

Antibacterial activity of ethanolic extract of leaves was reported against ten clinical bacterial strains, *Klebsiella pneumoniae* (16 mm), *Streptococcus pyogenes* (16 mm), *S. aureus* (18 mm), *Corynbacterium ulcerans* (16 mm), methicillin resistant *S. aureus* (14 mm), *Salmonella typhi* (20 mm), *P. aeruginosa* (18 mm), *Shigella dysentriae* (0 mm), *Proteus mirabilis* (0 mm) and *Pseudomonas fluorescence* (0 mm), by the disk diffusion and broth microdilution methods. The MIC values ranged from 1.25 to 2.5 mg/mL for all the organisms tested (Aliyu et al., 2011).

**Vernonia amygdalina Delile**

*V. amygdalina* is a species with wide medicinal use in African countries, to treat malaria, helminth infections, gastrointestinal disorders and fever (Hamill et al., 2000; Magadula and Erasto, 2009), to promote wound healing by decoction (Adetutu et al., 2011) and to treat microbial infections (Noumedem et al., 2013). Three sesquiterpene lactones were isolated from the ethyl acetate extract of the leaves: vernodalin, vernolide and hydroxyvernolide. In turn, vernodalol was isolated from the methanol extract. All were tested against strains of *B. subtilis*, *Micrococcus luteus*, *E. coli* and *Agrobacterium tumefaciens*, showing significant results, indicated by growth inhibition zone (Jisaka et al., 1993). Akinpelu (1999), isolated saponins, tannins and flavonoids of the methanol extract of the leaves. Sensitivity tests were performed by the agar diffusion and broth dilution methods for the bacteria *Klebsiella pneumoniae*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *Proteus vulgaris*, *Serratia marcescens*, *Shigella dysenteriae* and *Staphylococcus aureus*, along with antifungal testing against the yeast *Candida albicans*.

The results showed antimicrobial activity against all tested microorganisms except *S. marcescens*, *E. coli* and *C. albicans*. Several studies have investigated the antimicrobial potential of solvent extracts of the aerial parts against gram-positive and gram-negative bacteria, using methanol, ethanol and water (Otshudi et al., 1999); methanol and acetone (Kambizi and Afolayan, 2001); ethanol (Erasto et al., 2006); methanol (Cheruiyot et al., 2009); ethanol and water (Salawu et al., 2011); ethanol and water (Adetutu et al., 2011); methanol (Noumedem et al., 2013); and acetone (Muraina et al., 2010). There
are also reports of the antifungal activity of *V. amygdalina* against *C. albicans* (Akinpelu, 1999), as well as fungi of the genus *Fusarium*, which cause superficial and systemic human infections, in addition to food contamination by the mycotoxins produced (Suleiman et al., 2008).

**Vernonia anthelmintica** (L.) Willd.

*V. anthelmintica* [syn. *Centhraterum anthelminticum* (L.) Kuntze], native to Africa and Asia, has been studied to treat diabetes (Rao et al., 2010; Fatima et al., 2010) and helminth infections (Mali and Mehta, 2008). The methanol extract of the seeds was tested against strains of the bacteria *P. aeruginosa*, *Yersinia aldovae*, *Citrobacter*, *Shigella flexneri*, *E. coli* and *S. aureus*. The antifungal activity was also tested against *Saccharomyces cerevisiae*, *C. albicans*, *Aspergillus parasiticus*, *Macrophomina*, *Fusarium solani*, *Trichophyton rubrum* and *Trichophyton occidentalis*. The extracts showed antibacterial activity against all strains tested except *Y. aldovae*. However the antifungal assay showed only inhibitory action against the dermatophyte *T. rubrum* (Jahan et al., 2010).

**Vernonia auriculifera** Hiern.

*V. auriculifera* is a shrubby species whose height varies between 1 and 7.5 m. It is native to Africa and is used in folk medicine to relieve headaches (Kusamba, 2001), to treat conjunctivitis, fever, viral and bacterial infections (Muthaura et al., 2007), and for various purposes by decoction in Cameroon (Focho et al., 2009). Extracts using the organic solvents hexane, dichloromethane, ethyl acetate and methanol of roots, stems and leaves of *V. auriculifera* were conducted.

Eight triterpenes and one aminated sesquiterpene were isolated. All compounds were tested by the broth microdilution method, against *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *Stenotrophomonas maltophilia*, *S. aureus*, *B. subtilis*, *Enterococcus faecium*, *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*. The results showed moderate antibacterial activity (Kiplimo et al., 2011).

**Vernonia blumeoides** Hook. F.

*V. blumenoides* is a perennial herb native to Africa in “Hausa” known as “Bagashi”, used in traditional medicine for the treatment of various protozoans (malaria), diarrhea and other unspecified infectious diseases in Nigeria (Ibrahim et al., 2013; Aliyu et al., 2015). A study have investigated the antibacterial activity of two sesquiterpene lactones isolated and of crude extracts using hexane, dichloromethane and ethyl acetate of aerial parts against gram-positive bacteria *B. subtilis*, *S. pyogenes*, *Staphylococcus saprophyticus*, *Staphylococcus sciuri*, *Staphylococcus xylosus*, *S. aureus* and *S. epidermidis*, by the disk diffusion method. The sesquiterpene lactone blumeolidole - A demonstrated the better antibacterial activity (Aliyu et al., 2015). In a previous study, a phytochemical screening revealed the presence of secondary metabolites alkaloids, flavonoids, saponins, tannins, glycosides, cardiac glycosides, steroids and triterpenes (Aliyu et al., 2011).

In other studies in Nigeria, the ethanolic extracts of leaves was reported against the clinical bacterial strains, *K. pneumoniae* (22 mm), *S. pyogenes* (16 mm), *S. aureus* (22 mm), *C. ulcerans* (24 mm), methicillin resistant *S. aureus* (17 mm), *S. typhi* (18 mm) and *S. dysentriae* (14 mm), by the disk diffusion and broth microdilution methods. The MIC values ranged from 1.25 to 2.5 mg/ml for all the organisms tested (Aliyu et al., 2011).

**Vernonia Brasiliana** (L.) Druce.

*V. brasiliana* is a species found in north eastern Brazil whose antiplasmodial activity was investigated against *Plasmodium berghei* and *P. falciparum* (Carvalho et al., 1991). The essential oil of leaves, extracted by hydrodistillation, was tested against *P. aeruginosa*, *Enterobacter aerogenes*, *Salmonella choleraesuis*, *K. pneumoniae*, *S. aureus* and *B. subtilis*, by the disk diffusion method, finding broad-spectrum antibacterial activity against all tested microorganisms (Maia et al., 2010).

**Vernonia cinerea** (L.) Less.

*V. cinerea* is an herbaceous species with ethnopharmacological use in South America, Africa and Asia, to treat various diseases or ailments such as malaria (Jain and Puri, 1984; Moshi et al., 2009; Padal et al., 2010), helminths infections (Johri et al., 1995; Alagesabooopati, 2009) and skin infections (Maregesi et al., 2007; Moshi et al., 2009). In Asia it has been documented and recommended in Thai and Indian traditional medicine, as in other countries, for smoking cessation, and relief of urinary calculi, cough, fever, asthma, malaria, and arthritis (Leelarungrayub et al., 2010; Kitikannakorn et al., 2013). In India the undergrowth includes shrub species such as *V. cinerea* (Behera and Misra, 2006). Studies on the chemical composition of extracts of the aerial parts from *V. cinerea* revealed the presence of triterpenes like α-amyrin, β-amyrin and lupeol (Gaikwad et al., 2012; Sreedevi et al., 2011).

The methanol extract of the aerial parts was tested against the bacterium *P. aeruginosa*, a prominent
opportunistic pathogen in hospital infections. The disk diffusion and broth dilution methods were used. The extract showed dose-dependent antimicrobial activity against the tested bacterium (Latha et al., 2010). The antifungal activity was tested using the methanol extract of the aerial parts against strains of the yeasts C. albicans and Cryptococcus neoformans. Assays were performed using the disk diffusion method, showing activity against both yeasts, but better activity against C. albicans (Latha et al., 2011).

**Vernonia colorata (Willd) Drake.**

*V. colorata* is a shrub native to Africa especially found in Cameroon, Ivory Coast, Senegal, Togo, South Africa and Benin (Chukwujekwu et al., 2009), known as “Ibozane” in South Africa (Kelmanson et al., 2000). Senegalese and Togolese traditional medicine practitioners employ the decoction of *V. colorata* leaves to cure diabetes mellitus (Sy et al., 2004). It is used for the treatment of cough, fever, hepatitis, gastritis, stomach pain, gastrointestinal disorders, venereal diseases and skin eruptions (Hutchings et al., 1996; Cioffi et al., 2004). Its leaves are used in culinary art in Benin, Cameroon and Togo (Sy et al., 2005). Phytochemical studies reported the presence of sesquiterpene lactones vernolide, 11β, 13-dihydrovernolide and vernodalin (Rabe et al., 2002). Also identified were tannins and saponosides (Sy et al., 2005).

In a study with 14 plants used in traditional Zulu medicine for treatment of ailments of an infectious nature, the methanolic and ethyl acetate extracts of *V. colorata* revealed antibacterial activity against gram-positive bacterium *S. epidermidis, S. aureus, M. luteus and B. subtilis* and gram-negative *E. coli* and *P. aeruginosa*, by the disk diffusion and broth microdilution methods. The ratios of the inhibition zone were expressed between 0.52 to 1.36 mm. The MIC values ranged from 1.0 to 4.0 mg/mL for *S. aureus, M. luteus, B. subtilis and P. aeruginosa* (Kelmanson et al., 2000). In other study in South Africa, the sesquiterpene lactones vernolide, 11β, 13-dihydrovernolide and vernodalin were identified and tested against the bacterium *S. aureus, B. subtilis, E. coli* and *K. pneumoniae* by the direct bioautography and broth microdilution methods. The results showed that all sesquiterpene lactones were active at inhibiting bacterial growth (Rabe et al., 2002).

**Vernonia condensata Baker.**

*V. condensata* is a species found in Brazil and Africa with medicinal use reported in Nigeria against the snakebite (Pereira et al., 1994). Known in Brazil as “aluma” and “alcachofra”, and its leaves are mainly used to relieve muscle pain along with gastrointestinal and hepatic problems (Albuquerque et al., 2007b). A study of the methanol extract of the aerial parts showed antibacterial activity against *S. aureus* by the broth dilution method, with minimal inhibitory concentration greater than the positive control used, chloramphenicol (Brasileiro et al., 2006).

**Vernonia galamensis Less.**

*V. galamensis* is found in Africa and has added commercial value because the oil extracted from its seeds has important medicinal use for treating diabetes and other organic dysfunctions (Autamashih et al., 2011). These oils have high vernolic acid content, an unsaturated fat composed of triglycerides such as the trivernolin, present as the major constituent (Figure 2). The derivatives of fixed oils of *V. galamensis* such as aminated fats are important intermediate chemicals, also acting as additives for polyethylene films, water-based insect repellent and fungicidal compounds, among others (Watanabe et al., 1993).

A study of the fixed oil of the seeds isolated unsaturated fats, subsequently converted into aminated fats, vernolamide, tested the antimicrobial activity against the bacteria *E. coli, B. subtilis and S. aureus*, and the fungi *S. cerevisiae, Microsporum gypseum* and *Trichophyton mentagrophytes*. The authors have used the disk diffusion method and the results showed activity against strains of *E. coli* and *B. subtilis*, but not against the yeast and dermatophyte fungi tested (Mbogua et al., 2007).

**Vernonia glabra (Steetz) Oliv. & Hiern**

A herbaceous species found in Africa, *V. glabra* has therapeutic use in Kenya for gastrointestinal problems by drinking tea from decoction of leaves and roots (Johns et al., 1995). It is also used in the same country as an antidote for snakebite (Owuor and Kisangau, 2006). Dichloromethane and methanolic extracts of the aerial parts, stems and roots were tested against strains of the gram-positive bacterium *S. aureus* and gram-negative *E. coli* and fungi *C. albicans* and *Aspergillus niger* by the disk diffusion method. The results showed moderate antimicrobial potential against all tested microorganisms, dependent upon the plant part used (Kitonde et al., 2013).

**Vernonia guineensis Benth.**

This species is widely used in folk medicine in the Central African Republic, Congo and Cameroon. It has ethnopharmacological use of the roots as a stimulant, aphrodisiac, antimicrobial, snakebite antidote and to treat helminth infections (Tchinda et al., 2002; Noumi, 2010). Extracts obtained with dichloromethane, methanol and distilled water of the roots of *V. guineensis* var. *cameroonica* C. D. Adams were tested against the
Figure 2. Trivernolin, a triglyceride of vernolic acid (cis-12,13-epoxy-cis-9-octadecenoic acid).

bacteria Acinetobacter baumannii, E. coli, P. aeruginosa, Salmonella typhimurium, S. epidermidis and S. aureus as well as a methicillin-resistant strain of S. aureus. In turn, antifungal tests were conducted against Aspergillus fumigatus, C. albicans, C. neoformans and T. mentagrophytes. The authors used the microdilution broth method for determination of the minimal inhibitory concentration. All extracts showed antimicrobial activity against all tested strains (Toyang et al., 2012).

**Vernonia hymenolepis Vatke.**

Found in western Africa, the species *V. Hymenolepis* has medicinal use in Angola for treatment of respiratory system disorders and high blood pressure (Mengome et al., 2010). The methanol extract of the fresh leaves allowed isolation of alkaloids, phenols and flavonoids. The extracts were tested against the gram-negative bacteria Providencia stuartii, P. aeruginosa, K. pneumoniae, E. coli, Enterobacter cloacae and E. aerogenes. The assays were performed by the microdilution broth method, with promising results for all the tested strains of microorganisms (Noumedem et al., 2013).

**Vernonia lasiopus O. Hoffm.**

*V. lasiopus* is a species native to Africa, especially found in Central African countries such as in Kenya, Tanzania, Uganda and Rwanda. It shows biological potential, being used for the treatment of malaria and helminth infections, by decoction of leaves and bark (Kareru et al., 2007). The aqueous extract of the leaves and bark was tested against strains of E. coli, S. aureus and B. subtilis by disk diffusion method, with positive results for E. coli and B. subtilis, indicated by growth inhibition zone (Kareru et al., 2008).

**Vernonia oocophala Baker.**

Distributed across Northern part of Nigeria, *V. oocophala* is an erect perennial shrub used in the folk medicine to treat malaria and a number of unspecified infectious diseases (Aliyu et al., 2014). A study of phytochemical screening with native plants from Nigeria revealed the presence of secondary metabolites alkaloids, flavonoids, saponins, tannins, glycosides, cardiac glycosides, steroids and triterpenes (Aliyu et al., 2011).

The antibacterial activity of the ethanolic extracts of leaves of *V. oocophala* was reported against the clinical bacterial strains, K. pneumoniae (20 mm), S. pyogenes (16 mm), S. aureus (18 mm), C. ulcerans (20 mm), methicillin resistant S. aureus (20 mm) and P. mirabilis (22 mm) by the disk diffusion and broth microdilution methods. The MIC values ranged from 1.25 to 2.5 mg/mL for all the organisms tested (Aliyu et al., 2011).

**Vernonia polyanthes Less.**

*V. polyanthes* is native to Brazil. Extracts of its leaves and roots are used in folk for the treatment of rheumatism, bronchitis and cough (Lorenzi and Matos, 2002). The methanol extract of the leaves was found to contain alkaloids, triterpenoids, coumarins and flavonoids. The extracts were tested for antifungal and antileishmanial activity. In the antifungal assay, strains of the yeast C. albicans and C. neoformans were tested by the broth dilution and agar diffusion methods to determine the minimum inhibitory concentration. For the antileishmanial tests, the protozoa Leishmania amazonensis and L. chagasi were used. In all cases, the results were indicated by inhibition of parasite growth.
The results were satisfactory for leishmanicidal activity, though the extract showed no antifungal activity (Braga et al., 2007).

**Vernonia remotiflora Rich.**

*V. remotiflora* [syn. *Lepidaploa remotiflora*] is a species found in north eastern Brazil. Preliminary phytochemical studies allowed the identification and isolation of sesquiterpene lactones (Valdés et al., 1998) and a flavone (Jacobs et al., 1986). A study of the essential oil, extracted by hydrodistillation from the fresh leaves, investigated the antibacterial activity to *P. aeruginosa*, *E. aerogenes*, *Salmonella choleraesuis*, *K. pneumoniae*, *S. aureus* and *B. subtilis*, by the disk diffusion method. The essential oil inhibited the growth of all tested microorganisms (Maia et al., 2010).

**Vernonia scorpioide (Lam.) Pers.**

It is popularly known in Brazil by many names, *V. scorpioide* is a lianous, perennial, branched herb, considered to be a weed widely distributed throughout Brazil, usually growing in poor soils and deforested areas, behaving as ruderal parantropophyte plant (Rauh et al., 2011, Toigo et al., 2004). A study of its essential oil by gas chromatography and mass spectrometry indicated the predominant presence of sesquiterpene hydrocarbons, with the main constituents being β-caryophyllene, germacrene-D and bicyclogermacrene (Albuquerque et al., 2007a). Investigations of extracts of the aerial parts, using ethanol and hexane, allowed the isolation of triterpenes, steroids, a flavonoid and a polyacetylene lactone (Machado et al., 2013).

*V. scorpioide* is commonly used externally to treat a variety of skin disorders such as allergies, irritations, parasitosis, skin lesions, chronic wounds including ulcers of the lower extremities and itching (Rauh et al., 2011). It is also popularly used as an anti-hemorrhoidal and a potent antidiarrheal, employed in the form of teas for internal use and infusions for external use (Freire et al., 1996c). Evaluation of the antifungal activity of the chloroform extract of the stems of *V. scorpioide* against the fungus *Penicillium citrinum* showed the formation of growth inhibition zones up to 80 mm in diameter. Assays were performed by the agar diffusion method. The use of a low-polar solvent for the preparation of the extract allowed satisfactory obtainment of active extracts, supporting the possibility of the presence of sesquiterpene lactones, constituents with potent antifungal action (Freire et al., 1996a). A study with chloroform and hexane extracts of the stems and leaves demonstrated that the development of hyphae was incipient in the presence of extracts and that the extracts from the leaves showed less intensity, since the growth inhibition halos were smaller for both tested fungi, *Aspergillus alutaceos* and *P. citrinum* (Freire et al., 1996b).

Another study investigated the antimicrobial activity of the ethyl acetate extract obtained from fresh leaves of *V. scorpioide* for healing of excisional wounds in tissues of rats with inflammatory lesions caused by *S. aureus*. Rifamycin B diethylamide was used as positive control and saline as negative control. The results pointed to effective antimicrobial activity for wound healing in infected mice with increased wound contraction, smaller area of necrotic tissue, good development of granulation tissue, extensive array of extracellular matrix and epithelial regeneration (Kreuger et al., 2012). The ethanol extract of *V. scorpioide* showed antimicrobial activity against a strain of the yeast *C. albicans* and the bacterium *S. aureus*, with inhibition zones greater than 16 mm that were concentration dependent for gram-positive cocci and greater than 15 mm for the tested yeast. These tests were performed by agar diffusion method (Toigo et al., 2004).

**Vernonia smithiana Less.**

Native to the African continent, *V. Smithian*, which the common name in Tanzania is “umwanzuranya”, has therapeutic use for dysentery, gastrointestinal problems, eye diseases and urinary problems, mainly using extracts from the aerial parts and roots (Adjahoun et al., 1988). A study of the essential oil extracted from the aerial parts by hydrodistillation led to isolation of 39 volatile compounds. The main constituents were sesquiterpenes and monoterpenes.

The essential oil was tested by the agar dilution method, compared to a panel of microorganisms including the bacteria *S. aureus*, *E. coli*, *E. cloacae*, *K. pneumoniae*, *P. aeruginosa*, *Streptococcus mutans* and *Streptococcus viridans*, the latter two oral mucosal pathogens. For the antifungal assays, the yeasts *Candida glabrata*, *C. albicans* and *C. tropicalis* were used. The results showed excellent antimicrobial activity, inhibiting all the species of microorganisms tested (Vagionas et al., 2007).

**Vernonia tenoreana Oliv.**

*V. tenoreana* is a wild growing shrub found in African savannas, whose common name in the Yoruba language of Nigeria is “Ewuro Igbo”. The bark is used in some parts of Nigeria as emergency food (Sofowora, 1993). The antidiabetic biological activity was investigated in a prospective study through random biological screening (Taiwo et al., 2008). A phytochemical study of the extract of fresh leaves, using the organic solvents methanol, ethyl acetate, acetone and n-hexane allowed isolating alkaloids, tannins (including phlobaphenic tannins), cardiac glycosides and anthraquinones, which demon-
strates the phytochemical richness of this species, making it a promising target for subsequent biological studies (Ogundare et al., 2006).

The extracts above were used in antimicrobial susceptibility tests against bacteria and fungi by the agar diffusion method. The bacteria tested were *S. aureus*, *Streptococcus faecalis*, *B. subtilis*, *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *Bacillus cereus*, *Proteus vulgaris*, *Shigella dysenteriae* and *Serratia marcesens* and the fungi were *C. albicans*, *Aspergillus flavus*, *A. niger*, *Rhizopus stolonifer* and *Fusarium poae*. The results showed growth inhibition for all bacteria tested, but no activity against the fungi *R. stolonifer* and *F. poae* (Ogundare et al., 2006).

**CONCLUSION**

This review shows that the genus *Vernonia* is rich in species with extensive ethnomedicinal use, especially in Africa, and extracts obtained by various preparation methods are used, especially from the leaves, bark and roots. There are extensive studies of *V. amygdalina* and *V. scorpioides* pointing to their use in folk medicine and confirming their biological properties, and reafirms the genus importance in studies of the biology and chemistry of natural products.

Phytochemical tests have shown the genus to have a range of bioactive properties among the Asteraceae family, containing multiple chemical compounds, especially terpenoids, flavonoids, tannins, steroids and alkaloids. Several sesquiterpene lactones have been isolated with potential antifungal, cytotoxic, molluscicidal, insecticidal and antitumor activity. Thus, this review showed a profile of species of *Vernonia* with antimicrobial potential and their main ethnomedicinal uses, which corroborates other existing studies to the Asteraceae family and the genus *Vernonia*.

**ACKNOWLEDGEMENTS**

We are grateful to the Master’s Program in Natural Resources of State University of Ceará, the CAPES financing agency and to the Microbiology Laboratory of State University of Vale do Acaraú.

**Conflicts of interest**

The authors have declared that there is no conflict of interest.

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