Phytochemistry, Pharmacology and Agronomy of Medicinal Plants: *Amburana cearensis*, an Interdisciplinary Study

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1. Introduction

Plants are an important source of biologically active substances, therefore they have been used for medicinal purposes, since ancient times. Plant materials are used as home remedies, in over-the-counter drug products, dietary supplements and as raw material for obtention of phytochemicals. The use of medicinal plants is usually based on traditional knowledge, from which their therapeutic properties are oftenly ratified in pharmacological studies.

Nowadays, a considerable amount of prescribed drug is still originated from botanical sources and they are associated with several pharmacological activities, such as morphine (**I**) (analgesic), scopolamine (**II**) atropine (**III**) (anticholinergics), galantamine (**IV**) (Alzheimer's disease), quinine (**V**) (antimalarial), paclitaxel (**VI**), vincristine (**VII**) and vinblastine (**VIII**) (anticancer drugs), as well as with digitalis glycosides (**IX**) (heart failure) (Fig. 1). The versatility of biological actions can be attributed to the huge amount and wide variety of secondary metabolites in plant organisms, belonging to several chemical classes as alkaloids, coumarins, flavonoids, tannins, terpenoids, xanthones, etc.

The large consumption of herbal drugs, in spite of the efficiency of synthetic drugs, is due to the belief that natural products are not toxic and/or have fewer side effects, the preference/need for alternative therapies, and their associated lower costs. In developing countries, herbal medicine is the main form of health care. In Brazil, where there is one of greatest biodiversity of plants in the world, pharmaceutical assistance programs, such as "Living Pharmacies", have a prominent role in spreading the rational use of medicinal plants mainly for poor people, under recognition by World Health Organization (WHO). Furthermore, herbal medicines also represent a significant pharmaceutical market share in some industrialized countries like Germany.

On the flip side, herbal drugs are discredited by most of the health related professionals, owing to a lack of scientific research supporting its efficacy and safety. In general, physicians feel insecure in prescribing herbal medicines, as most of them do not undergo through clinical trials, phytochemical analysis, and their active principles not being

determined. Therefore, herbal medicines do not have a defined dosage, information on the chemical composition and warnings about possible risks. Additionally, the poor quality control of herbal drugs, which are subject to adulteration and intrinsic factors related to used raw material, do produce variables and inconsistent effects. Furthermore, most herbal drugs are produced from wild source, limiting the production at industrial level and putting the species used under threat of extinction.

Due to these aforesaid limitations, disadvantages and drawbacks of herbal medicine, we would like to present an updated review of chemical, pharmacological and agronomic studies of *Amburana cearensis* as a well succeeded example of a scientific research on wild plants and a model of a sustainable economic utilization of medicinal plants.
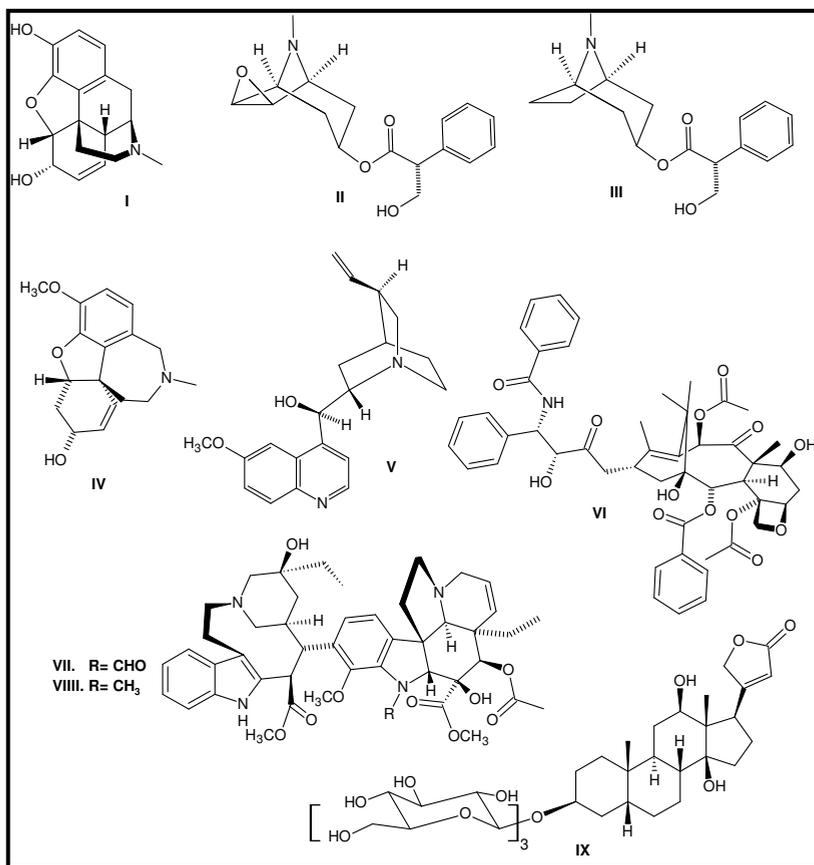

Fig. 1. Chemical structures of plant-derived drugs

2. Herbal drugs and phytopharmaceuticals

According to the WHO definition, herbal drugs are preparations containing plant parts (leaves, roots, seeds, stem bark, etc.) or whole plant materials in the crude or processed form, as active ingredients, besides some excipients. Herbal preparations can be found

under different forms: oral tablets, capsules, gel caps, syrup, extracts and infusions. In general, combinations with chemically defined active substances or isolated constituents, are not considered to be herbal medicines. (Calixto, 2000).

The information about the therapeutical properties and usage of medicinal plants are commonly based on the empirical knowledge of ancient people, which was passed over several generations and originated the traditional medicine systems, utilized all over the world (Traditional Chinese Medicine, Ayurvedic system, Western and African Herbalisms). An estimated quantity of 50 000 plant species are used for medicinal purposes, from which the stand out species of the following families, such as Apocynaceae, Araliaceae, Apiaceae, Asclepiadaceae, Canellaceae, Clusiaceae and Menispermaceae (Schippmann et al., 2002). From the total of 252 drugs in the WHO's essential medicine list, 11% are exclusively derived from plant origins (Sahoo et al., 2010)

Herbal drugs are consumed by three-quarters of the world's population in the treatment of mainly chronic diseases, particularly headache, rheumatological disorders and asthma (Inamdar et al., 2010). In the developing countries, the population relies basically on medicinal plants for primary health care, since modern medicine is expensive and not easily accessible. However, the consumption of herbal drugs is also large in developed countries. Phytotherapy is popular in many countries of Western Europe (Germany, France, Italy, etc.), since people believe that either herbal drugs are devoid of side-effects or seek a healthier life style. Americans usually buy herbal products as a dietary supplement in the United States, aiming at preventing aging and diseases like cancer, as well as diabetes (Calixto, 2000).

Herbal drugs have some features which distinguish themselves considerably from synthetic drugs. Herbal medicines are always formed from a complex mixture of chemical compounds (eg. *Scutellaria baicalensis* has over 2000 components), and they may be constituted by many plants, therefore herbal drugs show an ample therapeutic usage. It is quite common to find a medicinal plant with several therapeutic properties (Sahoo et al., 2010; Calixto, 2000). The combination of either many plants, containing diverse bioactive substances or a pool of structural analogs, can produce a synergistic action that results in a stronger effect, therefore, permitting a reduction of dosage, which implies in lower risks of intoxication and undesirable side effects. As some diseases (e.g. AIDS or various types of cancer) possess a multi-causal etiology and a complex pathophysiology, a medical treatment may be more effective through well-chosen drug combinations than a single drug. Ginkgolides A and B, isolated from *Ginkgo biloba*, duly demonstrated a greater effect on the thrombocyte aggregation inhibition, when used as a mixture as opposed to what would be expected from the sum of the two compounds separately (Wagner, 2011).

On the other hand, plant-based products do not possess a well-defined chemical composition, due partially to chemical complexity stated above. Hence, the active principles of herbal drugs are frequently unknown, in addition to their standardization and quality control, being hardly achieved (Calixto, 2000) owing to mainly chemical variability in raw material. Secondary metabolites are the bioactive components from herbal drugs and their contents are strongly influenced by several factors: genetic (genotypes, chemotypes), physiologic (circadian rhythm, phenology, age), environmental (climate, sunlight exposure, water availability, soil, agronomic conditions) and manufacturing conditions (harvesting, storage and processing) (Tab. 1), (Sahoo et al., 2010; Gobbo-Beto & Lopes, 2007).

Evaluated Effect	Plant	Compound (s)	Result
Seasonality	*Hypericum perforatum*	Hypericin	300x content larger in summer than winter. (Southwell & Bourke, 2001)
Harvesting time	*Ocimum gratissimum*	Eugenol	98 % at 17h, but it is 11 % at midday (Silva et al. 1999)
Age	*Papaver somniferum*	Morphine	6x content larger on the 75th day after germination than on the 50th day (Williams & Ellis, 1989)
Phenology	*Gentiana lutea*	Mangiferin/ isoorientin	Before flowering-↑ [Mangiferin] ; during flowering- ↑ [Isoorientin] (Menković et al., 2000)
Temperature	*Nicotiana tabacum*	Scopolamine	[Scopolamine] 4x larger after freezing (Koeppe et al, 1970)
Water availability	*H. perforatum*	Hyperforin/ Hypericin	Under water stress: ↑ 2x [Hyperforin] and ↓ [Hypericin] (Zobayed et al, 2007)

Table 1. Effects on the production of secondary metabolites

Furthermore, most herbal drugs are utilized and commercialized without having a proven efficacy and safety through well-controlled double-blind clinical and toxicological trials, as pharmaceuticals are usually tested prior to being marketed. The safety and efficacy of herbal drugs are supported by their long historical use. Nevertheless, it is known that various herbal drugs fail, after testing in clinical trials and there are numerous reports on intoxication cases associated with their consumption. The WHO database has over sixteen thousand suspected case reports, related to intoxication by herbal drugs. The most frequent adverse reactions are hypertension, hepatitis, convulsions, thrombocytopenia and allergic reactions. Cardiovascular problems with the use of ephedra, hepatotoxicity caused by the consumption of kava-kava and comfrey, as well as licorice-related water retention, are some side effects claimed by the pharmacovigilance authorities. In addition to intrinsic factors mentioned above, the herbal drugs efficacy and safety, may also be seriously affected due to botanical misidentification or intentional usage of fake plants, contamination with pesticide residue, toxic heavy metals, pathogens and mycotoxins, as well as adulterants added to increase potency (synthetic substances) or the weight of herbal products in order to reduce costs (Sahoo et al., 2010; Calixto, 2000).

For the purpose of overcoming or mitigating the aforesaid inconvenient issues, WHO has developed a series of technical guidelines and documents in relation to the safety and the quality assurance of medicinal plants and herbal drugs preparations, such as "Quality Control Methods for Medicinal Plant Materials" (a collection of recommended test procedures for assessing the identity, purity and content of medicinal plant materials), "Guidelines on good agricultural and collection practices for medicinal plants", as well as "WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues". In turn, pharmaceutical laboratories have been investing in the enhancement of the quality for herbal products, aiming at the approval by governmental regulatory agencies, as a strategy to offer more reliable products, therefore conquering the confidence of health care professionals and consumers (Sahoo et al., 2010).

The interest in herbal drugs is continuously growing and they account for a significant share in the pharmaceutical market. The global herbal pharmaceutical industry (including drugs from herbal precursors and registered herbal medicines) invoices approximately US$ 50 billion/year (2008). In 2006, the best selling herbal products were: Ginseng (>U$ 1 billion global sales), Ginkgo (U$ 1 billion), Noni (U$ 1 billion), Saw Palmetto (U$ 600 millions) Echinacea (U$ 500 millions), Valerian (U$ 450 millions), and Green Tea (U $ 450 millions) (Gruenwald, 2008- Entrepreneur). The United States, China, Japan, Germany, South Korea and India, are the largest market. Medicinal plants have also been utilized as source of phytochemicals for the pharmaceutical, cosmetic and agrochemical industries. The most successful examples are paclitaxel (an anticancer drug from *Taxus baccata*), artemisinin (an antimalarial agent from *Artemisia annua*), vincristine/vinblastine (anticancer substances from *Catharanthus roseus*). The Pharmaceutical industry is interested in phytochemicals, however, the availability of quantities of pure chemical substances is normally a limiting factor, since the market demand for phytochemicals, usually reaches a scale from hundreds to thousands of kilograms per annum. (McChesney, 2007)

3. Medicinal plants threatened by extinction

The increasing demand for medicinal plants has endangered several species, since the main source of herbal drugs is the wild plant and the amount required from plant materials invariably exceeds the supply available from its natural source. Although the Convention on Biological Diversity (CBD), held in 1992, has established as goals, the conservation of biological diversity, the sustainable use of its components as well as the fair and equitable sharing of the benefits from the usage of genetic resources, it is still estimated that slightly more than 4000 medicinal plant species are under threat of extinction. The Convention on International Trade of Endangered Species of Wild Fauna and Flora (CITES), being the principal tool for monitoring or restricting the international trade of species threatened by over-exploitation, has published a biannual list of medicinal species like: *Taxus wallichiana*, *Panax quinquefolius*, *Dioscorea deltoidea*, *Hydrastis canadensis*, *Prunus africana* , *Rauvolfia serpentina* and *Pterocarpus santalinus* (Schippmann et al., 2002).

The overexploitation of a certain wild medicinal plant and consequent depletion of its raw material affect inevitably the economic feasibility of any phytopharmaceutical business in medium or long-term, since the production cost tends to be higher and the product supply become discontinuous. Furthermore, the extractivism provokes loss of genetic diversity, becoming the remaining plant population more vulnerable to diseases/pests and diminishing the variability of genotypes with features of interest such as yielding, bioactive substance content and resistance to biotic and abiotic factors (Rao et al., 2004). Hence, agencies concerned with conservation policies are recommending that wild species be brought into cultivation systems in order to assure the economic and environmental sustainability of herbal medicines trade (Schippmann et al., 2002). *Ginkgo biloba* and *Hypericum perforatum* are some of the top-selling medicinal plants, however they are not endangered, because their plant materials have been obtained by cultivation for a long time (Canter et al., 2005).

4. Cultivation of medicinal plants

The cultivation of medicinal plants is advocated as a means for meeting current and future demands for large quantities of herbal drugs, but also as a way to relieve the pressure of

harvesting on wild populations (Schippmann et al., 2002). In China, one of the largest markets of herbal medicine, 380,000 ha of lands are utilized for farming of medicinal plants.

Medicinal plants are also cultivated for supplying phytochemicals. Bristol-Myers Squibb developed a system of production based upon isolation of a precursor of Taxol from the leaves or needles of cultivated *Taxus baccata* or *T. wallichiana* that provide the hundreds of kilograms of Taxol required per year for the treatment of cancer patients. (McChesney, 2007)

From the perspective of the market, domestication and cultivation provide a number of advantages over wild harvest for production of herbal drugs: (1) reliable botanical identification; (2) uniform and high quality raw material. As wild plants are dependent on many factors that cannot be controlled and the irregularity of supply is a common feature, the cultivation assures a steady source of raw material; (3) price and volume between farmer and pharmaceutical companies can be more easily negotiable, since the production forecast is more precise; (4) genetic breeding and biotechnology tools can lead to the development of plant materials with agronomically and commercially desirable features, permitting to optimize yield and to meet regulations as well as consumer preferences, respectively; (5) cultivated material can be easily certified as "organic product" (Schippmann et al., 2002; Canter et al, 2005).

Cultivated plants account for 60-90 % in terms of amount of plant material employed by Herbal medicines companies, but the number of wild species still is larger. Although the cultivation is apparently more advantageous than wild harvesting, only 130-140 species are cultivated in Europe, while just 20 out of 400 medicinal plants marketed in India are grown in field. Likewise, amongst 1000 plants more commonly used with medicinal purposes in China, only 100-250 species are sourced from cultivation. There are some reasons that can explain this low utilization of cultivated plants:

(1) Belief of that wild specimens are more potent than cultivated plants. Chinese believe that the physical appearance of wild roots to the human body symbolizes vitality and this feature is crucial for the potency of the ginseng roots, nevertheless cultivated roots do not exhibit this characteristic shape. Furthermore, some scientific studies support partly this hypothesis saying that secondary metabolites, the main responsible for therapeutic properties of herbal medicines, are biosynthesized by plants under particular conditions of stress and competition in their natural environments. Hence, perhaps the secondary metabolites would not be so expressed in monoculture conditions, therefore the active ingredient levels can be much lower in cultivated plant.

(2) Domestication of wild plant is not always technically possible. Many species are difficult to cultivate because of certain biological features or ecological requirements (slow growth rate, special soil requirements, low germination rates, susceptibility to pests, etc.).

(3) Economical feasibility. Domestication requires a long time of agronomical studies and high financial investment for the plantation. Generally, production costs through cultivation are higher than wild harvesting, thus few species can be marketed at a high sufficient price to make cultivation profitable, for instance *Garcinia afzelii, Panax quinquefolius, Saussurea costus* and *Warburgia salutaris.* Hence, many endangered medicinal plants only will bring into cultivation, if exists governmental incentive (Schippmann et al., 2002).

However, the cultivation of medicinal plant in agroforestry system can be a good alternative for more viable and environmentally sustainable farming. In China, ginseng (*Panax ginseng*) and other medicinal plants are grown in pine (*Pinus* spp.), *Paulownia tomentosa* and spruce (*Picea* spp.) forests; besides some medicinal herbs are often planted with bamboo (*Bambusa* spp.). In New Zealand, American ginseng showed better growth under *Pinus radiate.* The shade offered by forest species seems to favor the growth of medicinal plants. Likewise, quinine yields of *Cinchona ledgeriana* increase when it is protected by shade of other species, such as *Crotalaria anagyroides* and *Tephrosia candida*. In India, some medicinal plants that have also been successfully intercropped with fuel wood trees (e.g., *Acacia auriculiformis* and *Eucalyptus tereticornis*) and coconut. Intercropping gives some income to farmers during the period when the main trees have not started production. (Rao et al., 2004).

Application of traditional and biotechnological plant-breeding techniques can become the cultivation of medicinal plants a trade more attractive (eg. increasing the yielding) as well as it can improve features of the plant that affect the efficacy and safety of a herbal drug (eg. levels of bioactive compounds or presence of potentially toxic substances). *Mentha* spp (mints) have been engineered to modify essential oil production and to enhance the resistance of the plant to fungal infection and abiotic stresses. Genetic engineering allowed the enhancement of scopolamine and artemisinin in *Atropa belladonna* and *Artemisia annua*, respectively. (Canter et al., 2005)

5. Amburana cearensis

A. cearensis (Fabaceae) is a native tree from "Caatinga" (a kind of vegetation found in the Brazilian semi-arid region), where it is popularly known as "cumaru" or "imburana-de-cheiro" (Fig. 2). Because of these said popular names, *A. cearensis* is usually misidentified as *Dipteryx odorata* (Fabaceae) and *Commiphora leptophloeos* (Burseraceae). *A. cearensis* occurs widely in South America (from Peru to Argentina), along with another species of this taxon, *Amburana acreana*, which is found chiefly in the southwestern region of the Amazon Forest. *A. cearensis* can reach 15 m of height and 50 cm of diameter, but it is characterized by white flowers and dark pods containing only one seed each, besides its stem bark possessing reddish stains and a vanilla-like aroma of coumarin (**1**). At the early stage of development (seedlings), *A. cearensis* displays a hypertrophied and subterraneous tube-like structure, called xylopodium, which acts as a storage of water and nutrients, therefore it is considered an adaptive strategy for arid habitats (Lima, 1989; Cunha & Ferreira, 2003).

Given the various applications, *A. cearensis* has a great commercial importance in Northeastern region of Brazil. Its wood is used in the carpentry for the manufacturing of furniture, doors and crates, owing to its recognized durability, whereas the seeds are used as flavoring and insect repellents. The wood powder from it can be added to alcoholic beverage barrels for accelerating the aging process of sugar cane distilled spirits (cachaça) (Aquino et al, 2005). The seeds and stem bark are traditionally utilized for treating respiratory diseases, such as influenza, asthma and bronchitis due to anti-inflammatory, analgesic and bronchodilator properties. As far as folk medicine is concerned, *A. cearensis* is consumed as a homemade medication called "lambedô (a sugary drink), however in an industrial scale, the syrup is a pharmaceutical form, which is produced by the government and private laboratories (Fig. 3).

Fig. 2. A wild specimen of *Amburana cearensis* in its natural habitat.

The medicinal use of *A. cearensis* is based on scientific studies, which demonstrated that this plant possesses therapeutic properties that justify its recommendation for the treatment of respiratory illnesses. Preclinical tests demonstrated bronchodilator, analgesic and anti-inflammatory activities for the hydro-alcohol extract from the stem bark of the *A. cearensis*, which also showed to be free of toxicity in therapeutic doses. The chemical composition of the stem bark and seeds from it, consists basically of coumarin, flavonoids, phenol acids and phenol glucosides. Some of them were tested individually and showed pharmacological activities similar to the extract, hence they were considered the active principles of the *A. cearensis*.

However, the intense commercial use of *A. cearensis* has led to the threat of extinction for this specie. In order to ensure the conservation and the economic utilization of *A. cearensis*, we proposed the replacement of its stem bark of a wild adult plant for a young specimen, cultivated under controlled agronomic parameters. In an interdisciplinary study, ethanol extracts of cultivated plants were compared to the extracts of this wild plant through preclinical trials and phytochemical analysis.

Fig. 3. Syrups made from the trunk bark of *Amburana cearensis*.

5.1 Agronomical study of *A. cearensis*

The agronomical study of *A. cearensis* was carried out with seedlings obtained by seed germination. Each plot consisted of six regularly spaced rows of 20 cm, whose sowing density was 50 seeds/row. The seedlings were transplanted to four garden beds (1.2m×10 m), fertilized prior to an organic fertilizer (2.8 kg m^{-2}), containing each of them 20 young plants.

Fig. 4. Seedlings of *A. cearensis* harvested in 8th month of growth

Eight harvestings were performed monthly, starting on the 2nd month until the 9th month after the sowing. The plants harvested were evaluated with the following parameters: fresh plant weight, plant height, xylopodium diameter, root size, ethanol extract yield from the aerial part and xylopodium (Fig. 4). The fresh biomass production of *A. cearensis* seedlings increased almost eight-fold, during the 2nd through 9th month after the sowing. With reference to ethanol extract yield, there was a tendency of decrease for the extract weigh/xylopodium weight ratio over a period of time, while an oscillatory behavior was observed for yield of the ethanol extract from its aerial part, achieving a plateau on the 3rd and 7th month (Leal et al. 2011)

5.2 Phytochemistry of *A. cearensis*

5.2.1 Wild plant

While the pharmacological research about *A. cearensis* advanced, it was necessary to have a better understanding of the chemistry in this specie, previously limited to coumarin and a few phenol compounds, in order to discover its active principles. Bastos (1983), in her master thesis, found coumarin (**1**) as the most abundant component and described the isolation of isokaempferide (**2**), methyl 3,4-dimethoxy-cinnamate (**3**), afrormosin (**4**), 7-hidroxy-8,4'-dimethoxy-isoflavone (**5**), 24-methylenecycloartanol (**6**), β-sitosterol (**7**) and 6-hidroxy-coumarin (**8**). Additionally, the seeds presented a high oil content (23 %), which is composed of triglycerides of the following fatty acids: oleic acid (53 %), palmitic acid (19 %), stearic acid (8 %) and linoleic acid (7 %). Bravo et al. (1999) isolated amburosides A [(4-*O*-β-D-glucopyranosylbenzyl) protocatechuate] (**9**) and B [(4-*O*-β-D-glucopyranosylbenzyl) vanillate] (**10**) from the ethyl acetate extract of the trunk bark, utilizing just silica gel preparative Thin-Layer Chromatography (TLC) (Fig. 5)

Canuto & Silveira (2006) carried out a phytochemical investigation of the ethanol extract from the stem bark. The ethanol extract was partitioned with water and ethyl acetate. The aqueous phase showed to be very rich in sucrose (**11**), whereas the organic phase was dried and submitted to successive chromatographic columns on silica gel and dextran gels. These chromatographic separations led to the isolation of coumarin (**1**), two phenol acids [vanillic acid (**12**) and protocatechuic acid (**13**)], five flavonoids [isokaempferide (**2**), afrormosin (**4**), kaempferol (**14**), quercetin (**15**) and 4'-methoxy-fisetin (**16**)], amburoside A (**9**) and a mixture of β-sitosterol and stigmasterol glycosides (**17-18**). Later on, this same methodology was applied to isolate an isoflavone formononetin (**19**) and a novel coumarin 6-coumaryl protocatechuate (**20**) (Canuto et al., 2010) (Fig. 5). Continuing with the phytochemical study of *A. cearensis*, the seeds revealed high presence of phenol glucosides. Liquid-liquid partitioning from the ethanol extracts followed by chromatography on Sephadex LH-20 and a reversed-phase HPLC chromatography of the ethyl acetate fraction, resulted in the isolation of six new amburosides (C-H). 4-*O*-β-D-(6''-*O*-galloylglucopyranosyl)-benzyl protocatechuate (**21**), 4-*O*-β-D-(6''-*O*-acetylglucopyranosyl)-benzyl protocatechuate (**22**), 4-*O*-β-D-(6''-*O*-protocatechuoylglucopyranosyl)-benzyl protocatechuate (**23**), 4-*O*-β-D-(6''-*O*-feruloylglucopyranosyl)-benzyl protocatechuate (**24**), 4-*O*-β-D-(6''-*O*-vanilloylglucopyranosyl)-benzyl protocatechuate (**25**) and 4-*O*-β-D-(6''-*O*-sinapoylglucopyranosyl)-benzyl protocatechuate (**26**). Additionally, amburoside A (**9**), isokaempferide (**2**), vanillic acid (**12**), 6-hydroxycoumarin (**8**) and (*E*)-*o*-coumaric acid (**27**) were isolated from this same extract. (Canuto et al., 2010) (Fig. 5).

The isolation of 6-coumaryl protocatechuate (**20**) (trunk bark) and 6-hydroxycoumarin (**8**) (seeds) from *A. cearensis* presents an intriguing finding for biosynthesis of coumarins, since monoxygenated-coumarins are preferentially substituted at C-7 position (umbelliferone and its derivatives), according to biogenetic rules. This substitution pattern is due to the usual precursor of coumarins, *p*-coumaric acid, which is biosynthentized by the shikimate pathway from either tyrosine-deamination or *p*-hydroxylation of cinnamic acid (Dewick, 2002). Nevertheless, despite a large occurrence of simple coumarins oxygenated at the C-7 position, 6-hydroxycoumarin (**8**) was also found in some species like *Bidens parviflora* (Asteraceae), *Paeonia suffruticosa* (Paeoniaceae) and *Hydrangea chinensis* (Hydrangeaceae) (Tommasi et al., 1992; Wu et al., 2002; Khalil et al., 2003)
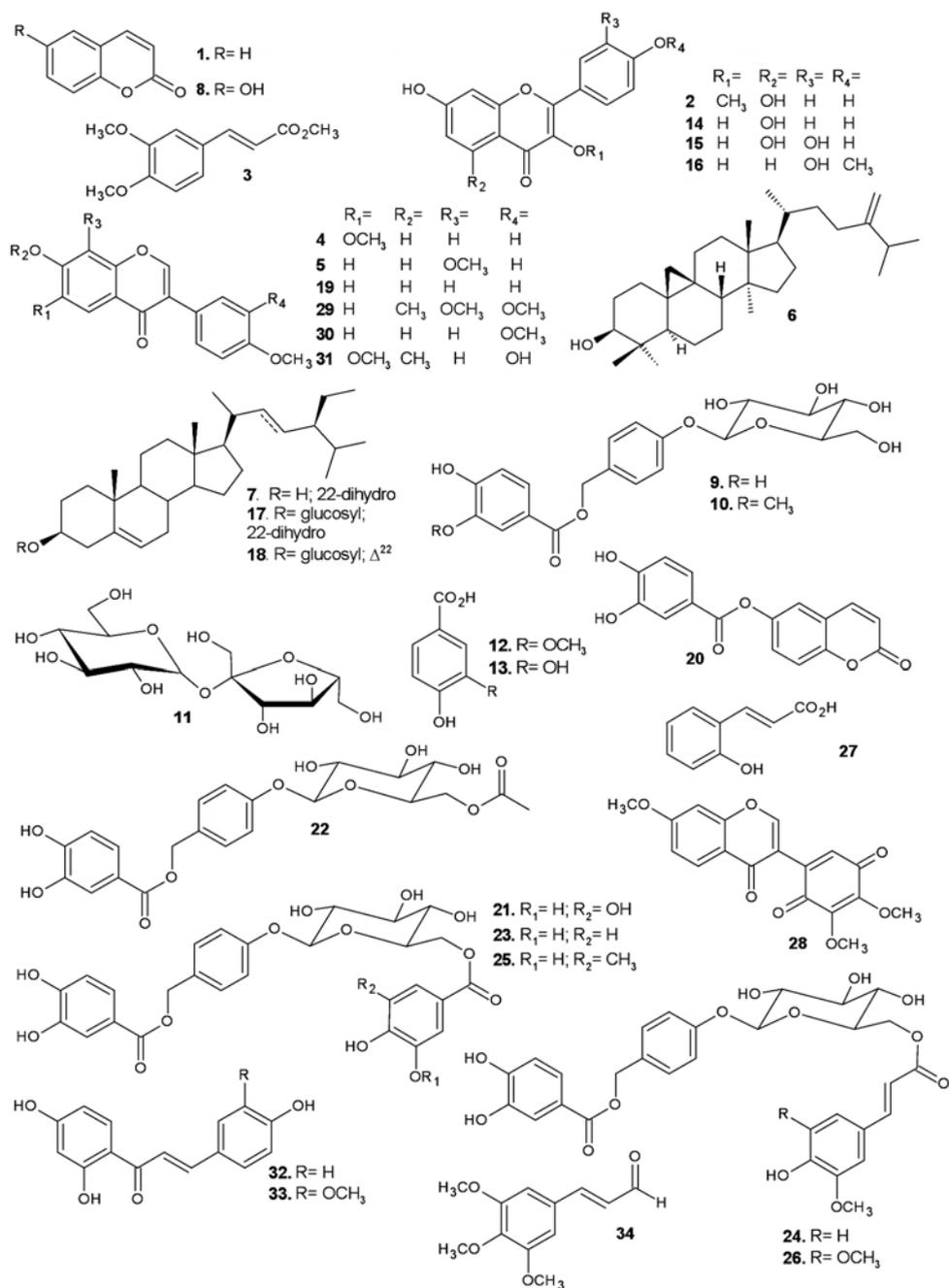
Fig. 5. Chemical structures of constituents isolated from wild *A. cearensis*.

Recently, Bandeira et al (2011) studied a resin exuded from the trunk of *A. cearensis* and found a flavonoid-rich material. The resin ethanol extract was partitioned with water and organic solvents, yielding an ethyl acetate fraction, which was chromatographed on silica gel. From this chromatography, a chloroform fraction was separated by a Sephadex LH-20, resulting in the isolation of a novel compound 3′,4′-dimethoxy-1′-(7-methoxy-4-oxo-4H-cromen-3-yl)-benzo-2′,5′-quinone (**28**), along with six known compounds:, 7,8,3′,4′-tetramethoxyisoflavone (**29**), 3′,4′-dimethoxy-7-hydroxyisoflavone (**30**) and 6,7,4′-trimethoxy-3′-hydroxyisoflavone (**31**), 4,2′,4′-trihydroxychalcone (**32**), 4,2′,4′-trihydroxy-3-methoxychalcone (**33**), 3,4,5-trimethoxycinnamaldehyde (**34**).

5.2.2 Cultivated plant

In order to seek a sustainable alternative for an economic utilization of *A. cearensis*, our research became focused on this *A. cearensis* cultivated plant. The chemical study of the cultivated *A. cearensis* was divided into two parts: (1) a Nuclear Magnetic Resonance (NMR) and the HPLC profiling of ethanol extracts obtained from the aerial part (EEAP) and xylopodium (EEX) of specimens cultivated according to the growing conditions described above; (2) A refined phytochemical analysis of EEAP and EEX extracts produced from specimens in 7 months of growth, where was chosen with basis on pharmacological results.

NMR profiling was performed with extracts of specimens from the 2nd through the 9th month of growth. ^{1}H NMR spectra, recorded in deuterated dimethylsulfoxide, revealed that the extracts from specimens with 2, 4, 7 and 9 months of growth, presented significantly different profiles, requiring further analysis. Hence, these extracts were duly analyzed comparatively with the wild plant extract by Photodiode Array detector (PDA)-HPLC profiling, utilizing constituents previously isolated from the wild *A. cearensis* as an analytical standard. The separations were performed on a C18 analytical column and the mobile phase was a gradient composed of H_2O (pH 3, H_3PO_4-Et$_3$N)/MeOH. The run time was 40 min and the chromatograms were observed at 254 nm. The qualitative analysis consisted of identification from analytical standards in the chromatograms of ethanol extracts, which were derived from the trunk bark (wild plant), xylopodium and the aerial part (cultivated plant) by retention time and UV spectra. Only 8 out of the 13 standards injected were detected in the samples. Coumarin (**1**) and vanillic acid (**12**) were the only substances present in all extracts of the *A. cearensis.* Amburoside B (**10**) and protocatechuic acid (**13**) were found in all extracts, except in the xylopodium extracts from specimens harvested in the 2nd and 4th month of growth, respectively. (*E*)-*o*-coumaric acid (**27**) and its glucoside (**35**), along with isokaempferide (**2**), afrormorsin (**4**) and kaempferol (**14**) were not detected in any extracts. Ayapin (**36**) and (*Z*)-*o*-coumaric acid glucoside (**37**) were found only in cultivated plants (Table 2).

A quantitative analysis was carried out for four major constituents of the *A. cearensis* [coumarin (**1**), amburoside A (**9**), vanillic acid (**12**), protocatechuic acid (**13**)] in ethanol extracts from trunk bark (EETB) and seeds (EES) of the said wild plant, as well as the whole plant (EEWP), xylopodium (EEX), as well as the aerial part (EEAP) of cultivated specimens in the four following selected months (2, 4, 7 and 9), accounting for 10 samples. The HPLC method was developed in chromatographic condition similar to the once described above and validated according to analytical parameters, defined by the Brazilian Health Surveillance Agency and the Brazilian Institute of Metrology, Standardization and

Substances	Retention time (min.)	Trunk Bark	Xylopodium (month)				Aerial Part (month)			
			2	4	7	9	2	4	7	9
(*Z*)-Coumaric acid glucoside	3,85	-	+	+	+	+	-	-	-	-
p-Hidroxi-benzoic acid	4,99	+	+	+	+	+	-	-	-	-
Protocatechuic acid	7,36	+	+	-	+	+	+	+	+	+
Vanillic acid	10,87	+	+	+	+	+	+	+	+	+
Coumarin	13,98	+	+	+	+	+	+	+	+	+
Amburoside A	14,65	+	+	-	+	+	-	+	+	+
Ayapin	14,85	-	+	+	+	-	+	+	+	+
Amburoside B	17,17	+	-	+	+	+	+	+	+	+

Table 2. Distribution of some constituents in *A. cearensis* (**+**, presence; **-** ,not detected)

Industrial Quality: linearity, selectivity, accuracy, precision as well as the limit of detection and the limit of quantification. As can be noticed on Table 3, amburoside A (**9**) was the most abundant component in EETB, followed by coumarin (**1**), protocatechuic acid (**13**) and vanillic acid (**12**). However, coumarin (**1**) was the only component detected in EES. Among cultivated plants extracts, vanillic acid (**12**) was the principal component in 3 out of 4 periods analyzed through EEW, while coumarin (**1**) appeared as the major compound in the 7th month. Protocatechuic acid (**13**) and amburoside A (**9**) were below the limit of quantification in all extracts, except in the 9th month, whereby amburoside A (**9**) had a considerable content (Leal et al., 2011). In EEAP, vanillic acid (**12**) was the main constituent evaluated in all seasons, reaching the highest concentration in the 7th month (8520 mg/100g ext). On the other hand, coumarin (**1**) was the major component in xylopodium (4th month: 3760 mg/100g ext), except in the last month, when amburoside A (**9**) was the most abundant (680 mg/100g ext). Protocatechuic acid (**13**) presented measurable levels only in the EEAP of 4 months (360 mg/100g ext). Coumarin (**1**) and vanillic acid (**12**) were found preferentially in the aerial part, while amburoside A (**9**) was present mainly in the xylopodium. In comparison with wild plants, EEX of 9 months was the only cultivated plant extract which had amburoside A as the major component like EETB, even so at a concentration being three-fold lower than in the latter.

EEAP and EEX extracts harvested in the 7th month of growth were submitted to partition H_2O/EtOAc, yielding aqueous and ethyl acetate fractions from each extract. Isokaempferide (**2**), amburoside B (**10**), vanillic acid (**12**), *p*-hidroxy-benzoic acid (**38**) and the coumarin ayapin (**36**) were isolated from the ethyl acetate fraction derived from EEAP after being chromatographed on silica and dextran gels, while the aqueous fraction of EEAP yielded (*E*)-melilotoside (**35**) and amburoside B (**10**), again, through C18 solid-phase extraction (SPE) and C18 HPLC. On the flip side, adsorption and exclusion chromatography of the ethyl acetate fraction derived of EEX, afforded the isolation of amburoside A (**9**) and protocatechuic acid (**13**), whereas (*Z*)-melilotoside (**37**) was isolated by Sephadex LH-20 followed with the purification through C18 HPLC (Fig. 6). (*E/Z*)- melilotoside (**35** and **37**) are trivial names for *o*-coumaric acid glucoside in allusion to the genus *Melilotus*, where these compounds were firstly identified. Interestingly, the *E* and *Z*-melilotosides were found in different parts of the *A. cearensis*: (*E*)-stereoisomer exclusively in the aerial part, while (*Z*)-

Extracts	Concentration (mg/100g extract)– *CV* (%)			
	Protocatechuic acid	Vanillic acid	Coumarin	Amburoside
Wild Plant				
Trunk bark	320 (6,3)	200 (5,6)	1340 (6,8)	2180 (5,5)
Seeds	ND	ND	23520 (3,9)	ND
Cultivated Plant				
Whole				
2 months	ND	1520 (1,3)	1020 (5,7)	ND
4 months	ND	2680 (13,4)	2000 (7,0)	ND
7 months	ND	3440 (4,3)	4060 (6,5)	ND
9 months	ND	1520 (6,1)	660 (1,8)	400 (5,7)
Aerial Part				
2 months	ND	4780 (10,6)	1540 (3,0)	ND
4 months	360 (3,3)	6120 (4,8)	1660 (2,0)	260 (8,7)
7 months	ND	8520 (1,0)	6060 (7,9)	ND
9 months	ND	3460 (6,3)	1500 (3,3)	ND
Xylopodium				
2 months	ND	780 (5,3)	1320 (1,0)	380 (5,3)
4 months	ND	760 (7,5)	3760 (0,9)	ND
7 months	ND	1380 (2,9)	2500 (4,2)	300 (10,4)
9 months	ND	540 (9,8)	420 (10,8)	680 (9,4)

Table 3. Concentrations of four major compounds of *A.cearensis* in different extracts.
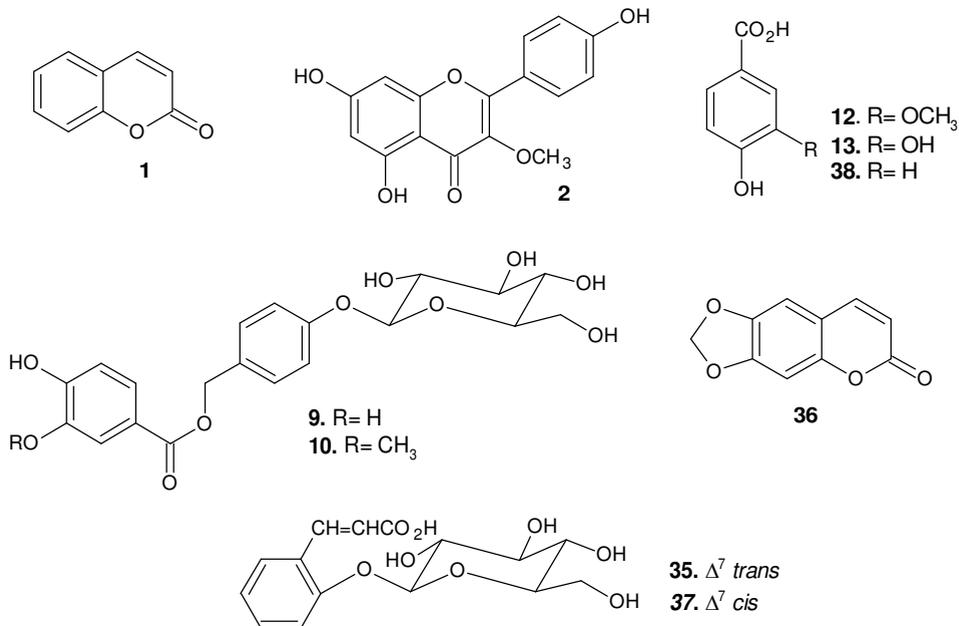
Fig. 6. Chemical structures of constituents isolated from cultivated *A. cearensis*.

stereoisomer was present only in xylopodium. In *Melilotus alba* (a legume), the melilotosides are considered the precursors of coumarin, being one of the major constituents of *A. cearensis*. (*E*)-melilotoside (**35**) exposed to UV radiation (sunlight) may be converted to the less stable stereoisomer (*Z*), which do undergo enzyme-catalyzed lactonization to yield coumarin (**1**) (Dewick et al., 2002).

As part of our effort for finding out which substances are responsible by medicinal properties of *A. cearensis*, isokaempferide (**2**), afrormosin (**4**), amburoside A (**9**), vanillic acid (**12**) and protocatechuic acid (**13**) obtained from this work were assayed in diverse pharmacological tests, which will be discussed briefly. The chemical structures of the new compounds were elucidated by means of spectroscopic techniques such as IR, HRMS, 1D and 2D NMR (COSY, HSQC, HMBC and NOESY).

5.3 Pharmacology of *A. cearensis*

The literature reports several toxicological and pharmacological studies carried out with the extracts and substances isolated from wild and cultivated *A. cearensis*. The focus of them is on the anti-inflammatory, antioxidant, smooth muscle relaxant, antinociceptive, neuroprotector and platelet antiaggregant effects (Leal, 1995; 2006; Leal et al., 1997; 2000; 2001; 2003ab; 2005; 2006ab; 2008).

A toxicological study carried out with the hydroalcoholic extract (HAE) from the trunk bark of the *A. cearensis* administered to rats by the oral route did not show any toxic effects (Leal et al., 2003). Further studies demonstrated that the HAE administered to rats daily for 50 days did not interfere with the pregnancy rate and development during the 1st as well as the 2nd generation of animals (Leal et al., 2003a, Leal et al., 2006a). The cytotoxicity of isokaempferide (**2**), kaempferol (**14**), amburoside A (**9**) and protocatechuic acid (**13**) from the *A. cearensis,* were evaluated on tumor cell lines and on the sea urchin egg development, as well as their lytic properties on mouse erythrocytes. The results showed that isokaempferide (**2**) and kaempferol (**14**), but not amburoside A (**9**) and protocatechuic acid (**13**), inhibited the sea urchin egg development, as well as tumor cell lines. However, only protocatechuic acid (**13**) induced lysis on mouse erythrocytes (Costa-Lotufo et al., 2003).

Previous studies (Leal et al., 1997; 2000) reported the antinociceptive, antiedematogenic and smooth muscle relaxant properties of HAE, coumarin (**1**), and the flavonoid fraction, from wild *A. cearensis*. The antiedematogenic activity was manifested in inflammatory process dependents on polimorphonuclear cells, while the antinociceptive effect of coumarin (**1**) and HAE seems to occur by a mechanism at least in part dependent on the opioid system. Nevertheless, the nitridergic system has also an important role in the coumarin nociception. Additional studies about the pharmacological potential of the HAE, coumarin (**1**) and the flavonoid fraction emphasized the anti-inflammatory potential of these species, which seems to be related to the presence of coumarin (**1**) in the plant (Leal et al., 2003).

Like other medicinal plants containing coumarin (**1**) such as *Justicia pectoralis, Pterodon polygaliflorus, Hybanthus ipecacuanha* and *Eclipta alba*, *A. cearensis,* also has a relaxing activity on isolated guinea pig tracheal muscles (Leal et al., 2000). Confirming this as particular effect, it was recently (Leal et al., 2006) demonstrated the relaxant action of the isokaempferide (**2**). The relaxation of the guinea-pig isolated trachea, induced by isokaempferide (**2**), was a direct and an epithelium-independent phenomenon, resulting

from several intracellular actions through a common pathway e.g., the opening of Ca^{2+} and ATP-sensitive K^+ channels.

Previous studies (Leal et al., 2003; Leal, 2006) showed that the anti-inflammatory activity of HAE, coumarin (**1**), isokaempferide (**2**) and amburoside A (**9**) from *A. cearensis,* seems to occur by an inhibitory action on the release of inflammatory mediators, and/or alternatively by interfering with a certain phase of the neutrophil migration into the inflammatory focus. Other data (Leal et al., 2008) corroborated this hypothesis showing that both the isokaempferide (**2**) and amburoside A (**9**) exert their anti-inflammatory activities mainly by inhibiting the lipopolysaccharide-induced release of TNF-α, although the involvement of other inflammatory mediators cannot be excluded. Furthermore, inhibitions of some biological functions of neutrophils, namely, accumulation of cells and activity of hydrolytic enzymes, as myeloperoxidase, may also play a role.

Amburoside A (**9**) showed a hepatoprotective property in the CCl_4-induced liver toxicity model in rats. This effect may be due to its capacity to modulate the oxidative stress, especially by reducing of the lipid peroxidation, as well as by a significant restoration to normal levels of the catalase activity and GSH contents as observed in CCl_4-intoxicated rats after the amburoside A (**9**) treatment (Leal et al., 2008).

The large-scale usage and demand for the wild *A. cearensis,* as a medicinal plant by communities in the Northeastern of Brazil, governmental programs of phytotherapy as well as the pharmaceutical industry, are contributing to decrease availability on these species, presently considered as endangered ones. In this sense, our laboratory has conducted comparative studies on the pharmacological profile of the ethanolic extract (EtOHE) or vanillic acid (**12**) from the wild and cultivated *A. cear*ensis, by evaluating their antinociceptive and antiedematogenic activities in several experimental models, such as the formalin test, carrageenan or dextran-induced edema and carragenan-induced neutrophil migration into the rat peritoneal cavity (Leal et al., 2010).

The acute treatment with both the EtOHE prepared from all parts of the cultivated *A. cearensis* (4, 7 or 9 months) or the wild *A. cearensis,* present antinociceptive and anti-inflammatory activities (Fig. 7). In addition, vanillic acid (**12**), which together with coumarin (**1**) are the major compounds present in cultivated *A. cearensis*, also showed an antinociceptive activity by inhibiting both phases of the formalin test in mice, and this effect was partially blocked by naloxone. Thus, the data suggest that antinociceptive effect of vanillic acid (**12**) occur by a mechanism at least in part dependent on the opioid system (Leal et al., 2010).

Coumarin (**1**) has been found in several Brazilian medicinal plants including *J. pectoralis*, *M. glomerata* and *A. cearensis*. It has been reported that the antinociceptive and the anti-inflammatory activities of these species seems to be related at least in part to the presence of coumarin (**1**) (Leal et al., 1997; 2003; Leal et al., 1997; Lino et al., 1997; Leal et al., 2000; Freitas et al., 2008). The biological effects of coumarin (**1**) include antibacterial, antiviral, antiedematogenic, antioxidant, lipoxygenase inhibition, lipid peroxidation inhibition, and scavenging of superoxide hydroxyl radicals (Hoult & Paya, 1996; Chang et al., 1996; Casley-Smith et al., 1993; Rajarajeswari & Pari, 2011).

Recently (Leal et al., 2010), it was also determined that the anti-inflammatory effect of vanillic acid (**12**) is isolated from the cultivated *A. cearensis.* This compound orally

administered to rats, was shown to significantly inhibit the carrageenan, but not the dextran-induced edema. It also reduced the accumulation of PMN into the peritoneal cavity of rats and this effect was comparable to that observed with dexamethasone, used as a standard drug (Fig. 7).

Vanillic acid (**12**) is a benzoic acid derivative that is used as a flavoring agent. It is an intermediate in the production of vanillin from ferulic acid (Prince et al., 2011; Kim et al., 2011). Previous studies have shown antifilarial, antibacterial, antioxidant, hepatoprotective and anti-inflammatory (Kim et al., 2011; Prince et al., 2011; Itoh et al., 2009) effects of the vanillic acid. This compound exerts its anti-inflammatory effect by suppressing the production of prostaglandin E2, nitric oxide and cytokines. Furthermore, it also suppressed the activation of nuclear-factor-kappa B and caspase (Kim et al., 2011; Itoh et al., 2009). These findings confirm the anti-inflammatory activity of vanillic acid (**12**) as demonstrated by our laboratory.
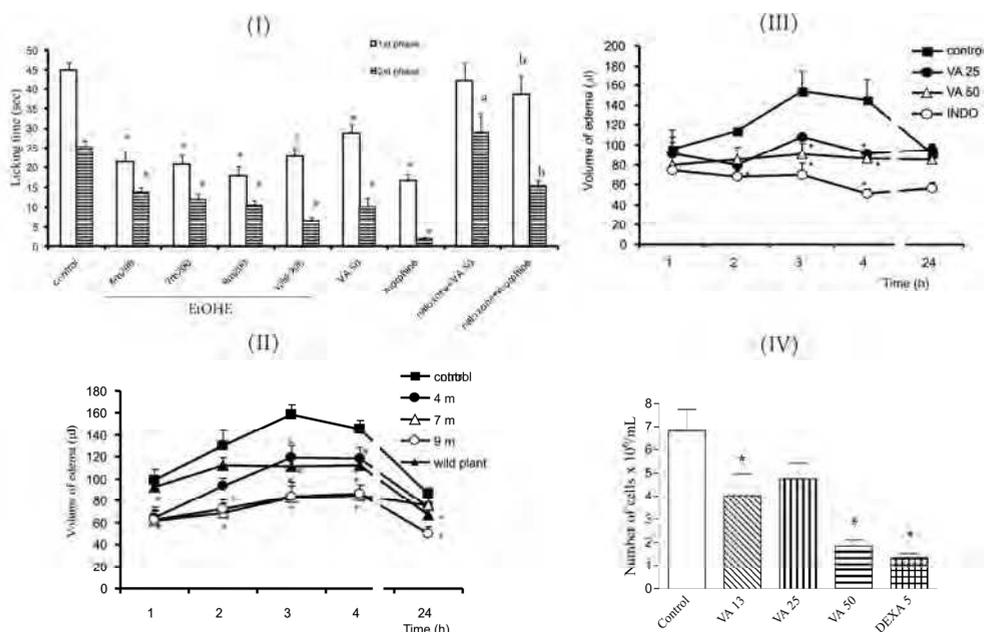
Fig. 7. Antinociceptive and anti-inflammatory effects of ethanolic extracts (EtOHE) and vanillic acid (VA) from *Amburana cearensis* in rodents.[1]

A growing body of evidence suggests that the extract and chemical constituents from the wild *A. cearensis* have pharmacological properties which justify at least in part its traditional use in the treatment of asthma. Among others, the anti-inflammatory activity is possible due

[1] I : effects of EtOHE (cultivated, (4, 7 and 9 months(m) or wild plants: 200 mg/kg, p.o.), VA (50 mg/kg, p.o.) or morphine (MP, 5 mg/kg, s.c.) on the formalin-induced nociception in mice (6-18 animals/group). II, III and IV: anti-inflammatory effects of EtOHE and VA on the carrageenan (Cg)-induced mice paw edema and Cg-inuced rat peritonitis.

to their capacity to modular several responses, especially those related to oxidative stress, the production of inflammatory mediators, and the accumulation and/or activation of inflammatory cells as neutrophils.

The preliminary pharmacological study of the cultivated *A. cearensis* (Leal et al., 2010) showed that both cultivated and wild plants have antinociceptive and anti-inflammatory activities in rodents. Coumarin (**1**) and vanillic acid (**12**) are possibly responsible for the pharmacological activities of the cultivated *A. cearensis* extracts, however the pharmacological importance of other chemical constituents present in the cultivated species cannot be ruled out.

6. Conclusions

The interdisciplinary study of the *A. cearensis* revealed that its ethanol extracts from cultivated and wild sources have similar phytochemical profiles, as consequence, both extracts possess similar pharmacological activities. Hence, these findings support the idea of the utilization of cultivated plants for the manufacturing of herbal drugs preparations by pharmaceutical laboratories, favoring the uniform and constant supply of high quality raw material, as well as the conservation of the wild specimens in the original biome. Indeed, this research indicates promising prospects for the rational use of the *A. cearensis*, however, it is still needed to be advanced in some issues concerning with agronomical, phytochemical and pharmacological knowledge of these species. The influence of some agronomic parameters (plant spacing, shading or sunlight exposure, water supply, etc) on the chemical composition will be performed. Chemical markers or a metabolomic approach will be developed in order to evaluate the influence of the aforementioned agronomic parameters on chemical composition. Pharmacological testing with other types of inflammation experimental models and clinical trials will be carried out aiming to elucidate the mechanisms of action of the *A. cearensis* active principles as well as to evaluate the efficacy in human beings. Additionally, an economic analysis should be performed in order to evaluate the economic feasibility in the production of *A. cearensis* herbal drug preparations from cultivated source.

7. Acknowledgements

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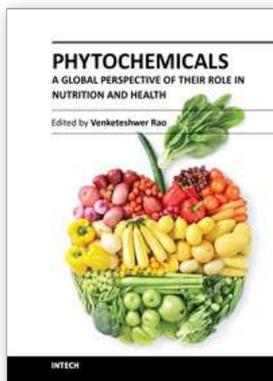
Phytochemicals - A Global Perspective of Their Role in Nutrition and Health

Edited by Dr Venketeshwer Rao

Phytochemicals are biologically active compounds present in plants used for food and medicine. A great deal of interest has been generated recently in the isolation, characterization and biological activity of these phytochemicals. This book is in response to the need for more current and global scope of phytochemicals. It contains chapters written by internationally recognized authors. The topics covered in the book range from their occurrence, chemical and physical characteristics, analytical procedures, biological activity, safety and industrial applications. The book has been planned to meet the needs of the researchers, health professionals, government regulatory agencies and industries. This book will serve as a standard reference book in this important and fast growing area of phytochemicals, human nutrition and health.

How to reference

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