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Anti-inflammatory property and redox profile of the leaves extract from Morinda citrifolia L.

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Morinda citrifolia Linn (Rubiaceae), popularly known as noni, is widely used in folk medicine in the form of decoction and infusion, particularly as anti-inflammatory, depurative, anti-rheumatic and antiulcer remedy. The infusion of M. citrifolia L. leaves is used in popular medicine in Northeast of Brazil to treat inflammatory and painful diseases. The present study was designed to evaluate the antioxidant potential and the anti-inflammatory effect of the aqueous extract from the dried leaves from M. citrifolia L. (EAMC). The free radical scavenging activities were determined for different concentrations using in vitro models, and the inflammatory processes were evaluated by carrageenan-induced pleurisy. The study results indicated a significant dose-dependent antioxidant effect by noni extract as evaluated by total antioxidant potential (TRAP) and total antioxid reactivity (TAR) assays. Noni extracts also exhibited modest catalase-like activity, and was able to inhibit the levels of proinflammatory cytokines. All experimental data indicate that the EAMC have not only remarkable antioxidant properties but also potential anti-inflammatory properties.

Key words: Morinda citrifolia, antioxidant, inflammation.

INTRODUCTION

Antioxidants are chemical substances that reduce or prevent cellular oxidation. These substances counteract the damaging effects of free radicals in tissues (Haliwell, 2008). An imbalance in the production and detoxification of reactive species in cells leads to widespread damage to biomolecules, resulting in conditions such as cancer, heart disease, inflammation and several other diseases (Bandyopadhyay et al., 2007). By counteracting the...
deleterious action of free radicals, antioxidants are believed to play a preventive role in such diseases (Haliwell, 2008).

Plants are potential sources of natural antioxidants. They absorb the sun’s radiation and generate high levels of oxygen as a secondary metabolite of photosynthesis. Oxygen is easily activated by ultraviolet (UV) radiation and heat from sunlight to produce, reactive oxygen species (ROS). Phytochemicals of plants consist of various antioxidant compounds to counteract these ROS in order to survive. Flavonoids and other polyphenols of foods and beverages have been associated with the decreased risk of age-related diseases in several epidemiological studies (Esmaeili and Sonboli, 2010). Flavonoids have powerful antioxidant activities in vitro, being able to scavenge a wide range of reactive oxygen, nitrogen, and chlorine species, such as superoxide $O_2^-$, hydroxyl radical $\cdotOH$, peroxyl radicals $RO_2^-$, hypochlorous acid (HOCl) and peroxynitrous acid (ONOOH) (Haliwell, 2008; Gasparetto et al., 2014).

Recent studies emphasize that recurrent or chronic inflammations associated with an oxidative stress have been implicated in various diseases such as cancer, diabetes, asthma and autoimmune diseases. The development of strategies for reducing inflammation and oxidation status could lead to effective treatments for these diseases. In this way, some natural products containing biological active molecules could participate to the prevention or the treatment of some of these diseases (Dussossoy et al., 2011). Herbal and natural products derived from plants have been used for centuries throughout the world in every culture. Recently, research has been intensified in Morinda citrifolia Linn (Rubiaceae) and its products as its benefits have become well known (Zin et al., 2002). Morinda citrifolia L., popularly known as noni, has been used in traditional Polynesian medicine for over 2,000 years. Morinda citrifolia is native from Southeast Asia to Australia and is cultivated in Polynesia, India, the Caribbean region, and Central and Northern South America. The infusion of Morinda citrifolia L. leaves is used in popular medicine in Northeast of Brazil to treat inflammatory and painful diseases (Wang et al., 2002; Chan-Blanco et al., 2006; Serafini et al., 2011).

The noni fruits have been used as a folk medicine for the treatment of many diseases including diabetes, high blood pressure, inflammation and cancer (Chan-Blanco et al., 2006). Noni leaves have been consumed as a vegetable food by multiple cultural groups. For this reason, it is included in the World Health Organization’s and Food and Agriculture Organization’s, food composition tables for East Asia and the Islands of the Pacific (West et al., 2007). It has been shown that noni leaves contain several phytochemicals including phenolic compounds (Serafini et al., 2011) such as flavonoids (Sang et al., 2001; Takashima et al., 2007; Deng et al., 2008). Regarding its biological activity, the noni juice and fruit demonstrated an antibacterial, anti-cancer, antioxidant, antinociceptive and anti-inflammatory activity (Wang and Su, 2001; Wang et al., 2002; Su et al., 2005; Dussossoy et al., 2011; Brown, 2012; Zin et al., 2012; Gupta and Patel, 2013). Other in-vivo studies include the reduction of blood glucose levels in mice with streptozotocin-induced diabetes, hypotensive effects in dogs by the intravenous injection of the water-soluble extract from the roots and an analgesic effect of the aqueous extract injected intraperitoneally in mice (Youngken et al., 1960; Younos et al., 1990; Yamaguchi et al., 2002; Nayak et al., 2007).

Whereas, noni juice and fruit have been well characterized pharmacologically, few data are available regarding the properties of Morinda citrifolia leaves. In this regard, this study aims at investigating the antioxidant and the anti-inflammatory effects of the aqueous extract from Morinda citrifolia leaves.

**MATERIAL AND METHODS**

**Plant material and preparation of the extract**

Morinda citrifolia leaves were collected from São Cristóvão in March 2013, Sergipe, Brazil (10°18’20.7“(S); 36°39’7.2”(W)). Herbarium voucher specimens (registry number 13503) were prepared, and deposited at the Department of Biology of the Federal University of Sergipe. To prepare the Morinda citrifolia leaves aqueous extract from Morinda citrifolia (EAMC), fresh leaves were dried in a ventilated oven (45°C). Previously powdered dried leaves were prepared by decoction in distilled water (7.5% (w/v) in 15 min; the solvent evaporated under reduced pressure and lyophilized.

**Drugs and reagents**

AAPH (2,2′-Azobis(2-methylpropionamide) dihydrochloride), luminol (5-amino-2,3-dihydro-1,4-phthalazinedione), 2-deoxyribose, glycine, Griess reagent, SNP (sodium nitroprusside), $H_2O_2$ (hydrogen peroxide), catalase and SOD (superoxide dismutase) were purchased from Sigma (USA). Indomethacine and Trolox were purchased from Sigma (Brazil).

**Animals**

Adult male albino Swiss mice (25 to 35 g) bred in animal house were used. Animals were housed at controlled temperature (22±2°C) with a 12 h light/dark cycle, standard lab chow and tap water ad libitum. Animals were habituated to the experimental room for at least 2 h before the experiments and used only once. All protocols employed have been approved by the Local Ethic’s Committee (process number: CEPA/UFS # 27/09) and are in accordance with the US guidelines for the care and use of Laboratory animals (NIH publication #85 to 23, revised in 1985). The number of animals used was the minimum necessary to demonstrate the consistent effects of the drug treatments.

**Total reactive antioxidant potential (TRAP) and total antioxidant reactivity (TAR)**

The total reactive antioxidant potential (TRAP) is employed to estimate the nonenzymatic antioxidant capacity of samples in vitro. This method is based on the quenching of luminol-enhanced...
Chemiluminescence (CL) derived from the thermolysis of AAPH as the free radical source (Lissi et al., 1992; Gasparotto et al., 2014). The background CL was measured by adding 4 ml of AAPH (10 mM) dissolved in glycine buffer (0.1 M, pH 8.6) to a glass scintillation vial. Then 10 μL of luminol (4 mM) was added to each vial and the CL was measured until constant light intensity. After this stabilization time, 10 μL of Trolox solution (water-soluble vitamin E analogue) or 10 μL of sample was added, and the CL was measured in a liquid scintillator counter working in the out of coincidence mode. The last count before the addition of Trolox or samples was considered as 100%. The count time was 10 s, and the CL emission was monitored for 3000 s after the addition of Trolox or samples. The luminescence emission was recorded in a Micro Beta luminescence counter (Perkin Elmer, Watham, LA). Graphs were obtained by plotting percentage of counts per minute (% cpm) versus time (s). The Area Under the Curve (AUC) was calculated using GraphPad Prism software. The total antioxidant reactivity (TAR) was also analyzed in the same samples used for TRAP readings. The TAR results were calculated as the ratio of light intensity in absence of samples (I0)/light intensity right after sample addition. Although TAR and TRAP evaluations are obtained in the same experiment, they represent different observations, since the TAR is more related to the antioxidant quality (reactivity, the scavenging capacity in a short-term period) and TRAP is more related to the antioxidant amount and kinetic behavior.

Catalase-Like activity
The capacity of EAMC to degrade hydrogen peroxide (H2O2) added in the incubation medium (“catalase-like” or “CAT-like” activity) was measured as described earlier (Aebi, 1984; Quintans-Junior et al., 2013). Catalase-like activity was monitored based on the rate decomposition of H2O2. Data were expressed as percentage of the rate decomposition of H2O2.

Superoxide-dependent adrenaline autooxidation (“SOD-Like” Activity)
The ability of EAMC to degrade hydrogen peroxide (H2O2) added in the incubation medium (“superoxide dismutase-like” activity or “SOD-like” activity) was measured as previously described (Bannister and Calabrese, 1987; Quintans-Junior et al., 2013). Superoxide production was determined by measuring spectrophotometrically by the inhibition of adrenaline autooxidation at 480 nm.

Carrageenan-induced pleurisy
Pleurisy was induced in mice by intrapleural injection of 0.1 ml of a carrageenan suspension (300 μg/cavity) diluted in sterile saline (NaCl 0.9%) as described by da Silva et al. (2014). Animals were pretreated with EAMC (100, 200 and 400 mg/kg orally), vehicle (saline) or indomethacine (10 mg/kg, i.p.) 60 min before the injection of phlogistic agent. Four hours after carrageenan injection, mice were killed by excess carbon dioxide and the pleural exudate was collected by pleural cavity lavage with 1 ml of PBS solution containing EDTA (10 mM). Several samples of the pleural fluid were collected for further determination TNF-α levels by ELISA or cells in a Neubauer chamber and cytocentrifuged.

Statistical analysis
Data are expressed as mean ± S.E.M. The obtained data were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's test. Data analyses were performed using the GraphPad Prism 5.0 software. Differences were considered significant if p <0.05.

RESULTS

Total reactive antioxidant potential (TRAP) and total antioxidant reactivity (TAR)
To further explore the redox profile of EAMC, the TRAP/TAR parameters were evaluated, which indicate the capacity of a given sample to act as a general antioxidant or prooxidant agent in a constant reactive species generating system. TRAP and TAR measurements showed a strong antioxidant capacity of the EAMC against the peroxyl radicals generated by the AAPH system (Figure 1). The EAMC at 100 μg/ml and 1 mg/mL had an antioxidant activity significantly stronger than Trolox (200 nM), which was used as a standard antioxidant reference compound.

Catalase-like activity
The study results showed a statistically significant, increase in CAT-like activity at the highest concentration of EAMC tested (Figure 2). However, due to the small extent of this increase, the study was not able to state whether this H2O2–scavenger activity would be significant in a physiologic context.

Superoxide-dependent adrenaline autooxidation
The study observed a decrease in SOD-like activity by EAMC at the highest concentration (1 mg/mL) when compared to the superoxide-generating system (Figure 3).

DISCUSSION
Oxidative stress is the result of an unbalance in reactive species production and antioxidant defense, and is a main component in cancer, infectious diseases, cardio-
Figure 1. (A) Total radical-trapping antioxidant parameter (TRAP) at different concentrations. (B) The total antioxidant reactivity (TAR) was calculated as the ratio of light intensity in absence of samples expressed as percent of inhibition (I₀/I). Values represent mean ± S.E.D., experiments in triplicate. * p < 0.001 different from system; ** p < 0.001 different from Trolox (ANOVA followed by Tukey).
Figure 2. Catalase-like (CAT-like) activity. CAT-like activity was measured in a catalase reaction buffer with H$_2$O$_2$. Values represent mean ± S.E.D., experiments in triplicate. ***p <0.001 different from control + NPS (ANOVA followed by Tukey).

Figure 3. Superoxide dismutase-like (SOD-like) activities. SOD-like activity was determined by following formation of adrenochrome in a SOD reaction buffer containing native purified catalase and adrenaline. Values represent mean ± S.E.D., experiments in triplicate. ***p <0.001 different from control + NPS (ANOVA followed by Tukey).
vascular disorders, and neurodegenerative conditions (Gelain et al., 2009). Pharmacological agents showing therapeutic efficiency against some diseases may exert antioxidant properties in target tissues, which may be related to their mechanism of action. *M. citrifolia* L. (Rubiaceae), the noni, has been used in traditional medicine in northeast Brazil to treat painful conditions. In the present work, a screening of redox activities and anti-inflammatory actions of the EAMC was performed.

In order to evaluate the antioxidant activity of a natural product, it is crucial to work with different assays, taking into consideration the various oxidation aspects in the systems under scrutiny (Esmaeili and Sonboli, 2010). In this context, the antioxidant activity of the EAMC were analyzed for the levels/activities of non-enzymatic antioxidants and compared with the activity of the well-known antioxidants. Due to this, the study examined the total antioxidant capacity of the EAMC on TRAP/TAR methods. The TRAP assay is widely used to determine the non-enzymatic antioxidant capacity in plant extracts, which is mostly dependent on the content on secondary metabolites with redox activity (Dresch et al., 2009).

The total antioxidant reactivity (TAR) index indicates the instantaneous decrease luminescence associated with the sample addition into peroxyl-generating system. While TRAP indicates the quantity of antioxidants pre-
sents in the plant extracts, the TAR indicates their antioxidant effectiveness (Gasparotto et al., 2014). The study results on TRAP/TAR assays showed that the EAMC had a significant antioxidant capacity can be attributed to the total phenolic content.

To confirm the antioxidant activity of the EAMC the study verified its ability to decompose hydrogen peroxide and oxygen. The primary scavenger of ROS is the enzyme superoxide dismutase (SOD), which converts superoxide to hydrogen peroxide and oxygen (Slesak and Miszalski, 2003). It has been proposed that SOD may play a vital role in the supplying H$_2$O$_2$ to peroxidase and preventing peroxidase inactivation by superoxide anion radicals (Zielinski et al., 2006).

The study observed a decrease in SOD-like activity by EAMC at the highest concentration when compared to the superoxide-generating system. Differences in the relative antioxidant potential of model compounds were observed when one compound is strongly antioxidant with one method and pro-oxidant with another (Moure et al., 2001). For such reason, the antioxidant activity of a compound must always be evaluated with different tests, in order to identify different mechanisms (Melo et al., 2011). To confirm the antioxidant activity of the EAMC the study evaluated the catalase-like activity. CAT constitutes the most efficient and elaborates system available in both plants and animals to control H$_2$O$_2$ concentrations. CAT catalyzes the dismutation of H$_2$O$_2$ to one molecule of H$_2$O and a half molecule of O$_2$. H$_2$O$_2$ is a normal product of mitochondrial electron transport, oxidation of fatty acids and photorespiration. The first reaction responsible for the generation of H$_2$O$_2$ is the transfer of electrons to molecular oxygen, which produces the superoxide radical (Montavon et al., 2007). Plant extracts exhibiting H$_2$O$_2$–scavenger activity may prevent a variety of deleterious effects derived from oxidative damage. The study results showed an increase in CAT-like activity at the highest concentration of EAMC. However, due to the small extent of this increase the study was not able to state whether this H$_2$O$_2$–scavenger activity would be significant in a physiologic context.

The results of this study, are in accordance with a study showing that the root methanol extract and the ethyl acetate partitions of all parts of the M. citrifolia tested had antioxidant activity, similar to the positive controls, using the ferric thiocyanate method and thiobarbituric acid test (Zin et al., 2002). Moreover, other study demonstrated antioxidant activity of the fruit juice from M. citrifolia in both lipid hydroperoxide and tetrazolium nitroblue assays (Wang and Su, 2001). Natural antioxidants present in medicinal plants are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. Natural products contain free radical scavengers such as polyphenols, flavonoids, and phenolic compounds. A number of scientific reports indicate that terpenoids, steroids, and phenolic compounds such as tannins, coumarins and flavonoids exert protective effects due to their antioxidant properties (Chandrakshar et al., 2006; Sreelatha and Padma, 2010). Several studies have shown that the antioxidant activity associated with medicinal plants is attributed to the total content of phenolic compounds.

The study results show an antioxidant and anti-inflammatory potential exhibited by the extracts in a dose-dependent manner. It has also been found in other studies that the EAMC contains polyphenols (Rasal et al., 2008; Yang et al., 2007; Dussossoy et al., 2011) and therefore, the antioxidant and anti-inflammatory effects of this extract may depend on its phenolic components. Also, flavonol glycosides, lipid glycodies, triterpenoids, polysaccharides, iridoids, alkaloids, lignans, trisaccharide, fatty acids esters, anthraquinones, scopoletin, vitamin C, minerals, octanoic acid, potassium, sitosterol, β-carotene, vitamin A, and linoleic acid have been isolated from noni fruits, roots and leaves (Chan-Hong et al., 2006; Rasal et al., 2008; Yang et al., 2007). Recently, the total phenolic content of the EAMC was reported to be 196.8 mg of phenolic equivalents (gallic acid) per gram of extract (Serafini et al., 2011). In addition to these, high-performance liquid chromatography (HPLC) fingerprint of the EAMC demonstrated pharmacologically important phyto-chemical, namely, the flavonoid rutin (Serafini et al., 2011).

It has been proposed that phenolic compounds are antioxidants and anti-inflammatory agents. Nevertheless, there is a relationship between the antioxidant and anti-inflammatory properties of phenolic compounds. Therefore, the biological effects of the leaf extract may depend on its phenolic components (Serafini et al., 2011). The anti-inflammatory property of phenolic compounds is associated with their ability to inhibit neutrophil degranulation. Indeed, studies have shown that certain flavonoids down regulate nitric oxide production in response to inflammatory stimuli. In addition, specific flavonoids are known to chelate iron, thereby removing a causal factor for the development of free radicals. Direct inhibition of lipid peroxidation is another protective measure. Selected flavonoids can reduce system complement activation, thereby decreasing the adhesion of inflammatory cells to the endothelium and in general resulting in a diminished inflammatory response.

Lipid mediators such as prostaglandins and leucotrienes and cytokines such as TNF-α, IL-1, IL-6 and IL-8 are involved in the carrageenan-induced pleurisy. These mediators promote accumulation of neutrophils and mononuclear cells from blood vessels and activate endothelial cells (Fröde and Medeiros, 2001). In addition, these mediators promote the plasmatic extravasation that contributes to the formation of pleural exudates and migration of leukocytes, which peaked at 4 h after administration of the phlogistic agent (Fröde et al., 2001; Menegazzi et al., 2008). The investigation of anti-inflammatory property of EAMC was evaluated in the
carrageenan-induced pleurisy. The study was able to detect a marked inhibitory effect of different doses of EAMC on neutrophil, leukocytes cells migration and TNF-α level without altering mononuclear cells migration in the pleural exudates. A possible explanation for these findings may be the fact that EAMC, through activation of PPAR α and γ, inhibits the COX-2 expression and prostaglandin synthesis that is involved in the exudates formation in the inflammatory process (Hotta et al., 2010). Dussossoy et al. (2011), also observed that several polyphenols belonging to the coumarin, flavonoids and phenolics acid groups, and two iridoid present in Noni juice have demonstrated reduced carrageenan-induced paw edema, directly inhibited cyclooxygenase COX-1 and COX-2 activities and inhibited the production of nitric oxide (NO) and prostaglandins E(2) (PGE(2)) in activated J774 cells, in a dose dependent manner. This study showed that Noni’s biological effects include anti-inflammatory action through NO and PGE pathways that might also be strengthened by anti-oxidant effects. Together, the results suggested that properties of M. citrifolia extract should be explored further in order to achieve newer tools for managing painful and inflammation conditions, including those related to oxidant states. Also, may be a source of new therapeutic candidates with a spectrum of activity similar to the current anti-inflammatory non-steroids such as indomethacin. Further studies are underway to investigate which compounds in the extract are responsible for the anti-inflammatory activity and the precise mechanism and site of action.

CONCLUSION

The data reported in this study show that M. citrifolia has important antioxidant and anti-inflammatory properties. The inhibition of proinflammatory cytokine TNF-α production seems to be a key event to these effects, as does the antioxidant effect, but further studies can clarify the exact mechanisms underlying the effects of M. citrifolia. Altogether, the results obtained in this study, along with previously reported data on M. citrifolia (Serafini et al., 2011), suggest that this plant may be an interesting candidate for the development of new therapeutic options for the treatment of inflammatory disorders.

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Conflict of interest

The authors declare no conflict of interest.

REFERENCES


A review of four common medicinal plants used to treat eczema

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Many medicinal plants are commonly used to treat skin diseases such as eczema, psoriasis, vitiligo, cellulitis, herpes and cancer. Herbal medicine is as old as civilization. Application of traditional herbal medicine is widespread in different regions of the world. It is more common in villages and desert areas where medical services are less accessible. Herbal treatments are generally perceived as effective and have few side effects. Research on herbal drugs in terms of controlled clinical trials in humans is still limited. Herbal clinical research optimistically opens new therapeutic avenues. Eczema is considered as a group of medical conditions that cause the skin to become inflamed or irritated. The treatment of eczema is complicated. Moreover, screening is essential to reduce any potentially harmful side effects on human skin and health. This review summarizes the published literature on four common medicinal plants, namely, aloe (Aloe vera), oat (Avena sativa), turmeric (Curcuma longa) and chamomile (Matricaria chamomilla) used for the treatment of eczema. The mechanism of action, therapeutic indications and side effects of these plants are described.

Key words: Medicinal plants, treatment, skin diseases, eczema.

INTRODUCTION

Many medicinal plant species worldwide are used in traditional medicine for treating different diseases. The world health organization (WHO) has estimated that about 80% of the population living in the developing countries depends tremendously on traditional medicine for their primary health needs. More than half of the world's population still depends exclusively on medicinal plants, and plants offer the active ingredients of most traditional medical products (Kumar and Navaratnam, 2013). Human skin is the largest organ in the body. It forms the first guard line. Its three main layers are epidermis, dermis and hypodermis (subcutaneous tissue). Each layer offers a distinctive role in the homeostasis of the skin. They vary in thickness throughout the body and from person-to-person (Tabassum and Hamdani, 2014).

Cutaneous inflammation is aggravated by pathogens, noxious mechanical and chemical agents, and immune/
autoimmune responses. It is a complicated process during which the body repairs tissue damage and protects itself against harmful stimuli. Inflammation is distinguished by symptoms such as redness, swelling, itching, heat and pain (Ikeda et al., 2008). Under the effect of an inflammatory factor, some intracellular biochemicals are released from cells. Monocytes and macrophages produce cytokines. Their basic function in inflammatory processes is to activate cells engaged in the inflammation (neutrophils, macrophages, and mast cells), allow communication between them, provoke the prostaglandin synthesis and affect the synthesis of the C-reactive proteins. Among cytokines one can differentiate pro-inflammatory (interleukins IL-1, IL-6, IL-8, IL-17, IL-18, α and β interferon, and TNF) from anti-inflammatory ones (for example, IL-4, IL-10, IL-13). Prevalence of the first type leads to the systemic inflammatory reaction while prevalence of anti-inflammatory cytokines results in the anti-inflammatory response (Karpel, 2001). Chronic inflammatory diseases appear as a response to the disorders of inflammatory processes and excessive production of pro-inflammatory mediators such as IL-1β, IL-6 and TNF-α initiating an inflammatory reaction cascade. Furthermore, in skin inflammatory diseases one may notice biosynthesis disturbance of eicosanoids in epidermis and instability of the neuroimmunological system in the skin, that is increased production of neurological mediators like P substance which stimulates nitrogen oxide synthesis (Aries et al., 2003; European Medicines Agency (EMEA), 2008).

One of the inflammation-based diseases is atopic dermatitis (atopic eczema), which is a chronic disease affecting people genetically tended to overreact to external factors. It is commonly found in association with allergic rhinitis, asthma, or other manifestations of atopy. Atopic dermatitis is a widespread dermatologic disease in children. The most commonly observed manifestations of atopic dermatitis are extreme skin dryness and itching, redness, scaly patches, and thickened lichenified plaques with excoriation. 

*Staphylococcus aureus* is being noticed to inhabit skin. Secondary impetiginization, with honey-colored crust, is general in infants. Atopic dermatitis origin is complicated. It is claimed that during the onset and in the course of the disease, the most significant are genetic factors and the effect of the external environment (Tay et al., 2002; Worm, 2002; Teplitisky et al., 2008). An immunological mechanism which participates in the pathogenesis of the atopic dermatitis and other skin diseases of the inflammatory origin is linked to activation of T lymphocytes and it is a result of complicated interactions of different cells: keratinocytes, endothelium cells, eosinophils, Langerhans cells and T lymphocytes, and many cytokines and mediators. In atopic skin diseases, skin cells generate interleukins initiating inflammatory reactions (Aries et al., 1999). In patients, great production of specific antigens IgE against low amounts of food and inhalant allergens, which are responsible for inflammation development, is noticed. Due to releasing the leukotrienes, prostaglandins and proteases, inflammation symptoms happen in different organs and systems. Extreme skin dryness, characteristic of atopic dermatitis, is linked to activity change of Δ6-desaturase – an enzyme catalyzing transformation of the linolenic acid into the γ-linolenic one.

Patients affected with this disease exhibit a low level of essential fatty acids (EFA) and disorder of lipid production in epidermis, which are of importance during formation and persistence of dermal changes. In patients suffering from atopic dermatitis, an elevated value of trans epidermal water loss (TEWL) is noted in dry skin areas and not affected by inflammation, and in the clinically healthy skin, which may be linked to a lowered concentration of lipids in skin, particularly that of ceramides, and the loss of ingredients of a natural moisturizing factor (NMF) (Murata et al., 1996; Schürer, 2002; Pytkowska, 2003). In atopic dermatitis treatment, plant substances with anti-inflammatory activities and the ability to regulate the synthesis of lipids in epidermis would be employed.

The paper of Pan et al. (2014) offers a review of the history and status quo of Chinese, Indian, and Arabic herbal medicines regarding their significant contribution to the health promotion in the present-day over-populated and aging societies. Medicinal plants have been used for centuries all over the world, and many people still depend on indigenous medicinal plants for their primary health care requirements. Several researchers including Rahman et al. (2004), El-Ghazali et al. (2010) and Daur (2012) have demonstrated that Saudi Arabia has valuable medicinal plants and its natural stress conditions of drought and heat are considered as positive factors for medicinal plants. Many herbs are used to treat various skin diseases including eczema (Khiljee et al., 2011; Zari, 2012; Dawid-Pać, 2013; Tabassum and Hamdani, 2014; Radha and Laxmi Priya, 2015). In this review, we summarize the scientific data published on four common medicinal plants, namely, *Aloe vera, Avena sativa, Curcuma longa* and *Matricaria chamomilla* used for the treatment of eczema. The results of different studies on each plant, possible mechanism of action, their chemical composition and toxicity data have been presented.

**METHODOLOGY**

There are many medicinal plants used to treat eczema, however, we selected in this review four common medicinal plants, namely, *Aloe vera*, oat (*Avena sativa*), turmeric (*Curcuma longa*) and chamomile (*Matricaria chamomilla*) used for the treatment of eczema. These plants were chosen for this study based on: first, previous literature reviews and second, ethnobotanical information. In addition, it seems that these four plants are relatively more effective in treating eczema and have minimal side effects compared to most other plants. The current review was achieved using an organized search of the scientific data published on four medicinal plants used for the treatment of eczema. The searches were carried out using various
Aloe vera (Aloe barbadensis) — Aloe barbadensis Mill. syn. A. vera (L.) Burm. f. (Barbados aloe, cape aloe) is known in Saudi Arabia as ‘Sabar’. It is a perennial plant. It is commonly found in arid climate areas (Figure 1). It is native to Africa but it has been commonly cultivated throughout the world. Naturalised stands of the species occur in the southern half of the Arabian Peninsula, through North Africa, as well as Sudan and neighbouring countries. It is widely naturalized elsewhere (Akinyele and Odiyi, 2007) and large-scale agricultural production is undertaken in different countries to supply the cosmetics industry with A. vera gel. It is a stemless succulent plant growing up to 60 to 100 cm tall. Its flowers are produced in summer and are pendulous with yellow tubular corolla, 2 to 3 cm long (Yates, 2002; McLeod, 2002). It is juicy with bright yellow tubular flowers and thick and fleshy, 30 to 50 cm long, pea-green leaves. The leaf edge is serrated and spiky (World Health Organization, 1999). Its various photocomponents have different biological properties that help to improve health and prevent disease conditions. It is one of the richest natural sources of health for human beings coming. Its chemistry has revealed the presence of more than 200 different biologically active substances. Many biological properties linked with Aloe species are contributed by the inner gel of the leaves. The genus Aloe has more than 400 species but few, such as A. vera, Aloe ferox, and Aloe arborescens, are worldwide used for trade (Radha and Laxmi Priya, 2015).

A. vera has anti-inflammatory, antioxidant, antimicrobial, anticancer, immune boosting and hypoglycemic properties. It is used traditionally to treat many diseases. It is applied externally for wound healing, soothing inflamed skin and psoriasis (Vogler and Ernst, 1999). It is also used internally (Eshun and He, 2004) for relief of heart burns and indigestion (Langmead et al., 2004), liver disease like hepatitis (Bottenberg et al., 2007), and diabetes mellitus (Bunyapraphatsara et al., 1996). A. vera extracts possess antifungal and antibacterial activities (Ferro et al., 2003). Its gel is applied directly to the eczematous skin. Because of its moisturising effect, the skin becomes softer and wounds heal rapidly. Many patients reported decrease of the symptoms of eczema such as skin dryness, scaling and improved skin quality. In addition, its antibacterial activities prevent secondary infection. In a randomised, double-blind clinical study by Syed et al. (1996) A. vera cream was compared with the placebo in 60 patients having mild to moderate chronic psoriasis. The cure rate was 83% with A. vera cream as compared to 7% with placebo.

A. vera is used in traditional medicine as a multipurpose skin treatment such as in Ayurvedic medicine Quattrochi (2012). Early records of A. vera use appear in the Ebers Papyrus from the 16th century BC, and in Dioscorides’ De Materia Medica and Pliny the Elder’s Natural History - both written in the mid-first century AD (Barcroft and Myskja, 2003). It is also written of in the Juliana Anicia Codex of 512 AD (Reynolds, 2004). It is used generally in traditional herbal medicine of many countries (Boudreau and Beland, 2006). Records have been found dating back over two thousand years listing A. vera as a skin treatment for eczema. Research has revealed that it contains a wound healing and anti-inflammatory property (Subramanian et al., 2006) which is why it is considered to be effective on eczema.

Many active ingredients including aloesin, aloemodin, acemannan, aloeride, methylchromones, flavonoids, saponin, amino acids, vitamins, and minerals have been identified from inner gel of leaves. Active ingredients in fresh aloe leaves are also carbohydrates (mannose-6-phosphate, acemannan - acetylated-1,4 polymer of mannose), glycoproteins, sterols (lupeol, β-sitosterol) and enzymes (bradykinase). Gel is prepared from fresh leaves and it is an antranoid-free preparation (Schulz et al., 2004; Choi et al., 2001; Femenia et al., 1999). A. vera leaves have also phytochemicals such as polymannans, anthraquinone C-glycosides, anethrones, other anthraquinones, such as emodin, and various lectins (Eshun and He, 2004; Boudreau and Beland, 2006).

Fresh aloe gel considerably reduced acute inflammation in rats (carrageenin-induced paw oedema), though no effect on chronic inflammation was noticed. Enzymes, carbohydrates and sterols contribute to anti-inflammatory activity of the aloe gel. Bradykininase inhibited thromboxane B2 and prostaglandin F2 activity in vitro, and mannose-6-phosphate, acemannan and sterols (mainly lupeol) decreased inflammation induced experimentally in vivo (Choi et al., 2003; Jia et al., 2008). Its gel is used for external treatment of minor wounds and inflammatory skin disorders, minor skin irritations including burns, bruises and abrasions. The application of freshly prepared gel is recommended because of its sensitivity to enzymatic, oxidative or microbial degradation. The gel possesses characteristics that are harmful to certain bacteria and fungi. A cream containing 0.5% aloe for 4 weeks lessened the skin “plaques” linked with psoriasis (Syed et al., 1996). Gel application assisted in the improvement of partial thickness burns (Kauffman et al., 1988). When the gel is applied to the skin, it appears to aid the skin to survive frostbite injury (Miller and Koltai, 1995). It may delay wound healing by improving blood circulation and stopping cell death around the wound. The effects of Scutellariae radix and A. vera gel on spontaneous atopic dermatitis (AD)-like skin lesions in mice were investigated. The results showed that the group receiving only A. vera gel in a dose of 0.8 mg/kg p.o gave relief in AD due to decrease of interleukin (IL)-5 and IL-10 levels (Kim et al., 2010).


**ALOE VERA (COMMON NAME: BARBADOS ALOE; FAMILY: XANTHORRHOEACEAE)**
alternative medicine industries, being marketed as variously having rejuvenating, healing, or soothing properties. However, there is insufficient scientific evidence of the efficiency or safety of A. vera extracts for either cosmetic or medicinal purposes, and what positive evidence is available (Boudreau and Beland, 2006) is frequently contradicted by other studies (Vogler and Ernst, 1999; Ernst, 2000). A. vera has possible toxicity, with side effects occurring at some dose levels either when ingested or applied topically. Oral ingestion of A. vera might cause diarrhea, while topical application may induce contact dermatitis, erythema, or phototoxicity (Boudreau et al., 2013). Cases of contact allergy have been seldom reported (WHO, 1999).

AVENA SATIVA L. (COMMON NAME: OAT; FAMILY: POACEAE)

It is native to the warm Mediterranean region. It is an annual plant. A. sativa is known in Saudi Arabia as 'Shofan' (Figure 2). Oat is cultivated in Europe, North America and Asia for its yield of grain. It has a distinctive inflorescence - a composite panicle, unlike wheat, rye and barley (Blumenthal et al., 2000). Active components of oat fruit are mucilage polysaccharides (β-glucan), proteins (glutelin and avenin), and flavonoids (European Medicines Agency (EMEA), 2008). It has been used as a relaxing herb for a long time in order to alleviate itching and irritation. Oats have compounds called avenanthramides, which are powerful anti-inflammatory agents and also display anti-oxidant activity (Sur et al., 2008). Oats are commonly considered "healthy", or a health food, being advertised as nutritious. The established property of their cholesterol-lowering effects has led to acceptance of oats as a health food (Whitehead et al., 2014). Oat grass has been used traditionally for medicinal purposes, including to help balance the menstrual cycle, treat dysmenorrhea and for osteoporosis and urinary tract infections (Duke, 2002).

Different clinical studies have been undertaken to investigate the effect of oats on eczema and these have all showed a significant decrease in skin redness, dryness, scaliness, itching and erythema after application of oat extracts. These results were observed in adults and children (Nebus et al., 2012). In vitro, a colloidal oat extract demonstrated anti-inflammatory activity – inhibited releasing of the arachidonic acid from phospholipids and the subsequent metabolism into prostaglandin and leukotrienes. In addition, it inhibited the expression of phospholipase A2 (PLA2) and cyclooxygenase (COX-2) (Aries et al., 2003). A colloidal oat extract stimulated production of the anti-inflammatory transforming growth factor β1 (TGFβ1) by keratinocytes, and inhibited production of interleukins (Aries et al., 1999). About 20 and 30% colloidal extracts of oat (in petrolatum), under occlusion for 2 h, protected the skin from irritation induced by sodium lauryl sulfate which caused skin redness and increased the cutaneous blood flow (improvement of both parameters was noticed) (European Medicines Agency, 2008). Colloidal oatmeal was used to treat 11 patients with drug – induced skin rash. Out of 10 patients evaluated, 6 demonstrated a complete response and 4 a partial response, with no toxic effects noticed (EMEA, 2008).

A. fructus is a traditional, herbal medicinal product used to treat minor skin inflammations such as sunburn, and it is used as an aid in the healing of minor wounds. Skin reactions may happen in atopic patients and in patients with contact dermatitis (EMEA, 2008). Oat straw comprises polysaccharides (β-glucan) and silicon dioxide in a soluble form – as esters of the silicic acid with polyphenols, and monosaccharides and oligosaccharides. β-Glucan stimulates immune functions in vitro and in vivo. Silicon controls skin and subcutaneous metabolic processes. Oat straw is applied for inflammatory and seborrhoeic skin diseases; particularly those that come with itching (Blumenthal et al., 2000; Weiss and Fintelmann, 2000).

Oat in colloidal form is a centuries-old topical treatment for different skin conditions, including skin rashes, erythema, burns, itch and eczema but few studies have examined the precise mechanism of action for the anti-inflammatory activity of colloidal oatmeal. Colloidal oatmeal extracts diminished pro-inflammatory cytokines in vitro and the colloidal oat skin protectant lotion demonstrated significant clinical improvements in skin dryness, scaling, roughness, and itch intensity. These results reveal that colloidal oat extracts display direct anti-oxidant and anti-inflammatory activities, which may provide the mechanisms for observed dermatological benefits while using the colloidal oatmeal skin protectant lotion (Reynertson et al., 2015).

CURCUMA LONGA L. (COMMON NAME: TURMERIC; FAMILY: ZINGIBERACEAE)

C. longa is known in Saudi Arabia as 'Kurkum' (Figure 3). The genus named Curcuma is the latinized form of the Arabic Al-Kurkum. It is a small rhizomatous perennial herb (Migahid, 1978). It is native in southwest India, and requires temperatures between 20 and 30°C and a great amount of annual rainfall to thrive (Prasad and Aggarwal, 2011). Turmeric grows wild in the forests of South and Southeast Asia. It has been used in Asia for thousands of years and is a main part of Siddha medicine. It was first used as a dye and then later for its medicinal properties (Chattopadhyay et al., 2004).
It is used as a main ingredient of cooking in Asian countries. Because of its yellow colour it is also employed as a dye. It has been widely investigated and found to have many applications. Important applications are in cancer, diabetes, asthma, anemia and intestinal disorders. In dermatology, it has wonderful wound healing activity. Furthermore, it improves skin complexion. It has antioxidant, anti-inflammatoriy, antiviral, antibacterial and antiseptic properties (Satoskar et al., 1986; Ramirez-Bosca et al., 1995; Khilji et al., 2011).

In the Siddha system, turmeric was a medicine for a range of diseases and conditions, including those of the skin, pulmonary, and gastrointestinal systems, aches, pains, wounds, sprains and liver disorders. A fresh juice is commonly used in many skin conditions, including eczema, chicken pox, shingles, allergy, and scabies. It has been used traditionally in India for thousands of years as a remedy for stomach and liver ailments, as well as topically to heal sores, basically for its believed antimicrobial property (Chaturvedi, 2009).

For over four thousand years, it has been widely used in Asian traditional medicine for the treatment of loss of appetite, jaundice, liver problems, gall bladder disorders, and arthritis. Hepatoprotective effect of turmeric has been attributed to its antioxidant (Ramirez-Bosca et al., 1995) and anti-inflammatory (Satoskar et al., 1986) properties. In addition, sodium curcuminate, a salt of curcumin, exerts choleretic effects by increasing biliary excretion of bile salts, cholesterol and bilirubin, supporting its application for cholelithiasis treatment (Al-Asmari et al., 2014).

The most important turmeric chemical constituents are a group of compounds called curcuminoids, which include curcumin (diferuloylmethane), demethoxycurcumin, bisdemethoxycurcumin. Curcumin, which constitutes about 3.14% of powdered turmeric (Tayyem et al., 2006). Furthermore, other important volatile oils include turmerone, atlantone and zingiberene. Some common components are sugars, proteins, and resins (Nagpal and Sood, 2013). The active compound curcumin is supposed to have many biological effects including anti-inflammatory, antioxidant, antitumour, antibacterial and antiviral activities, which show potential in clinical medicine (Aggarwal et al., 2007). It is generally used by people for eczema treatment. It appears that the active ingredient curcumin present in turmeric has anti-inflammatory and bactericidal properties, which may assist to treat skin inflammation linked with eczema. The healing effect of turmeric is attributed to polyphenolic curcuminoinds including curcumin I, curcumin II, and curcumin III. A medicinal paste of turmeric (turmeric powder mixed with sweet lime juice and salt) is applied on swellings. This paste provides rapid and long lasting relief. Turmeric powder is scattered on wounds/ulcers for fast healing. It is an antiseptic and stops bleeding and cures the cut or burn (Khilji et al., 2011). Turmeric does not aggravate the stomach as do many Cox-2 inhibitors (Gruenwald et al., 2007; Aggarwal, 2007).

Research conducted on male Swiss albino mice in which skin cancer was induced by topical application of 7,12-Dimethylbenz(a)anthracene (DMBA), demonstrated a considerable reduction in number of tumors per mouse in the group receiving 1% curcumin obtained from rhizomes of C. longa (Limtrakul, 1997). Toxicity studies on turmeric in animals demonstrated no adverse effect up to 2.5 g/kg b.w. (Shankar et al., 1980). Large doses in humans may cause gastric irritation. The review of Calapai et al. (2014) focuses on contact dermatitis as an adverse effect of a selection of topically used herbal medicinal products for which the European Medicines Agency has completed an evaluation up to the end of November 2013 and for which a Community herbal monograph has been produced. Part 1: Achillea millefolium L., Curcuma longa L., turmeric and other spices are usually sold by weight, the potential presently exists for powders of toxic, cheaper agents with a similar color to be added, such as lead(II)oxide, giving turmeric an orange-red color instead of its original gold-yellow (Kaul, 2015).

**MATRICARIA CHAMOMILA, MATRICARIA RECUTITA OR CHAMOMILLA RECUTITA (COMMON NAME: CHAMOMILE; FAMILY: ASTERACEAE)**

Matricaria chamomilla L., generally known as chamomile, is an annual plant. Chamomile is known in Saudi Arabia as 'Babunaj' (Figure 4). It is a well-known and generally used medicinal herb. *M. chamomilla*, a member of the Asteraceae family, is one of the oldest medicinal plants, widely used worldwide for diverse healing applications. It may be found near populated areas all over Europe and temperate Asia, and it has been broadly introduced in temperate North America and Australia. It frequently grows near roads, around landfills, and in cultivated fields as a weed. It is used in herbal medicine for a sore stomach, irritable bowel syndrome, and as a gentle sleep aid. In addition, it is used as a mild laxative and is anti-inflammatory (Bhaskaran et al., 2011) and bactercidal (Tayel and El-Tras, 2009). Its recommendations, derived from both traditional and modern medicine, include many diseases such as inflammation, ulcers, wounds, gastrointestinal disorders, stomach ache, pharyngitis, rheumatic pain. Extracts and decoctions made from chamomile are frequently recommended for treatment of many skin diseases for example, inflammation, wounds and itching. The review of Rügge et al. (2010) explores the evidence base of the dermatological effects of chamomile. Although many beneficial effects of chamomile have been suggested, no studies have so far been able to prove these claims significantly.

The work of Kolodziejczyk-Czepas et al. (2015) is focused on the biological activity of chamomile polyphenolic–polysaccharide conjugates – their antioxidant properties in the protection of blood plasma components against *in vitro* oxidative stress. Their results indicate that polyphenolic–polysaccharide conjugates isolated from *M. chamomilla* substances possess antioxidant properties. The *M. chamomilla* macromolecular glycoconjugates may be useful in the creation of new natural-based medications or dietary supplements, helpful in the prevention and treatment of oxidative stress-mediated disorders. Chamomile is a daisy like herb. It is well-known for its tea which is used in sleep disorders. It is traditionally claimed to be efficient in the treatment of cardiovascular disorders, common cold, sleep, cancer and gastrointestinal disorders. It is found to be efficient in wound healing and skin inflammatory conditions, consequently used in allergic conditions, atopic dermatitis and eczema. Flowers are used to make tea and liquid extracts, capsules and tablets. It is applied to skin in the form of ointment or cream (Blumenthal et al., 2000). Aertgeerts et al. (1985) performed a clinical study in 161 eczema patients using a cream made from chamomile extract. When compared with steroidal and nonsteroidal creams, it was similarly effective as steroidal cream and more effective than non-steroidal cream. Chamomile helps in skin cell regeneration and works as an antioxidant, fighting free radical damage on the skin. Allergies have been reported and those with daisy allergies may discover themselves allergic to chamomile (Renu, 2010).

It possesses flower heads with white internal linguiform flowers and the external tubular – yellow, typical of the Asteraceae family. It contains the essential oil (its main components are α-bisabolol and its oxides A, B and C, matricin, which is converted to chamazulene by distillation and en-ym-dicycloethers) and flavone derivatives such as apigenin, luteolin, and apigenin-7-glucoside (ESCP Monographs, 2003). It has antibacterial, anti-fungal, anti-inflammatory and antiseptic properties. It is also believed to be hypoallergenic with the ability to neutralize skin irritants. Most studies have been performed in Germany using a chamomile cream or ointment. Chamomile was found to have an effect that was 60% as active as 0.25% hydrocortisone when applied topically in humans. In another study, the chamomile ointment was effective in...
decreasing dermatitis following a single application of sodium lauryl sulfate (Brown and Dattner, 1998).

The dried flowers of chamomile contain many terpenoids and flavonoids contributing to its medicinal properties. Chamomile preparations are usually used for many human diseases such as hay fever, inflammation, muscle spasms, menstrual disorders, insomnia, ulcers, wounds, gastrointestinal disorders, rheumatic pain, and hemorrhoids. Essential oils of chamomile are used widely in cosmetics and aromatherapy. Many different preparations of chamomile have been developed, the most popular of which is in the form of herbal tea. Srivastava et al. (2010) reviewed the use of chamomile in traditional medicine and evaluated its curative and preventive properties.

Matricaria flower extracts revealed anti-inflammatory activity by inhibition of prostaglandins and leukotrienes synthesis in vitro. α-bisabolol and apigenin inhibited cyclooxygenase and 5-lipoxygenase activity, chamazulene inhibited only 5-lipoxygenase (Ammon et al., 1996). A dry extract of matricaria flower, used locally, inhibited croton oil-induced oedema in vivo, comparably to benzydamine (anti-inflammatory synthetic drug) (ESCOP Monographs, 2003). Intradermal use of liposomal apigenin-7-glucoside inhibited skin inflammations induced in rats. Topical use of either the total chamomile extract or the flavonoid fraction was effective in decreasing inflammation in a mouse model for croton oil-induced dermatitis. Apigenin and luteolin were more active than indometacin and phenylbutazone (non-steroidal anti-inflammatory synthetic drugs). Activity reduced in the following order: apigenin, luteolin, quercetin, myricetin, apigenin-7-glucoside, rutin (WHO, 1999).

In humans, an ointment containing matricaria flower extract was more efficient than 0.1% hydrocortisone (anti-inflammatory synthetic drug) in reduced chemically-induced toxic dermatitis. Creams containing matricaria flower extract decreased UV-induced erythema (ESCOP Monographs, 2003). Anti-inflammatory activity of ointment containing matricaria flower extract (treatment of patients suffering from inflammatory dermatoses on hands, forearms and lower legs) was similar to that of 0.25% hydrocortisone, and superior to 0.75% fluocortin butyl ester and 5% bufexamac (non-steroidal anti-inflammatory synthetic drugs) (Blumenthal et al., 2000). In another study, after 2 weeks of treatment of patients with medium-degree atopic eczema, effectiveness of cream containing matricaria flower extract was superior to that of 0.5% hydrocortisone cream regarding the symptoms of pruritus, erythema and desquamation (ESCOP Monographs, 2003).

Studies with animals suggest antispasmodic, anxiolytic, anti-inflammatory and some antimutagenic and cholesterol-lowering effects for chamomile (McKay and Blumberg, 2006). It has sped healing time of wounds in animals (Nayak et al., 2007; Jarrahi, 2008). In vitro chamomile has shown moderate antimicrobial and antioxidant properties and significant antiplatelet activity, as well as preliminary results against cancer (McKay and Blumberg, 2006; Srivastava and Gupta, 2007). Chamomile essential oil was demonstrated to be a potential antiviral agent against herpes simplex virus type 2 (HSV-2) in vitro (Koch et al., 2008). The active ingredients of its essential oil are the terpene bisabolol, farnesene, chamazulene, flavonoids (including apigenin, quercetin, patuletin and luteolin) and coumarin (McKay and Blumberg, 2006). The essential oil of chamomile and α-bisabolol
revealed bactericidal and fungicidal activity in vitro (chiefly against Gram-positive bacteria, *Staphylococcus aureus*, *Bacillus subtilis* and fungi *Candida albicans*) (WHO, 1999; Schulz et al., 2004). Matricaria flower is externally applied for skin inflammations and irritations, bacterial skin diseases, nappy rash and cradle cap, eczema, wounds, abscesses, frostbite and insect bites (Weiss and Fintelmann, 2000; WHO, 1999; Blumenthal et al., 2000). Matricaria flower is used for baths, compresses or rinses and poultice (ESCOP Monographs, 2003; Blumenthal et al., 2000). Chamomile, a relative of ragweed, may cause allergy symptoms and can cross-react with ragweed pollen in persons with ragweed allergies. Cases of contact allergy have been seldom reported (ESCOP Monographs, 2003).

**DISCUSSION**

Eczema is considered as a group of medical conditions that cause the skin to become inflamed or irritated. Eczema was estimated as of 2010 to affect about 230 million people worldwide (3.5% of the population) (Hay et al., 2014). In the United States approximately 10% of children have the condition, while in the United Kingdom approximately 20% are affected (McAleer et al., 2012). Al Shobaili (2010) conducted a study of all Saudi patients attending the Qassim University Medical College-affiliated dermatology clinics of the Ministry of Health for a period of 12 months from 1 March, 2008 to 28 February, 2009. The study included 3051 patients comprising 1786 (58.5%) males and 1265 (41.5%) females. Their mean age was 25.3 years. About 71% of the patients were between 5 and 34 years of age. The top five skin diseases were eczema/dermatitis (19.5%), viral infections (16.6%), pilosebaceous disorders (14.4%), pigmentary lesions (11.2%) and hair disorders (7.6%). The main disorder in males was viral skin infections (20.0%), while eczema/dermatitis (20.7%) constituted the most widespread skin disease in females.

The cause of eczema is unknown; however, it is thought to be related to an overactive response by the body's immune system to an irritant. Both endogenous and exogenous factors can cause increase to this inflammatory response (Ronald, 1992). Though there is no cure, most people can efficiently manage their disease with medical treatment and by avoiding irritants and frequent skin moisturizing. The aim of eczema treatment is to relieve and prevent itching, which can lead to infection. Because the disease makes the skin dry and itchy, ointments and creams are recommended to keep the skin moist. Medicines such as over the counter hydrocortisone 1% cream, or prescription topical steroid creams and ointments are frequently prescribed to decrease inflammation along with oral antihistamines to reduce and control the associated itching.

Moreover, if the affected area becomes infected, topical or oral antibiotics may be prescribed to kill the infection-causing microbes. Furthermore, creams based on calcineurin inhibitors might be used (Carr, 2013). Other treatments include tar treatments (chemicals designed to lessen itching), phototherapy (ultraviolet light therapy applied to the skin), and the drug cyclosporine or oral steroids for persons whose condition does not respond to other treatments. Corticosteroids are a significant treatment, however, side effects caused by long-term and excessive use heavily concern patients. These side effects could be decreased by integrating certain topical herbals to the treatment regimen. Chen et al. (2015) indicated that lower use rate of corticosteroids can be found after traditional Chinese medicine treatment, which can be considered as an integrative therapy for this disease.

In this review, we gathered publications on four common medicinal plants aloes (*A. vera*), oat (*A. sativa*), turmeric (*C. longa*) and chamomile (*M. chamomile*) used to treat eczema and addressed the question whether the treatment of eczema with these medicinal plants is efficient in humans. Though *in vivo* and *in vitro* investigations play a significant role in the evaluation of safety and efficacy of medicinal plants in preclinical trials, there is no perfect denouncement for their final success as human drugs. In addition, there are some conflicting clinical trials reported. Thus, the efficiency of these plants requires to be further clarified.

Many traditional medicines used in folk medicine are reported to have antieczema activity, however, only some have been investigated systematically *in vitro* or/and *in vivo*. Table 1 shows the four medicinal plants utilized in this review and their traditional uses for treating skin diseases. Though several *in vitro* studies have demonstrated the antieczema activity of plant extracts and phytochemicals, there is insufficient evidence in humans. The clinical trials and their highlighted results are limited. In addition, many of these phytochemicals have not been tested for their cytotoxicity, acute toxicity, or/and long-term toxicity in normal cells and animals, which seriously limits *in vivo* investigations. The clinical effectiveness and safety should be examined simultaneously for medicinal plant extracts and compounds. Though good progress has been lately accomplished, the impact of medicinal plants on eczema requires to be explored in more detail.

**CONCLUSION**

Medicinal plants have a great potential to cure different diseases. Many people worldwide use various plant based products for treating skin problems. These herbs are a rich source of active ingredients and can be safer and more cost effective for the treatment for different skin diseases. Inflammation is a complicated process, necessary for the host defense system. Extreme production of some inflammatory mediators may cause chronic diseases. Plant raw substances can possess an anti-inflammatory action affecting different stages of the inflammation process. They inhibit formation of cytokines.
Table 1. List of four medicinal plants utilized in this review and their traditional uses for treating skin diseases.

<table>
<thead>
<tr>
<th>Herb scientific name</th>
<th>Common name, local name</th>
<th>Family</th>
<th>Habit</th>
<th>Parts used</th>
<th>Selected traditional uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aloe vera L.</td>
<td>Aloe, Sabar</td>
<td>Xanthorrhoeaceae</td>
<td>Perennial herb</td>
<td>Leaf gel</td>
<td>Skin diseases including eczema, irritation, burns, wounds, bruises, abrasions, psoriasis, cuts, scrapes, cold sores, sun burns, inflammation, hair loss, rejuvenating, complexion improvement, cosmetic uses, microbial skin diseases</td>
</tr>
<tr>
<td>Avena sativa L.</td>
<td>Oat, Shofan</td>
<td>Poaceae</td>
<td>Annual herb</td>
<td>Colloidal oat extract, decoction</td>
<td>Skin diseases including eczema, wounds, irritation, inflammation, erythema, burns, itching, sunburn</td>
</tr>
<tr>
<td>Curcuma longa L.</td>
<td>Turmeric, Kurkum</td>
<td>Zingiberaceae</td>
<td>Perennial herb</td>
<td>Rhizome paste, powder</td>
<td>Skin diseases including eczema, wounds, burns, cuts, chicken pox, shingles, allergy, scabies, sores, infection, microbial skin diseases, complexion improvement</td>
</tr>
<tr>
<td>Matricaria chamomilla L.</td>
<td>Chamomile, Babunaj</td>
<td>Asteraceae</td>
<td>Annual herb</td>
<td>Flower extracts, decoctions, oil</td>
<td>Skin diseases including eczema, wounds, itching, irritation, inflammation, allergic conditions, dermatitis, erythema, bacterial skin diseases, nappy rash, frostbite, cosmetics uses</td>
</tr>
</tbody>
</table>

and eicosanoids, stop the inflammatory reaction cascade from starting, and reduce skin burn, itching or extreme exfoliation. The use of most herbs in treatment of inflammatory skin diseases is based on clinical and pharmacological trials in vitro and experiments in vivo. However, the use of some of them is based only on their longstanding traditional application in folk medicine. Though these herbs are generally safe to use on the skin, some people can be allergic or sensitive to certain plants, which can cause irritant contact dermatitis or allergic reactions. We constantly need to test new ingredients out before integrating them into any type of skin care regime.

Therefore, there is a need for more in vitro and in vivo research to evaluate and confirm the efficiency and safety of various herbs in the current era of evidence-based medicine. The present review could constitute a good basis for further investigation in the potential discovery of new natural bioactive compounds. Therefore, eczema treatment is complicated. Moreover, screening is essential to reduce any potentially harmful side effects on human skin and health.

**Conflict of Interest**

The authors have not declared any conflict of interest.

**REFERENCES**

Aertgeerts P, Albring M, Klaschka F (1985). Comparison of Kamillosan (TM) cream (2 g ethanolic extract from chamomile flowers in 100 g cream) versus steroid (0.25% hydrocortisone, 0.75% fluorocin butyl ester) and non-steroidal (5% bufexamac) dermatics in the maintenance therapy of eczema. Z. Hautkr. 60:270-277.


Foliar bioactive compounds in *Amburana cearensis* (Allemao) A.C. Smith seedlings: Increase of biosynthesis using mycorrhizal technology

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*Amburana cearensis* (Allemao) A.C. Smith is a widely used legume by the population due to its medicinal properties. This species establish symbiosis with the arbuscular mycorrhizal fungi (AMF) that can increase the production of secondary metabolites, a fact which has not been clarified for this plant. Therefore, the aim of this study was to examine the contribution of the AMF in the production increase of foliar bioactive compounds in *A. cearensis* seedlings. The experiment which under-goes protected roofing was carried out using four inoculation treatments: non-inoculated control treatment, inoculated with *Gigaspora albida*, inoculated with *Claroideoglomus etunicatum* and inoculated with *Acaulospora longula*. After 160 days, the following was examined: dry matter of the aerial part, chlorophylls *a*, *b* and total, soluble carbohydrates, total proteins, total phenols, total flavonoids and total tannins. *A. cearensis* seedlings inoculate with *C. etunicatum* accumulated more dry matter of the aerial part (78.38%), total chlorophylls (24.28%) and chlorophylls *b* (53.63%), total phenols (47.82%), total flavonoids (32.28%) and total tannins (61.58%) in relation to the control treatment. Mycorrhizal technology using the *C. etunicatum* fungus is an alternative to increase the levels of foliar bioactive compounds in *A. cearensis* seedlings.

**Key words:** Caatinga, phenolic compounds, arbuscular mycorrhizal fungi (AMF).

**INTRODUCTION**

Arbuscular mycorrhizal fungi (AMF) are inhabitants of the soil and belong to the Phylum Glomeromycota (Schubler et al., 2001). Such organisms are obligatory symbionts because they complete their life-cycle only in the presence of a host plant (Souza et al., 2008). After the fungus has established on the root, the AMF absorb
water and nutrients from the soil and in exchange the phytobiont makes about 20% of carbon available for the development of the fungus (Smith and Read, 2008).

Various studies relate the benefits of mycorrhizal association with legumes and point out the increased vegetable growth and optimized production of primary (Manoharan et al., 2010) and secondary metabolites (Silva et al., 2014a; Nisha and Rajeshkumar, 2010; Kapoor et al., 2004).

The increase in the production of secondary compounds in plants associated with AMF may be due to the increased nutritional supply (Toussaint et al., 2007), hormonal changes, enzymatic activation (Zhang et al., 2013) and increased activity of plastidal and mitochondrial pathways (Lohse et al., 2005), however, the effects seem to be somatoty and multifactorial (Toussaint et al., 2007).

The Caatinga is a biome that is rich in leguminous species with medicinal properties that are widely used by the local population as phytotherapeutic drugs (Agra et al., 2007, 2008). Amburana cearensis is found among the medicinal plants of the Caatinga, a legume that is used by the local population. Parts of this plant, such as the stem, the seeds and bark are used in the production of pastilles, syrups and teas for the treatment of various diseases due to their antioxidant (Leal et al., 2003), anti-inflammatory (Leal et al., 2008), antifungal (Santos et al., 2009), antibacterial (Figueiredo et al., 2013) and antineoplastic (Costa-Lotufo et al., 2003) properties. Such therapeutic benefits have been attributed to the presence of secondary compounds, especially phenolic compounds (Canuto and Silveira, 2006; Bravo et al., 1999). However, it is unknown in mycorrhizal that inoculation influences the increase in the production of secondary metabolites in A. cearensis seedlings. Therefore, the following hypothesis was tested: inoculation with AMF increases the production of bioactive compounds in A. cearensis with the benefits depending on the fungus that was tested. The aim of this study was to examine the efficiency of the AMF in increasing the production of foliar bioactive compounds in A. cearensis seedlings.

MATERIALS AND METHODS

Plant, AMF and experimental implementation

A. cearensis seeds were disinfected with 20% of NaClO (2% of active chlorine) for 2 min, washed in distilled water and put to germinate in plastic pots containing sterilized soil (autoclave at 121°C/30 min/2 consecutive days).

Three AMF isolates were tested: Acaulospora longula Spain & N.C. Schenck (UFPE 21), Claroideoglomus etunicatum (W. N. Becker & Gerdemann) C. Walker & A. Schussler (UFPE 06) and Gigaspora albida N.C. Schenck & G.S. Sm. (UFPE 01). The inoculums were supplied by the Department of Mycology from the Federal University of Pernambuco, Brazil, multiplied on millet (Panicum miliaceum L.) and stocked at 4°C, for 26 months, until the moment of inoculation.

Black polyethylene pots were filled with non-sterilized soil, which was collected from the Caatinga region and showed the following chemical characteristics: organic material, 3.21 g kg⁻¹; pH, 5.2; electric conductivity, 3.53 dSm⁻¹; P, 12.68 mg dm⁻³; K, 0.26 cmolc dm⁻³; Ca, 2.7 cmolc dm⁻³; Mg, 1.6 cmolc dm⁻³; Na, 0.49 cmolc dm⁻³; Al, 0.05 cmolc dm⁻³. The following AMF were identified in this soil: Appendiciglomus appendiculata Spain, Sieverd & Shenck, Acaulospora scrobiculata Trappe, Acaulospora sp.1, Glomus macrocarpum Tul. & Tul., Glomus sp.1 and Scutellospora sp.1 (Lima, 2014).

Plantlets with two definite leaves were transferred to the pots and inoculated at the root region with soil-inoculum of the tested AMF (200 glomerospores + colonized roots + hyphae). A. cearensis seedlings remained under experimental roofing for 160 days at the University of Pernambuco – Campus Petrolina, Brazil, under ambient temperature conditions (minimum: 21.7°C and maximum: 29.7°C), relative air humidity (42%) and an average global radiation (461.8 J/h/day).

Evaluation of the experiment and preparation of the extract

The experiment was evaluated 160 days after inoculation. Chlorophylls (total, a and b) were tested in vivo, using the CFL1030 – an electronic chlorophyll level meter ClorofiLOG (Silva et al., 2014a). After examining chlorophyll, the aerial part was separated from the roots and dried (45°C) for 3 consecutive days to determine the dry matter of the aerial part. The subterranean part was removed from the substrate and the fine roots were separated from the stylodium, washed and preserved in ethanol (50%) until examination.

Aliquots (100 mg) of the leaves were punctured and put in amber flasks containing 20 ml of ethanol (95% v/v) and maceration lasted 12 days at 25°C. After this period, the extract was filtered with gauze and refiltered with qualitative paper filter and stocked in amber flasks (- 4°C) (Brito et al., 2008). The extract was used to quantify the biomolecules.

Analysis of soluble carbohydrates and total proteins

Total proteins were quantified by a modification of the Bradford (1976): 50 µl of the extract was added to 2.5 ml of Bradford reagent and readings were taken with a spectrophotometer (595 nm) with a standard BSA curve (Bovine Serum Albumin). Total soluble carbohydrates were determined by a modification of the Dubois et al. (1956) method. The following was added to a test tube: 20 µl of the plant extract, 95 µl of distilled water, 50 µl of 80% phenol (w/v) and 2 ml of sulfuric acid. Readings were taken with a spectrophotometer (490 nm) and glucose was used to prepare the standard curve.

Analysis of phenols, flavonoids and total tannins

Total phenols were determined by a modification of the Folin-Ciocalteu method (Monteiro et al., 2006). The following was added to 100 ml volumetric balloons: 1 ml of the plant extract, 5 ml of the Folin-Ciocalteu reagent (10%, w/v) and 10 ml of sodium carbonate solution (7.5%, w/v) and the volume was completed with distilled water. Readings were taken with a spectrophotometer (760 nm) and tannic acid was used to prepare the standard curve.

Total flavonoids were quantified by a modification of the Araújo et al. (2008) method. The following was added to 25 ml flasks: 1 ml of the plant extract, 0.6 ml of glacial acetic acid, 10 ml of pyridine-methanol solution (2.8, v/v) and 2.5 ml of aluminum chloride (5% w/v, in absolute methanol) and the volume was completed with distilled water. Readings were taken with a spectrophotometer
Table 1. Analysis of variance for the studied variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter of the aerial part</td>
<td>**</td>
</tr>
<tr>
<td>Total chlorophyll</td>
<td></td>
</tr>
<tr>
<td>Chlorophyll a</td>
<td>ns</td>
</tr>
<tr>
<td>Chlorophyll b</td>
<td>**</td>
</tr>
<tr>
<td>Mycorrhizal colonization</td>
<td>**</td>
</tr>
<tr>
<td>Concentration of total proteins</td>
<td>ns</td>
</tr>
<tr>
<td>Content of total proteins</td>
<td></td>
</tr>
<tr>
<td>Concentration of soluble carbohydrates</td>
<td>ns</td>
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<tr>
<td>Content of soluble carbohydrates</td>
<td></td>
</tr>
<tr>
<td>Concentration of total phenols</td>
<td></td>
</tr>
<tr>
<td>Content of total phenols</td>
<td>**</td>
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<tr>
<td>Concentration of total flavonoids</td>
<td>**</td>
</tr>
<tr>
<td>Content of total flavonoids</td>
<td>**</td>
</tr>
<tr>
<td>Concentration of total tannins</td>
<td></td>
</tr>
<tr>
<td>Content of total tannins</td>
<td>**</td>
</tr>
</tbody>
</table>

*p ≤ 0.05; **p ≤ 0.01; ns: Non-significant.

(420 nm) and rutin was used to prepare the standard curve.
Analysis of total tannins was carried out with a modification of the Monteiro et al. (2006) method: 3 ml of the plant extract was mixed with 0.5 g of casein and the mixture was kept under agitation for 3 h at 25°C (160 rpm). After this period, the material was filtered with qualitative paper filter and the resulting volume was transferred to 25 ml volumetric balloons and completed with distilled water. Analysis of the remaining phenols was carried out by the Folin-Ciocalteu method and the concentration of total tannins corresponded to the difference between the levels found in this analysis and those found during quantification of total phenols.

Mycorrhizal colonization

For examination, the roots were bleached with KOH (10%, w/v, for 22 h), hydrogen peroxide (H₂O₂ 10% v/v, for 20 min), acidified (HCl 1% v/v, for 5 min) and stained with Trypan blue (0.05% in lactoglycerol w/v, for 22 h) (Phillips and Hayman, 1970). A physical examination is carried out using the intersection of quadrants method (Giovannetti and Mosse, 1980).

Reagents and equipment used

The following reagents were used: glacial acetic acid, sulfuric acid, ethyl alcohol, methyl alcohol, sodium carbonate, glycine and hydrogen peroxide (F Maia, Cotia, Brazil); bovine serum albumin and rutin hydrate (Sigma-Aldrich, São Paulo, Brazil); lactic acid, tannic acid, casein, aluminum chloride, Coomassie blue G-250, Trypan blue, glucose, phosphoric acid, phenol, pyridine (Vetec, Duque de Caxias, Brazil) and Folin-Ciocalteu reagent (Merck, Rio de Janeiro, Brazil).

The following equipment was used: a vortex shaker (Vision Scientific, Korea), a magnetic stirrer with heating (Quimis, Diadema, Brazil), a vertical autoclave (Phoenix, Araraquara, Brazil), semi-analytic scales (Bel Engineering, Italy), a digital spectrophotometer (Biospectro, Curitiba, Brazil), a drying oven (Biopar, Porto Alegre, RS, Brazil), an electronic chlorophyll content meter – ClorofiLOG -CFL 1030 (Falker, Porto Alegre, Brazil) and an orbital (Marconi, Piracicaba, Brazil).

Experimental outline and statistical analysis

The experimental outline was entirely randomized with four inoculation treatments (AMF control, inoculated with G. albida, inoculated with A. longula or inoculated with C. etunicatum), with five repetitions, totaling 20 experimental units. The data were submitted for analysis of variance (ANOVA) and the means were compared by the Tukey test (5%) using the Assistat program (2013).

RESULTS AND DISCUSSION

The mycorrhizal treatments had no effect on the chlorophyll a content, on the concentration of total proteins and on the concentration and content of total carbohydrates (Table 1).

The dry matter of the aerial part (DMAP) increased when the seedlings were colonized by G. albida (52.70%) and C. etunicatum (78.37%), in relation to the non-inoculated control treatment (Table 2), which means that the mycorrhization with G. albida and C. etunicatum was beneficial for the growth of A. cearensis. Similar results were found by Araim et al. (2009), Baslam et al. (2011) and Touissant et al. (2007), for Echinacea purpurea, in varieties of Lactuta sativa and in Ocimum basilicum, respectively.

Increased values of mycorrhizal colonization were found in the roots of inoculated plants in relation to the control (Table 2), which supports the results obtained for other Leguminosae, such as Libidibia ferrea (Silva et al., 2014a).
Inoculation with *G. albida* and *C. etunicatum* increased by 24.06 and 24.28% the concentration of total chlorophyll in relation to the non-inoculated control, respectively. Similar results were obtained for chlorophyll *b* (Table 2). On the other hand, the benefits of inoculation for chlorophyll *a* (Table 2) were not documented. As was suggested by Singh et al. (2012), the increase in chlorophyll content may be related to the increased nutrient absorption, taking into consideration that various studies indicate maximization in the production of photosynthetic pigments in terms of mycorrhization, which leads to an improvement of the nutritional status of the host (Selvaraj et al., 2009; Singh et al., 2012).

Mycorrhization did not alter the concentration and the total foliar protein content and soluble carbohydrates in *A. cearense* (Table 3); on the other hand, there are situations in which inoculation with AMF favors the accumulation of proteins and plant sugars, as was documented by Ratti et al. (2010) and Baslam et al. (2011). There are situations in which the increase in the content of primary metabolites directs the synthesis of secondary compounds (Oliveira et al., 2013), a fact that has not been documented in this study (Tables 2, 3 and 4).

Mycorrhization with *C. etunicatum* increased in relation to the non-inoculated control, the production of total foliar phenolic compounds in the *A. cearense* seedlings, both in content (198.92%) and concentration (47.82%) (Table 4). Levels of phenolic compounds also varied because of mycorrhizal inoculation, as was documented by Arain et al. (2009), Ceccarelli et al. (2010) and Singh et al. (2012), which makes the use of mycorrhizal technology an alternative to increase the production of such compounds with pharmacological importance.

The use of *C. etunicatum* maximized the content of total foliar flavonoids in relation to the non-inoculated control (Table 4). Possibly, mycorrhization lead to an increased absorption of nutrients, increasing the synthesis of production precursors of such compounds, such as the enzyme Chalcone synthase (*Chs*), which regulates the biosynthesis of this group of phenols (Zhang et al., 2013). An increase in the production of this group of phenolic compounds was also found in other situations (Antunes et al., 2006; Larose et al., 2002), as well as in other Leguminosae species in the Caatinga (Pedone-Bonfim et al., 2013).

Inoculation increased the production of total tannins when *C. etunicatum* was used (Table 5). Nisha and Rajeshkumar (2010) also observed an increase in the biosynthesis of tannins in *Wedilla chinensis* seedlings when inoculated with *Glomus aggregatum*. It is probable that intermediaries of biosynthetic pathways of the tannins, such as gallic acid have optimized the production through mycorrhization, as has been recently documented for the Leguminosae *L. ferrea* (Silva et al., 2014b).

Various mechanisms, nutritional and non-nutritional, have been suggested to explain the effects of mycorrhization on the increase in the biosynthesis of secondary compounds (Mandal et al., 2013; Zhang et al., 2013). Taking into consideration that mycorrhization did not alter the production of primary metabolites (Table 3), it is probable that the mechanisms that are involved in the
Table 4. Concentration of total foliar content of phenols and flavonoids in *Amburana cearensis* seedlings, inoculated or non-inoculated with arbuscular mycorrhizal fungi, 160 days after inoculation under experimental roofing.

<table>
<thead>
<tr>
<th>Inoculation treatment</th>
<th>Total phenols</th>
<th>Total flavonoids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration (mg g plant⁻¹)</td>
<td>Content (mg plant⁻¹)</td>
</tr>
<tr>
<td>Control</td>
<td>7.11ᵇ</td>
<td>4.67ᵇ</td>
</tr>
<tr>
<td><em>Acaulospora longula</em></td>
<td>8.99ᵃᵇ</td>
<td>6.23ᵇ</td>
</tr>
<tr>
<td><em>Gigaspora albida</em></td>
<td>7.53ᵇ</td>
<td>8.50ᵇ</td>
</tr>
<tr>
<td><em>Claroideoglomus etunicatum</em></td>
<td>10.51ᵃ</td>
<td>13.96ᵃ</td>
</tr>
</tbody>
</table>

Means followed by the same letter do not differ from the Tukey test (5%).

Table 5. Concentration of total foliar content of tannins in *Amburana cearensis* seedlings, inoculated or non-inoculated with arbuscular mycorrhizal fungi, 160 days after inoculation under experimental roofing.

<table>
<thead>
<tr>
<th>Inoculation treatment</th>
<th>Total tannins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration (mg g plant⁻¹)</td>
</tr>
<tr>
<td>Control</td>
<td>6.09ᵇ</td>
</tr>
<tr>
<td><em>Acaulospora longula</em></td>
<td>8.33ᵃᵇ</td>
</tr>
<tr>
<td><em>Gigaspora albida</em></td>
<td>6.61ᵇ</td>
</tr>
<tr>
<td><em>Claroideoglomus etunicatum</em></td>
<td>9.84ᵃ</td>
</tr>
</tbody>
</table>

Means followed by the same letter do not differ from the Tukey test (5%).

Foliar phenols increase in *A. cearensis* are non-nutritional as was suggested by Toussaint et al. (2007). Such mechanisms involve an increase in the enzymatic activity, increase in the gene expression, maximized activation of the metabolic pathways and optimized biosynthesis of signaling in mycorrhizal plants (Walter et al., 2000; Lohse et al., 2005; Zhang et al., 2013). Furthermore, it is probable that the inoculated AMF increased the absorption of P, a fact that is well documented for mycorrhizal plants (Smith and Read, 2008), which is an important requirement for the biosynthetic pathways of phenolic compounds (Heldt, 2005).

Benefits of the mycorrhizal technology for the production of bioactive compounds were found for other plants from the Caatinga, as was referred to by Pedone-Bonfim et al. (2013), Oliveira et al. (2013) and Silva et al. (2014a), for *Anadenanthera colubrina*, *Myracrodruon urundeuva* and *L. ferrea*, respectively. Such benefits were also observed for the first time in *A. cearensis*, which confirms the initial working hypothesis.

The mycorrhizal technology, employing selected AMF, favored the production of the phytomass of *A. cearensis* with an elevated concentration of bioactive compounds, which possess various therapeutic properties. Therefore, the fungus *C. etunicatum* is recommended as a biotechnological alternative to maximize the production of foliar bioactive compounds in *A. cearensis* seedlings. This way, a low cost biotechnological protocol was established to maximize the production of plant biomolecules that are important to the phytotherapeutic industry. Other experiments have to be carried out to elucidate the benefits under field conditions and to determine whether there is a specific increase of molecules that are of industrial interest, such as vanillic acid.

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Conflict of interest

The authors do not have any conflicts of interest.

REFERENCES

Lima CS (2014). Tecnologia micorrízica para maximização da produção de biomoléculas foliares em mudas de umburana-de-cambão e de ingaínea. Dissertação de Mestrado, Universidade de Pernambuco, Brasil.
Lima CS (2014). Tecnologia micorrízica para maximização da produção de biomoléculas foliares em mudas de umburana-de-cambão e de ingaínea. Dissertação de Mestrado, Universidade de Pernambuco, Brasil.
