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PHYTOCHEMICAL AND PHARMACOLOGICAL ASPECTS OF MELIACEAE AND *Azadirachta indica* A. JUSS.

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Abstract – The use of medicinal plants by world's population owns a large medical history keeping the interest on natural products research. In this context, Azadirachta indica A. Juss (Meliaceae), popularly known as neem, is widely used in folk medicine due its numerous therapeutic properties. From this plant a limonoid tetranortriterpenoid, azadirachtin, has been linked to several biological activities. Additionally, this compound shows significant importance over ecological aspects including insect pests control and also repellent, antifeedant and insecticidal effects. Current analysis on ethnopharmacologic, chemical and phytopharmacological of medicinal plants focusing upon Azadirachta indica A. Juss. species.

Keywords - Meliaceae; *Azadirachta indica* A. Juss.; Azadirachtin; Ethnobotany; Phytochemistry; Pharmacology.

I. INTRODUCTION

The family Meliaceae is widely known to provide plants wood of great commercial value depending on the quality and relative ease cultivation in the reforestation areas. The main plant species of this family are mahogany, cedar, rose cedar and santa barbara (a Brazilian native tree) [1]. Meliaceae is rich bioactive limonoids family for which antiparasitic, cytological, antifungal, antibacterial, antiviral and insecticide properties were comproved [2]. In that context, stand out azadirachtin, a complex tetranortriterpenoid obtained from *Azadirachta indica* A. Juss (Meliaceae) (Fig. 1) that intensificated studies regarding to limonoids applications such as repellent, antifeedant and insecticide [3, 4].

Many other limonoids have been tested for inhibitor insect growth and antifeedant and none showed exceptional activity such as azadirachtin. In fact, the insecticidal property of this biocompound was confirmed in about 300 insect different species. It was already demonstrated its interference+ in the endocrine glands function that control insect metamorphosis, preventing the development of the ecdysis process [5].

Azadirachta indica A. Juss known in the vernacular as "Neem", is widely distributed in Asia, Africa and other tropical parts of the world [6]. Scientific studies of this plant led to the isolation and structural elucidation of more than 300 compounds which were found on seed and leaves from this species, among them azadirachtin [7]. Speciffficaly, this compound could also be isolated from *Melia azedarach* L. From *A. indica* it is found in all plant parts, foremost higher percentages found in its seed fruit (about 3.5mg g^{-1}) [8, 9]. Despite of the azadirachtin bioactive importance, this tetranortriterpenoid could not be synthesized yet due to its complex chemical struture [6]. Bisides that *A. indica* for food propose is compromised due the strong azadirachtin insecticidal property [9, 10].

In the other hand, there are avaliable commercially products containing azadirachtin, for example, Margosan-O® [5].The present topical paper emphasize phytochemistry and pharmacology aspects of *Azadirachta indica* A. Juss and its family (Meliaceae) standing out on biodiversity, ethnobotany and medicinal plants importances, carrying the responsibility for answering in sequential fashion critical questions.

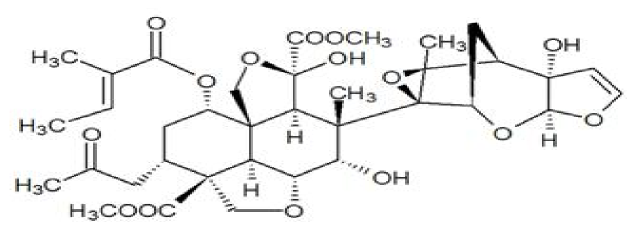

Fig. 1 Chemical structure of the tetranortriterpenoid azadirachtin.

II. MEDICINAL PLANTS ASPECTS

Plants components applied on treatment and cure of diseases is as old as the human species and become stronger accompanying the biotechnological development of the natural products [11]. Nowadays, economy, social factors and alternative medicine are primary reasons to natural medicine continuously be used on therapeutic care for people who lives in developing countries as well as in countries where conventional medicine is predominant [11-14]. In fact,

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approximately, 25% of drugs in the pharmaceutical market have in their composition extracts originating from plant sources [14].

Among the 252 drugs considered as basic and essential by the World Health Organization, 11% is exclusively originating from plants such as taxol, a natural compound with significant anticancer activities, initially isolated from the *Taxus brevifolia* Nutt and later from several *Taxus* species; etoposide and teniposide (*Podophyllum peltatum*); irinotecan, topotecan and camptothecin (*Camptotheca acuminate*); vinblastine and vincristine (*Catharanthus roseus*); homoharringtonine (*Cephalotaxus harringtonia*); digoxin (*Digitalis spp.*) and vincristine citing a few classical examplos [14-16].

It is estimated that 25 000 species of plants are used in the traditional medicine preparations. The Brazil possesses greater biodiversity in the world and its wide variety of natural environment has approximately 60 000 species of plants which corresponds to about 20% of the world's flora and no less than 75% of all the species in the great forests [17]. In the other hand, facing the limited knowledge about the complex biodiversity in the tropical forests such as Amazon region of Brazil, becomes obvious that the safe industrial use of plants and its isolated natural products still fragmented and sparse [17]. Although, worldwide a multidisciplinary team has been involved on natural product scientific development getting the research done in order to identify and confirm the effectiveness of the bioactive natural metabolites [11, 14, 16].

Among new discoveries on bioactives compounds from natural sources it is known that some plant-derived compounds can be synthesized in the laboratory, however this procedure is highly complex, often resulting in low yield and economically infeasible to the industrial production [11, 14, 18]. Just quoting some examples, one of the first substances which were isolated from a plant was the morphine (analgesic), followed by aconitine and emetine (amebacide/emetic); atropine (anticholinergic); digitoxin (cardio tonic); vincristine (antitumor); reserpine (antihypertensive); quinine and artemisinine (antimalarial). Nowadays, many of these substances are synthesized and traded in a large scale. Along the years, natural medicines had been gradually substituted for analogues or semi-synthetic derivatives. A well known example is the acetylsalicylic acid (aspirin), a substitute for the salicylic acid, which was isolated from *Salix alba*, a commonly used specie in the malaria treatment during the 19th century [14].

Furthermore, some compounds also originating from plants can not be or have never been chemically synthesized. Therefore, biotechnological procedures arise to assist process of exploration, identification, characterization, evaluation and application of the world biodiversity [11, 14, 17, 18].

Facing the modern scientific medicine giant strides it is possible to evidence that ethnobotany still enjoying unparalleled prestige and so much interest should be taken in traditional medicine reports. The term ethnobotanical was first used in 1895 by Harshberger. Even though it had not been entirely described in that time, this term pointed out ways in which it could be useful for scientific research [11, 19]. Since then, several definitions have been found for ethnobotany such as 1) ethnological science that studies the influence of vegetation in the culture [20]; 2) discipline in charge of the study and judgments concerning the vegetal kingdom developed by any society, which revolves around the social group rating plants and how they are put to use them [11, 21, 22]; 3) true scientific botany focusing upon the habitat and use of a specific ethnic group, performed by a scientific botany expert who would eventually associate the eastern scientific classifications with the local one [21]; 4) the science of botany holding a specific ethnic group, seeing the culture of a society as everything one has to know or believe in so as to act accordingly with their members [21].

Conclusively, ethnobotany is the science of relations between humans and plants, since exist a reciprocal influence. Thus, it helps the population in the conceptualizing the relevant aspects, encompassing both the way a social group classifies the plants as well as they use them.

In this sense, the knowledge of the ethnobotany of medicinal plants contributes useful information for the development of pharmacological, phytochemical and agronomic studies. In addition, it can also serve to provide new uses of medicinal plants already known popularly or even plants unused until the present moment for therapeutic application. Ethnobotany applied to the study of medicinal plants work hand in hand with ethnopharmacology which consists in the interdisciplinary scientific exploration of biologically active agents, traditionally applied or observed by determined human group [22].

Natural product research involving ethnobiology, ethnobotany, pharmacology and even chemistry, become an ethnopharmacology approach and is cited in the literature as an alternative method that provide both efficient and successful results contributing to the discovery of new bioactive natural products [11]. Specifically, ethnopharmacology dealing with medical practices, especially remedies used in tradicional medicine systems and consists in the combination of several acquired information including at least a botanist, a chemist and a pharmacologist with each carrying their responsibilities to develop studies in specialized laboratories [5, 11, 22].

In this context, arise many ethnopharmacology studies developed with Meliaceae plants in order to research the bioactivity of several metabolites, especially limonoids [2].

III. MELIACEAE FAMILY

A common chemical characteristic of the Meliaceae family is the presence of oxygenated triterpenes, known as limonoids or also meliacins, due to bitter taste. The biosynthetic pathway in plants provides the eufol or tirucalol as triterpene precursor, giving rise to tetranortriterpenoids by the loss of four carbon atoms from the original precursor (Fig. 2). This biosynthetic pathway involves several reaction steps leading to the formation of the various structures but almost invariably contains 26 carbon atoms [5]. Limonoids from species of Meliaceae were divided into eleven main groups according to the standard carbon skeleton (Fig. 3) [23]. Besides Meliaceae, the limonoid compounds have also been found in Ruteceae and Cneoraceae families which belong to the plant kingdom [2].

As cited above limonoid metabolity type has great potential as pesticide and antifeedant being that the most relevant properties reported to plants from Meliaceae [23]. In the large diversity of limonoids are included azedarachins, sendanins and trichilins that exhibit a chemistry C-seco ring. These limonoids are restricted to the genus *Azadirachta* and *Melia* sp. [24].

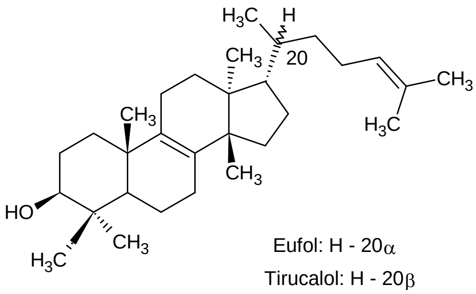


Fig. 2 Chemical structure of the eufol or tirucalol as triterpene precursor, giving rise to tetranortriterpenoids.

An other example of antifeedant sample is cedrelona (Fig. 4) isolated from various plants of Meliaceae as *Trichila catigua* A. Juss, known as "Catiguá" [25]. The genus *Trichilia* is very abundant in tropical regions of America with over 230 known species and is a rich source of insecticidal trichilin compounds [25].

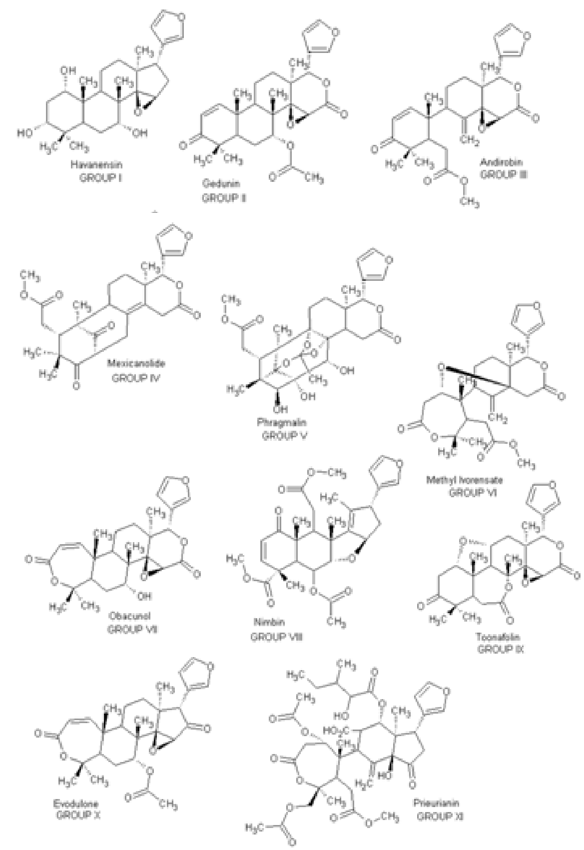


Fig. 3 Main limonoids of the Meliaceae family [23].

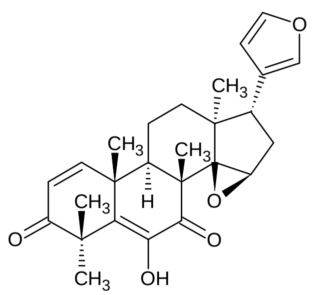


Fig. 4 Chemical structure of cedrelona.

Studies have shown methodologies for limonoids isolation such as cedrelone (Fig. 4) from *Trichilia catigua* and 11β-acetoxy-obacunone acetato (Fig. 5), methyl angolensato (Fig. 6) and an epimeric mixture of fotogedumine (Fig. 7) from *Trichilia elegans* [23, 25].

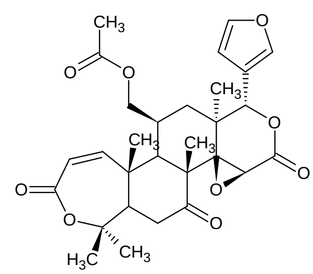


Fig. 5 Chemical structure of 11β-acetoxy-obacunone acetato.

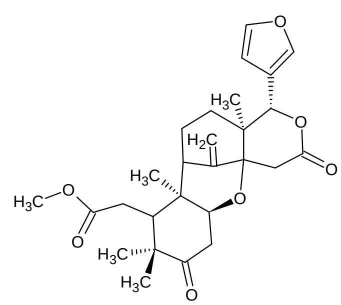


Fig. 6 Chemical structure of methyl angolensato.

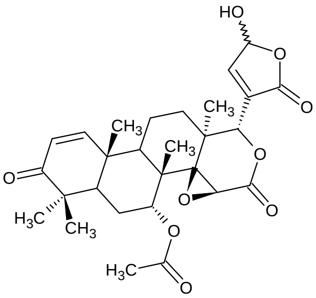


Fig. 7 Chemical structure of fotogedumine.

IV. THE VEGETAL SPECIES *Azadirachta indica* A. JUSS.

Azadirachta indica A. Juss (syn. *Melia Azadirachta* L.), a genus of two species of trees in the mahogany family (Meliaceae). Numerous species have been proposed for this genus but only two are currently recognized, *Azadirachta excelsa* and the economically important tree *A. indica* (Fig. 8). The latter is communly called the "Neem tree" as well as "Neem oil". Both species are native to the Indomalaysian region, and *A. indica* also widely cultivated and naturalized outside its native range such as United States, Australia, African and Central America countries. *A. indica* has been used over 2 000 years in India to control insect pests (whitefly, leaf miner, brasileirinho, tick, caterpillars) and pests of stored grain, nematodes, some fungi, bacteria and virus, and also for human and animal medicine, manufacture of cosmetic, reflorestation, as wood for construction, compost and landscaping [5-8].

Fig. 8 Tree sample of *Azadirachta indica* A. Juss.

Azadirachta indica A. Juss. is originally from tropical climate, thus grow ideally at temperatures above 20 °C, can reach 10 to 20 meters in height, its wood is hard, tough, and reddish-brown in color. Its leaves are alternate with clustered frequency at the ends of branches simple and intense bright green color (Fig. 9). Presenting white inflorescence and yellow stamens (Fig. 10). Its fruit is early an oval berry bright green color, subsequently become yellowish with soft pulp (Fig. 11). Pictures on figures 8 to 11 were taken by D. P. Emerenciano.

This plant is used to urban tree planting in streets and squares, and in the fields on farms for shade and as a windbreak. Its wood is resistant to termites and has been applied in the manufacture of furniture, fence posts, stakes, among other uses [26]. In Brazil, the first planting of "Neem tree" occurred in 1986 year by IAPAR (Agronomy Institute of Paraná City, Londrina state of Brazil) by seeds obtained from the Philippines in order to research *A. indica* insecticidal properties [26]. From that, *A. indica* trees grows up in tropical and subtropical areas of Brazil. In the first five years, this plant can grow up 4 to 7 meters of height and life cycle can reach up 200 years [7, 26 , 27].

Its flowering usually starts in the second year of age and fruit production becomes significant after three years of planting. The fruit production in Brazil, about 8 kg of fruit per tree, starts in December in the Central, North and Northeast regions and in the Southeast region, the production predominates between February to April and for the South region runs to May until June [27].

Fig. 9 Leaves sample of *A. indica* A. Juss.

Fig. 10 Flowers sample of *A. indica* A. Juss.

Fig. 11 Fruits sample of *A. indica* A. Juss.

Speaking of comprehensively, almost all parts of *A. indica* are used for indigenous medicine proporses, especially to combat bacteria and fungi [28]. As cited above, more than 300 compounds were identified from *A. indica*, among them azadirachtin was identified as the most toxic metabolite [10]. This bioactive limonoid was originally isolated by Butterworth and Morgan in 1968 [5, 29]. In 1975, Zanno *et al.* [5, 30] suggested the chemical structure which was corrected by Kraus *et al.*, in 1985 [5, 31]. Physical chemical studies on the structure of azadirachtin, due to its complexity were extended for 18 years. This substance is soluble in polar organic solvents, very sensitive to ultraviolet rays and is strong acidic or basic solutions. Azadirachtin (AZ) is rapidly biodegradable maintaining the maximum antifeedant effect for two weeks. It consists of a closed isomers compounds ranged from AZ-A to AZ-G, been the isomer AZ-A the most important component present in the Neem seed extract [32].

The insecticidal oil is extracted by pressing the seeds obtaining a maximum oil yield of 47% enriched with about 10% of azadirachtin. The seeds residue is very rich in AZ and can be dried and subsequently used for the preparation of insecticides extracts, after mixing with water and filtration. In addition showing nematicide effect and can be used as organic fertilizer [32]. There is no detailed information about specific doses to kill insect species. However, the following doses have shown efficacy in the control of vegetable pests [32].

Green leaves to ticks control for bovines: 1 250g/100 L of water, and for dogs: 500g/3 L of water. In that, the leaves should be left to infuse for 24 h, filtered and appled by spraying over the animals. Dried powder leaves for vegetable pests should be dried under shade and triturated (30g - 40 g/L of water standing for 24 h); followed by filtering and them spray on vegetals. Oil seeds residue (5mL/L of water) as nematicide, could become from 100g - 300g/plant for seeds oil extraction.

Among the wide diversity of biological functions from the chemical constituents present in different parts of *A. indica*, anti-ulcerogenic, anti-inflammatory, anticancer, hypolipidemic and hepatoprotective activies were comproved [33]. In Africa and Caribbean, users of this plant, especially children, eat ripe fruits of Neem. In India, since ancient times the leaves of Neem are consumed for food and tea preparations and domestic animals were also fed with Neem leaves [34]. Despite *A. indica* be known by its pesticide properties there is no records of Neem toxicity to humans, probable by avoid higher doses. In fact, it was observed that, toxic effects of Neem oil in mammals occur only at higher doses. The LD50 values of this oil was found to be 14mL/kg of rats and 24mL/kg of rabbits [35] that sould correspond 31.95g/kg for LD50 values [36]. In rats doses up to 80mL/kg body weight cause respiratory dysfunction, seizures and death. The LD50 of azadirachtin toxicity (in rats) raging from 2g/kg to 5g/kg (oral ingestion and dermal application, respectively). This toxicity is not lower compared to the natural compound rotenone (largely used as a broad-spectrum insecticide, piscicide and pesticide) which has an oral LD50 of 1.5g/kg whithout dermal toxicity [37]. However azadirachtin has a lower dermal toxicity when compared to permethrin (LD50 oral < 4g/kg and dermal LD50 > 4g/kg) a common synthetic chemical widely used as insecticide, acaricide and also insect repellent [35, 37].

Crude leaves extract of *A. indica* and its fractions were investigated against MCF-7 cell line in cytotoxic essay [38]. MTT assay was used to evaluate reduction in the viability of MCF-7 breast cancer cell line. Cell viability was inhibited by different extracts of *Azadirachta indica* in a dose dependent manner. Aqueous extract at the rate of 10mg/mL showed maximum inhibition (60%) than the minimum (30%) reported for hexane extract at the same rate. Phytochemical analysis of crude and fractionated leaves extracts confirmed the presence of flavonoids, saponins, coumarins and tannins [38, 39]. Recent studies report the isolation of triterpenoids and tetranortriterpenoid [40, 41], and limonoids such as meliacinanidrido, zafaral and odoratone [28, 42], as well 14-15-β-epoxynimonol [43], l,7-diacetoxy-apotirucall-14-ene-3,21,22,24,25-pentaol and 2,3,4-trihidroxy-pregnan-16-one [28] (Fig. 12).

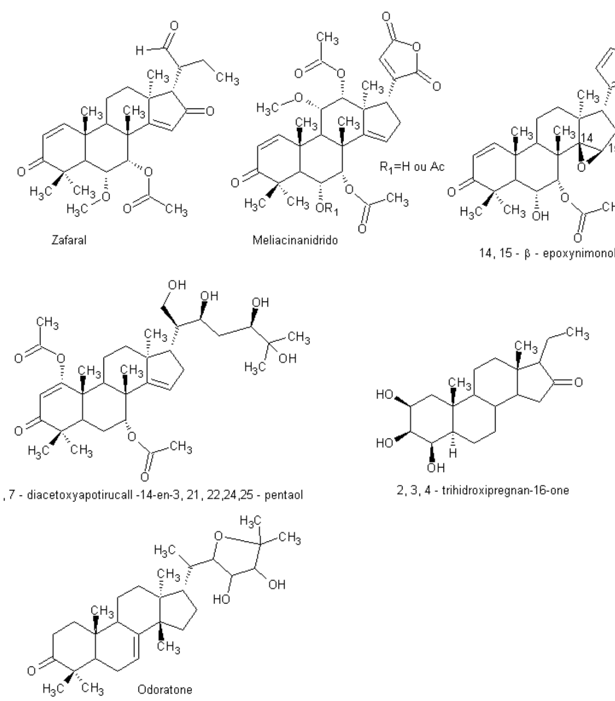


Fig. 12 limonoids present in *Azadirachta indica* A. Juss.

According to Morgan [3] the first compound isolated from the seed oil of *A. indica* was azadiradione (Fig. 13). Although, this compound is very abundant in the seeds of this species, the most abundant polar limonoid found in all parts of the plant is azadirachtin followed by the extraction together of the salannin. The limonoid 3-tigloilazadiractol was found in smaller quantities in the seeds (Fig. 14).

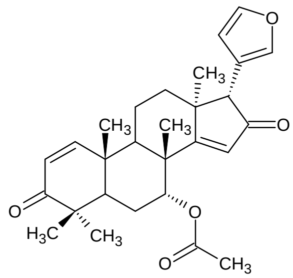


Fig. 13 Chemical structure of azadiradione.

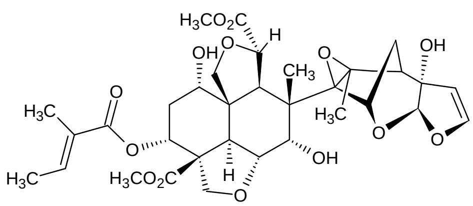
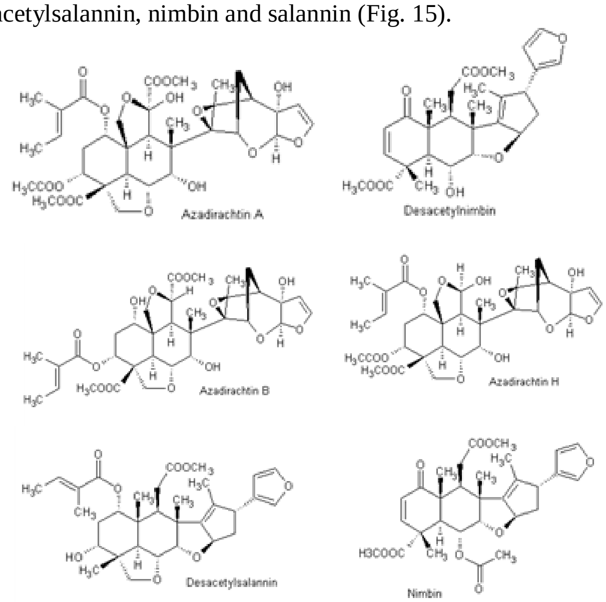


Fig. 14 Chemical structure of 3-tigloilazadiractol.

Silva *et al.* [44] purified seven tetranortriterpenoids (limonoids) from the seeds of *A. indica* A. Juss ussing countercurrent chromatography. Biphasic solvent system (n-hexane:butanol:methanol:water at v/v of 1:0.9:1:0.9, respectively) was used for the isolation of some bioactive compounds such as azadirachtin A (known as azadirachtin), desacetylnimbin, azadirachtin B, azadirachtin H, sesacetylsalannin, nimbin and salannin (Fig. 15).



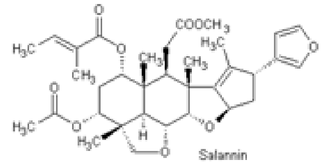


Fig. 15 Chemical structure of some bioactive components isolated from seeds of *Azadirachta indica* [44].

The isolation and purification of azadirachtin and other limonoids present in the Neem seed oil can be made using chromatographic methods. In this context, initially it is carried out at extraction procedure on a biphasic mixture of hydroalcoholic solvents with hexane or petroleum ether. The crude extract contains a quantity of 2% - 6% of limonoids soluble in polar or medium-polarity solvents [45]. In that, a variety of chromatographic procedures were used aiming at isolation and purification of azadirachtin which maximum content for leaves 11% (1kg of leaves produces 111mg of azadirachtin) and 0.075% for the total seed mass [43-48]. The amounts of other limonoids isolated from different parts of *A. indica* are very lower for seeds (0.03%), bark (0.009%), leaves (0.005%), roots (0.003%) and wood (0.002%). Interference effects of climate, soil, and collection time, among others are unknown in the limonoids concentration but it is known that seed storage cause variations on limonoid contents (0.01% to 0.9%) [3].

For Neem high polarity compounds (water soluble) the extensive composition still undetermined despite its presence in almost all parts of this vegetal. The Neem unkown components showed to be effective in the treatment of various diseases such as anti-ulcer, anti-inflammatory, anticancer, antiseptic, hypolipidemic and hepatoprotective [33, 49-51].

Research focusing on the performance of the bioactive azadirachtin revealed the induction of mutations and sterility for generations of harmful insects and can be optimized to combat pests [51]. As an example, the species of phlebotomines of different genera (*Phychodopygus, Lutzomya*) known as the insect vector of leishmaniasis (promastigotes form found in the digestive tract).

The use of Neem can reduce the population of insects, as well as combat this amastigote forms in the tissues of vertebrate hosts (humans and other higher animals). Therefore, the number of new cases of leishmaniose afflicting the worldwide could be reduced by the use of Neem oil [52, 53].

V. FINAL COMMENTS

Finally, it can be concluded that the medicinal specie *Azadirachta indica* is a plant that has become important in alternative herbal therapy being a rich source of bioactive metabolic that may produce phytochemicals and mainly agrochemical agents that do not harm the environment. In this context, azadirachtin has a significant interest in accordance with their antifeedant and insecticide properties that together play an important ecological control [54-56].

The most common way of using *A. indica* is shaped hydroalcoholic extract from leaves was discribed in the patent PI 1003892-2 A2 [57] in which an extract eliminates eggs and larvae forms of the mosquito that transmits dengue (*Aedes aegypti*). This invention results in a viable insecticide, easy to use and can be marked in insect control campaings with proven elimination of 100% and 76% for the eggs and larvae forms, respectively. Among the insectides products available in the market, there is the Margosan-O® commercialized in the United States of America and containing azadirachtin as bioactive principle [5].

Studies envolving methanolic extract of *A. indica* leaves to develop antimicrobial wound dressings impregnated with herbal nanocapsules, which was achieved by preparations with the help of chemical reactions between sodium alginate and calcium chloride cross linked with chitosan and evaluation of antimicrobial activity of these dressings against the wound pathogens in *in vitro*. In that, nanocapsules neem based were used to increase the durability of dressings by sustained release of compounds and recorded 100% bacterial reduction against all the test pathogens. The tested Neem-nanocapsules were formulated by cation induced controlled gelification method and showed a zeta potential above (+/-) 30mV been stable in suspension [58].

The other application for extract and oil from *Azadirachta indica*, consist in an anticorrosive nanoemulsion type formulation, reported in the patent BR 102014022875-6 [59], which was associated with the development of clean technologies. Specifically, refers to the process of preparation and application of a colloidal system nanoemulsion type with an oil phase obtained from seed of *Azadirachta indica*. This system showed good solubilization for a hydroalcoholic extract prepared from leaves of *Azadirachta indica* and was applied on corrosion control. The obtained *A. indica* colloidal product showed good inhibition on the corrosion phenomena which demage is one of the biggest challenges in the environmental technology [59]. This patent corroborate with nanotechnological innovations and could contribute with production of new green corrosion inhibitors.

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