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Andressa Finkler Staudt

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ETHNOPHARMACOLOGY VERSUS BIOLOGICAL ACTIVITY: A REVIEW WITH EMPHASIS ON THE TRITERPENE CLASS

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ABSTRACT

Ethnopharmacological relevance: In recent years, several studies aim to validate the traditional medicinal use of a variety of plant species, through evaluation of extract activity and/or isolation of active constituents. We discuss herein the latest studies on the ethnopharmacology of species with active secondary metabolites from the triterpene class.

Aim of the review: This review aimed to verify, through bibliographic research, the correlation between traditional medicinal use and biological activity of natural sources, with emphasis on the triterpene class of active secondary metabolites.

Methods: Literature research was done in ScienceDirect® database, using the keywords ethnopharmacology and triterpenes, from the period of 2015 and 2016. From 182 studies evaluated, 32 studies were selected. The selected studies were the ones relating the activity of a triterpene, isolated from a natural source extract, to its traditional medicinal use, or the studies relating the traditional medicinal use of a natural source and the activity of its extract, which may be due to the presence of a triterpene.

Results: Twenty studies on the *in vitro / in vivo* activity of isolated triterpenes are reported here, and 12 studies where the activity of the extract might be related to the presence of triterpenes. The most cited activities for the class include antitumoral, anti-mycobacterial, antiplasmodial, antibacterial and anti-inflammatory. The medicinal parts mostly used for extraction were the leaves. The most cited triterpene was betulinic acid, presenting anxiolytic activity *in vivo* and antimycobacterial, antitumoral, and antiplasmodial activities *in vitro*.

Conclusion: The relationship between traditional medicinal use and biological activity was demonstrated for a wide variety of natural source extracts as well as isolated compounds. Nevertheless, future studies are necessary focusing on understanding the pharmacological basis of the traditional use of some natural sources, as well as on generating conclusive data on their efficacy and safety, in order to validate their use in traditional medicine.

Keywords: bioactivity, medicinal use, traditional medicine, natural products, triterpenoids.

1. Introduction

Historically, traditional medicine has been used in health maintenance and in diseases prevention and treatment, mainly for chronic diseases (WHO, 2013). Traditional medicine is defined as "the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness" (WHO, 2000). The practices of traditional medicine are different depending on the country, region and philosophy. In some countries, the traditional or indigenous forms of healing are firmly rooted in their history and culture, and this practice is related with the experience passed on from generation to generation. The use over centuries, suggests the safety and efficacy of traditional medicine. Nevertheless, some health professionals are wrestling with issues regarding the safety, effectiveness, quality, availability, preservation and regulation of traditional medicine (WHO, 2013, 2000).

Since ancient times, worldwide, plants are used in traditional medicine for treatment of a wide variety of ailments, promoting health, and well-being. However, scientific research is needed to provide real evidence of its safety and efficacy (WHO, 2000). Thus, the need to prove the action of traditional medicine have lead to an increase in the number of publications and citations per year relative to traditional medicine and biological activity in the last 10 years (Figure 1) (http//apps.webofknowledge.com).

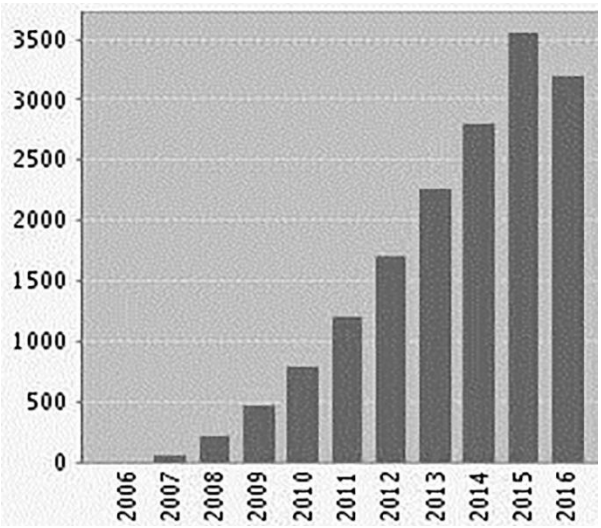

Figure 1. Number of citations per year (2006-2016). Source: Web of Science (http://apps.webofknowledge.com) – November 2016. Keywords: traditional medicine* AND biological activity.

Ethnopharmacology is the intersection of the medical, natural, and social sciences, through fieldwork with traditional societies. Most researches have been based on the combination of the chemical, biological, and pharmacological sciences. At date, some significant advances have been occurring in the technical domain, including the characterization of plant phytochemicals and activities and a better understanding of the interactions among elements of complex botanical, as well as between plants and pharmaceuticals (Etkin, 2001; McGonigle, 2016; Reyes-García, 2010).

The chemical diversity of secondary metabolites from natural sources is very impressive and natural products are a rich source of bioactive molecules. Even today, they are used in the search for new drugs (Newman and Cragg, 2016). The cost of the development of novel molecules with potential to become drugs is an obstacle, and natural products arise as an inexpensive alternative. In this context, based on ethnopharmacology studies, new compounds could be investigated. As example the drugs taxol, quinine, ephedrine, and digoxin, which are natural products discovered from ethnopharmacological studies (McGonigle, 2016).

Among various classes of natural products that have been studied, terpenes are a vast family of naturally occurring compounds with large structural diversity. Terpene structures result from condensation of isoprene units (C_5) and its classification are according to the number of isoprene units, which are normally combinations multiples of five (Silvestre and Gandini, 2008). Triterpenes, specially, are derived from 6 isoprene units (C_{30}), and are one of the wide classes of plant-derived secondary metabolites, with more than 20,000 different triterpenes reported. The majority of triterpene diversity is found in the plantae kingdom, although other organisms also produce triterpenes. These metabolites class are known to accumulate in the epicuticular and intracuticular wax layers of stem and leaf surfaces, and they have an important role in protection against dehydration and herbivores (Thimmappa et al., 2014).

Several studies have demonstrated the potential of triterpenes as leading compounds (Gamo et al., 2010; Sami et al., 2006; Sun et al., 2006). The traditional use of plants containing these secondary metabolites has been described and some researchers have been working to verify the relation of this use with the biological activity *in vitro* and *in vivo* (H. Li et al., 2015; Noundou et al., 2016; Wang et al., 2016). Therefore, the aim of this study was to demonstrate the relationship between traditional medicine and biological activity of active compounds from the triterpene class. Thus, we discuss the recent studies on the ethnopharmacology of species with bioactive secondary metabolites from triterpene class.

The survey was conducted based on information from ScienceDirect® database, using the keywords: ethnopharmacology and triterpenes, from the years of 2015 and 2016. The 182 studies found were categorized and placed on a table, from where 32 studies were selected. The selected studies shown in table 1 were the ones relating the activity of a triterpene, isolated from a natural source, to its traditional medicinal use. The selected studies shown in table 2 were the studies relating the traditional medicinal use of a natural source and the activity of its extract, which may be due to the presence of a triterpene.

2. Results

It was found 20 studies reporting the *in vitro* or *in vivo* activities of isolated triterpenes, from various natural sources (Table 1). Studies were mainly hypothesis-driven by ethnopharmacological reports on the use of these natural sources from communities in different parts of the world. Table 2 shows the 12 studies conducted using plant extracts. In these studies, triterpenes were identified as major constituents and/or probable responsible active constituents of the plant, based on their previously reported activities in the literature; however, no isolation of active compounds was accomplished.

Table 1. Reported activities of isolated triterpenes from natural sources.

	Natural source species	Medicinal part	Uses in traditional medicine	Isolated triterpene	Activity	Model	Reported activity	Reference
1	*Alchornea cordifolia* (Schumach. & Thonn.) Müll.Arg.	Leaves and stem bark	Gastrointestinal, respiratory and urinary tract infections; Wound infections	Friedelin	Antibacterial	*In vitro*	Gastrointestinal pathogens: MIC = 37.5 µM for *E. coli* ATCC 25922 strain Urinary tract pathogens: MIC = 18.7 µM for *P. mirabilis* ATCC 43071 strain MIC = 4.7 µM for *S. saprophyticus* ATCC 15305 strain	Noundou et al., (2016)
				Friedelane-3-one-28-al	Antibacterial	*In vitro*	Gastrointestinal pathogens: MIC = 34.0 µM for *E. coli* ATCC 25922 Urinary tract pathogens: MIC = 18.2 µM for *P. mirabilis* ATCC 43071 MIC = 4.5 µM for *S. saprophyticus* ATCC 15305	
				3-O-acetyl-aleuritolic acid	Antibacterial	*In vitro*	Gastrointestinal pathogens: MIC = 30.1 µM for *E. coli* ATCC 25922. Urinary tract pathogens: MIC = 32.1 µM for *P. mirabilis* ATCC 43071 MIC = 4.0 µM for *S. saprophyticus* ATCC 15305	
				3-O-acetyl-erythrodiol	Antibacterial	*In vitro*	Urinary tract pathogens: MIC = 33.0 µM for *P. mirabilis* ATCC 43071 MIC = 4.1 µM for *S. saprophyticus* ATCC 15305	
2	*Sarracenia purpurea*	Whole plant	Tuberculosis	Betulinaldehyde	Antimycobacterial	*In vitro*	MIC = 450.0 µM; IC_{50} = 98.0 µM for M. *tuberculosis* H37Ra	Morrison et al., (2016)
				Betulinic acid	Antimycobacterial	*In vitro*	MIC = 950.0 µM; IC_{50} = 169.0 µM for M. *tuberculosis* H37Ra	
				Ursolic acid	Antimycobacterial	*In vitro*	MIC = 450.0 µM; IC_{50} = 93.0 µM for M. *tuberculosis* H37Ra	
3	*Taraxacum mongolicum* Hand.-Mazz; *Taraxacum*	Whole herb	Hepatitis, upper respiratory tract infections, bronchitis,	Taraxasterol	Anti-arthritic	*In vivo*	FCA-induced arthritis: Significant inhibition of paw swelling on a dose-dependent manner and decrease on the arthritis index	Wang et al., (2016)

	sinicum Kitag,		pneumonia					
4	*Chrysophyllum cainito* L.	Leaves	Inflammatory diseases, as rheumathoid arthritis	3β-Lup-20(29)-en-3-yl acetate	Inhibition of induced hypersensitivity	*In vivo*	Epinephrine induced hypersensitivity: inhibition of 52 ± 6% LPS-induced hyperalgesia: inhibition of 26 ± 6%	Meira et al., (2016)
				Lup-20(29)-en-3β-O-hexanoate	Inhibition of induced hypersensitivity	*In vivo*	PGE_2-induced hypersensitivity: inhibition of 39 ± 5% Epinephrine induced hypersensitivity: inhibition of 32 ± 7% LPS-induced hyperalgesia: inhibition of 53 ± 4% FCA-induced hypersensitivity: inhibition of 28 ± 6%	
5	*Dillenia suffruticosa* (Griffith ex Hook. F. And Thomson) Martelli	Roots	Cancer	Katonic acid	Antitumoral	*In vitro*	IC_{50} = 64.2 ± 3.3 µM for MDA-MB-231 cells	Foo et al., (2016)
				Betulinic acid	Antitumoral	*In vitro*	IC_{50} = 9.6 ± 1.1 µM for MDA-MB-231 cells	
				Koetjapic acid	Antitumoral	*In vitro*	IC_{50} = 77.1 ± 2.1 µM for MDA-MB-231 cells	
6	*Scutia buxifolia* Reissek	Leaves and stem bark	Heart failure; Hypertension	Ursolic acid	Cardiotonic	*In vitro*	Inhibition of Na^+K^+-ATPase; Heart samples: IC_{50} = 26.3 µM Brain samples: IC_{50} = 120.4 µM	Carvalho et al., (2016)
7	*Parkia biglobosa* (Jacq.) G. Don	Leaves	Diabetes mellitus	Lupeol	Decrease in post prandial hyperglycemia	*In vitro*	Non-competitive inhibition of α-glucosidase (IC_{50} = 105.7 ± 3.5 µM) Uncompetitive inhibition of α-amylase (IC_{50} = 601.0 ± 37.8 µM)	Ibrahim et al., (2016)

8	*Cleistochlamys kirkii* (Benth) Oliv.	Root barks	Wound infections; Tuberculosis; Rheumatism	Polycarpol	Antibacterial	*In vitro*	*S. aureus* (MRSA ATCC 9144) Synergistic activity of polycarpol at 68.07 µM: - Reduction of oxacillin MIC from 311.4 µM to 3.7 µM - Reduction of amoxicillin MIC from 648.2 µM to 20.5 µM *S. aureus* (VISA CIP 106760) Synergistic activity of polycarpol at 68.07 µM: - Reduction of oxacillin MIC from 622.8 µM to 37.4 µM - Reduction of amoxicillin MIC from 648.2 µM to 164.2 µM - Reduction of vancomycin MIC from 2.8 µM to 0.04 µM	Pereira et al., (2016)
9	*Cissus quadrangularis* L.	Dried stem	Bone fractures	Squalene	Anti-osteoporotic	*In vitro*	Stimulation of ALP activity in MC3T3-E1 cells at 24.3 µM	Pathomwichaiwat et al., (2015)
10	*Alnus incana*	Barks	Tuberculosis	Betulin	Antimycobacterial	*In vitro*	MIC = 28.3 µM; IC_{50} = 5.4 µM for *M. tuberculosis* (H37Ra	H. Li et al., (2015)
				Betulinic acid	Antimycobacterial	*In vitro*	MIC > 900.0 µM; IC_{50} = 183.1 µM for *M. tuberculosis* (H37Ra)	
				Betulone	Antimycobacterial	*In vitro*	MIC = 909.0 µM; IC_{50} = 129.1 µM for *M. tuberculosis* (H37Ra)	
11	*Combretum leprosum* Mart.	Leaves	Skin diseases	3β,6β,16β-trihydroxylup-20(29)-ene	Healing of cutaneous wounds	*In vivo*	Surgically induced skin lesions in mice: Improvement in the formation of new blood vessels, structuring the extracellular matrix for migration of keratinocytes and increasing tensile strength of the injury, leading to a faster and more effective closure	Do Nascimento-Neto et al., (2015)
12	*Souroubea sympetala; Souroubea gilgii*	Leaves	Wichcraft; Fever; Ulcer; Dysentery	Betulinic acid	Anxiolytic	*In vivo*	Elevated plus-maze (EPM) test: Significant increase in time spent in the open arms of the elevated plus maze at 0.5 mg/kg daily, for 3 days prior the test	Puniani et al., (2015)
13	*Dillenia suffruticosa* (Griffith ex Hook.	Roots	Cancer	Katonic acid	Antitumoral	*In vitro*	IC_{50} = 85.9 µM for MCF-7 cells	Foo et al., (2015)
				Betulinic acid	Antitumoral	*In vitro*	IC_{50} = 16.5 µM for MCF-7 cells	

	F. And Thomson) Martelli			Koetjapic acid	Antitumoral	*In vitro*	IC_{50} = 95.1 µM for MCF-7 cells	
14	*Alstonia scholaris* Linn. R. Br.	Stem bark	Malaria; Hepatitis; Leprosy; Menstrual disorder; Rheumatism; Tuberculosis; Cancer and others	α-Amyrin	Hepatomodulator	*In vivo*	Liver oxidative stress induced by CCl_4 in rats: All biochemical parameters (serum-markers GGT, AST, ALT, LDH, ALP, ACP, SDH, GDH, total bilirubin, total protein, GSH, ceruloplasmin, β-carotene, vitamin C and vitamin E and hepatic-antioxidants SOD, CAT, GPx, GR, GST, 5´-nucleotidase, acid ribonuclease, glucose-6-phosphatase, succinic dehydrogenase and cytochrome-P-450 in liver tissue, as well as lipid peroxidation in both serum and liver contents) were recovered to almost normal level at 20.0 mg/kg body weight/day, orally	Singh et al., (2015)
15	*Uapaca paludosa*	Trunk bark	Malaria; Wound infections; Boils; Rheumatism; Skin diseases; Toothache	Squalene	Antiplasmodial	*In vitro*	IC_{50} = 1.7 µM for P. *falciparum* FcM29-Cameroon strain	Banzouzi et al., (2015)
				Samvisterin	Antiplasmodial	*In vitro*	IC_{50} = 10.4 µM for P. *falciparum* FcM29-Cameroon strain	
				Betulin	Antiplasmodial	*In vitro*	IC_{50} = 6.8 µM for P. *falciparum* FcM29-Cameroon strain	
				Betulinic acid	Antiplasmodial	*In vitro*	IC_{50} = 3.7 µM for P. *falciparum* FcM29-Cameroon strain	
16	*Neoboutonia macrocalyx*	Stem bark	Malaria	3-O-Acetyloleuritolic acid	Antiplasmodial	*In vitro*	IC_{50} = 30.9 µM for D6 strain (CQ sensitive) IC_{50} = 9.0 µM for W2 strain (CQ resistant)	Namukobe *et al.* (2015)
17	*Salvia urmiensis* Bunge	Aerial parts	Medicinal use; Spice; Perfumery	Urmiensolide B	Antitumoral	*In vitro*	IC_{50} = 2.8 µM for MCF-7 cells IC_{50} = 12.1 µM for A549 cells	Farimani et al., (2015)
				Urmiensic acid	Antitumoral	*In vitro*	IC_{50} = 1.6 µM for MCF-7 cells IC_{50} = 10.9 µM for A549 cells	
18	*Stelleta tenius* Lindgren (Marine sponge)	Whole sponge	Cancer	Stellettin N	Antitumoral	*In vitro*	IC_{50} = 4.5 µM for AGS cells IC_{50} = 21.5 µM for A549 cells IC_{50} > 80.0 µM for U-251 MG cells	Y. Li et al., (2015)

				Stelletin O	Antitumoral	*In vitro*	IC_{50} = 9.6 µM for AGS cells IC_{50} = 43.9 µM for A549 cells IC_{50} > 80.0 µM for U-251 MG cells	
				Stellettin P	Antitumoral	*In vitro*	IC_{50} = 7.4 µM for AGS cells IC_{50} = 56.0 µM for A549 cell IC_{50} > 80.0 µM for U-251 MG cells	
19	*Anopyxis klaineana*	Stem bark	Arthritis; Sexually transmitted infections; Skin diseases; Bronchitis; Wound infections	3,23-dioxotirucalla-7,24-dien-21-oic acid	Anti-inflammatory	*In vitro*	PGE_2 competitive enzyme immunoassay: IC_{50} = 3.6 µM	Mireku et al., (2015)
20	*Ilex latifolia*	Leaves	Hyperlipidemia; Hypertension	β-kudinlactone	Inhibition of triglyceride accumulation	*In vitro*	Oleic acid/palmitic acid induced triglyceride accumulation in HepG2 cells: Inhibition of triglyceride accumulation on a dose-dependent manner	Wang et al., (2015)

2.1 Reported activities for isolated triterpenes

2.1.1 Antibacterial activity

Noundou et al., (2016) has shown the antibacterial activity of four isolated triterpenes from the leaves and stem bark methanolic extract of *Alchornea cordifolia* (Schumach. & Thonn.) Müll.Arg. Traditional use of the plant for the management of gastrointestinal, respiratory and urinary tract infections, among other ailments, and involves decoctions and infusions using palm wine. The activity was determined *in vitro,* using gastrointestinal and urinary-tract pathogens (Table 1, entry 1). Friedelin, friedelane-3-one-28-al, 3-O-acetyl-aleuritolic acid and 3-O-acetyl-erythrodiol had outstanding antibacterial activity in *Escherichia coli* (ATCC 25922), *Proteus mirabilis* (ATCC 43071) and *Staphylococcus saprophyticus* (ATCC 15305) strains, with minimum inhibitory concentrations (MICs) of 4.0 μM for all tested compounds against *S. saprophyticus.* No cytotoxicity studies were reported by authors.

Cleistochlamys kirkii (Benth) Oliv. is traditionally used to treat wound infections, tuberculosis and rheumatism in Mozambique. Bioassay-guided fractionation of root bark methanolic extract of *C. kirkii* led to the isolation of active constituents, among them, the tetracyclic triterpene, polycarpol (Pereira et al., 2016). Polycarpol did not show significant activity alone, but showed strong synergistic effects at a concentration of 68.07 μM (Table 1, entry 8) against two resistant strains of *Staphylococcus aureus* (ATCC9144 and CIP 106760) when in combination with antibiotics, demonstrating it might be able to restore antibiotics effectiveness against *S. aureus* resistant strains. No cytotoxicity studies were reported by authors.

2.1.2 Antimycobacterial activity

Sarracenia purpurea (Table 1, entry 2) is a plant species widely used in traditional medicine in Easter Canada for the treatment of tuberculosis. Morrison et al., (2016) performed a bioassay-guided fractionation of *S. purpurea* methanolic extract, which led to the isolation and identification of three active triterpenes, betulinaldehyde, betulinic acid and ursolic acid. These compounds presented half-maximal inhibitory concentration (IC_{50}) of 98.0, 169.0 and 93.0 μM against *M. tuberculosis* (H37Ra), respectively, but authors did not perform any cytotoxicity studies.

Alnus incana is also traditionally used by Canadian First Nations communities as a treatment for tuberculosis-like symptoms, and antimycobacterial activity of triterpenes present on the species was recently demonstrated (H. Li et al., 2015). Betulin, betulinic acid and betulone were identified as the major antimycobacterial constituents in the bark of *A. incana*, with IC_{50} values of 5.4 μM, 183.1 μM and 129.1 μM against *M. tuberculosis* (H37Ra), respectively (Table 1, entry 10). *In vitro* cytotoxicity studies were performed using human embryonic kidney 293 (HEK293) cells, and revealed that whilst betulin had only moderate cytotoxic effects, with a therapeutic index of 16, betulinic acid was almost eight-fold more toxic to human cells than for the mycobacterial cells. The IC_{50} for HEK239 cells were 87.1, 24.1 and 248.2 μM for betulin, betulinic acid and betulone, respectively.

2.1.3 Anti-arthritic activity

Wang et al., (2016) performed studies on the anti-arthritic potential of Taraxacum (dandelion), the whole herb of *Taraxacum mongolicum* Hand.-Mazz, *Taraxacum sinicum* Kitag, or same genus plants, which are used in the Chinese traditional medicine as a remedy for inflammatory diseases. The triterpene taraxasterol had been previously isolated and identified as the main active constituent from Taraxacum (dandelion) (Schütz et al., 2006), and Wang et al., (2016) evaluated the *in vivo* anti-arthritic effect of taraxasterol on arthritis induced by Freund's complete adjuvant (FCA) in rats (Table 1, entry 3). Among other parameters evaluated, significant inhibition of paw swelling in a dose-dependent manner was observed when taraxasterol was administered orally at 2.0, 4.0 and 8.0 mg/kg/day. Significant decrease on the arthritis index was also noticed, at the doses of 4.0 and 8.0 mg/kg/day. Wang et al., (2016) study suggests that taraxasterol may be useful in the treatment of rheumatoid arthritis, although no toxicity studies were conducted.

2.1.4 Inhibition of induced hypersensitivity

Meira et al., (2016) evaluated the effects of two triterpenes isolated from *Chrysophyllum cainito* L. leaves on acute inflammatory pain models induced by prostaglandin E2 (PGE_2), epinephrine, lipopolysaccharide (LPS) and Freund's complete adjuvant (FCA) (Table 1, entry 4). *C. cainito* is commonly used in traditional medicine for diabetes mellitus and inflammatory diseases, such as rheumatoid arthritis. The triterpene 3β-lup-20(29)-en-3-yl acetate (1.0 mg/kg, i.p.) showed inhibition of 52 ± 6% on the epinephrine

induced hypersensitivity model, and 26 ± 6% on LPS-induced hyperalgesia model, while the triterpene lup-20(29)-en-3β-O-hexanoate (1.0 mg/kg, i.p.) showed inhibition of 39 ± 5% on PGE_2-induced hypersensitivity, 32 ± 7% on epinephrine induced hypersensitivity, 53 ± 4% on LPS-induced hyperalgesia and 28 ± 6% on FCA-induced hypersensitivity. Besides the activity of the isolated compounds, authors do not discard the effect of countless compounds present in the crude extract, acting synergistically. No toxicity studies were reported by authors.

2.1.5 Antitumoral activity

Dillenia suffruticosa (Griffith ex Hook. F. And Thomson) is a commonly used medicinal plant in Malaysia for the treatment of cancerous growth, including breast cancer (Foo et al., 2016, 2015). Foo et al., (2016) assessed the toxicity of the dichloromethane extract as well as three isolated triterpenes from the roots of *D. suffruticosa* against human breast cancer (MDA-MB-231) cell line (Table 1, entry 5). Betulinic acid was the most active constituent, with IC_{50} of 9.6 ± 1.1 µM while katonic acid and koetjapic acid presented IC_{50} of 64.2 ± 3.3 µM and 77.1 ± 2.1 µM, respectively. In another study by Foo et al., (2015), cytotoxicity of the same triterpenes extracted from *D. suffruticosa* was evaluated against human breast adenocarcinoma (MCF-7) cell line (Table 1, entry 13), and IC_{50} were 85.9 µM for katonic acid, 16.5 µM for betulinic acid and 95.1 µM for koetjapic acid. In both studies, evaluation of cytotoxicity on normal cells were not reported.

Two triterpenes (urmiensolide B and urmiensic acid) were isolated from the aerial parts of *Salvia urmiensis* Bunge (Table 1, entry 17), and the cytotoxicity against human breast adenocarcinoma (MCF-7) and human alveolar lung epithelial carcinoma (A549) cells were evaluated, showing outstanding activity (Farimani et al., 2015). Urmiensolide B presented IC_{50} values of 2.8 µM against MCF-7 cells and 12.1 µM against A549 cell, while urmiensic acid showed an IC_{50} of 1.6 µM against MCF-7 and 10.9 µM against A549 cells. *S. urmiensis* is an endemic species from Iran, belonging to a genus rich on structurally diverse triterpenoids, and is popularly used as a medicinal plant, as well as a spice and for production of essential oils. Authors did not report cytotoxicity studies on normal cells.

Marine sponges are known producers of secondary bioactive metabolites. Y. Li et al., (2015) isolated three new isomalabaricane-type triterpenes from the marine sponge *Stelleta tenius* Lindgren. Isomalabaricane-type triterpenes are rare structures and have been found to exhibit cytotoxic activity. Stellettin N, Stellettin P and Stellettin O were isolated from the

tetrachloromethane extract of *S. tenius* and had their activity evaluated *in vitro* against human gastric cancer (AGS), human alveolar lung epithelial carcinoma (A549) and human glioblastoma (U-251MG) cells lines (Table 1, entry 18). Although the authors did not report cytotoxicity studies on normal cells, all three compounds showed significant activity against AGS cells, with IC_{50} of 4.5, 9.6 and 7.4 µM for stellettin N, stellettin O and stellettin P, respectively (Y. Li et al., 2015).

2.1.6 Cardiotonic activity

Carvalho et al., (2016) investigated the compound responsible for *Scutia buxifolia* Reissek medicinal properties, in order to support its traditional use as a cardiotonic, anti-hypertensive, and diuretic agent in Argentina, Uruguay and the southern region of Brazil. Given the importance of Na^+, K^+-ATPase on the treatment of heart failure, the research group decided to assess the inhibitory activity of extract, fractions and isolated compounds from the leaves and stem bark of *S. buxifolia.* Among the isolated compounds, the triterpene ursolic acid showed the most prominent inhibitory activity, with IC_{50} of 26.3 µM on heart samples and 120.4 µM on brain samples (Table 1, entry 6). Compared to the tested fractions, ursolic acid did not have the most efficient inhibitory effect; therefore, authors claim the extract activity may result from a synergistic effect between ursolic acid and a compound yet to be identified. The study supports the popular use of the plant as a cardiotonic agent.

2.1.7 Anti-osteoporotic activity

Pathomwichaiwat et al., (2015) aimed to identify the compound responsible for *Cissus quadrangularis* L. previously reported anti-osteoporotic activity, through bioassay-guided fractionation of the plant stem hexane extract. *C. quadrangularis* is commonly used in Southeast Asia to treat bone fractures and swelling. The isolated compounds were subjected to *in vitro* assays with osteoblastic (MC3T3-E1) cells, and the stimulation of alkaline phosphatase (ALP), a bone differentiation marker, was assessed. Among the isolated compounds, the triterpene squalene was able to stimulate ALP activity at a concentration of 24.34 µM (Table 1, entry 9), which was as high as the concentration of the active hexane extract, and higher than the concentration that should be found in the extract, suggesting a probable synergistic effect. The authors claim that it is a beneficial characteristic that the

crude extract requires a similar dose than the isolated compound to exert the same effect, since it reduces the risk of toxicity.

2.1.8 Healing of cutaneous wounds

Combretum leprosum Mart., a native species of the semi-arid region of Brazil, is commonly used on the treatment of skin diseases. In a study by Do Nascimento-Neto et al., (2015) the healing potential of ethanolic extract (100.0 µg/ml in saline) as well as the isolated triterpene 3β,6β,16β-trihydroxylup-20(29)-ene (100.0 µg/ml in saline) was evaluated *in vivo,* using surgically induced skin lesions in mice, with microscopic and histopathologic assessment of wounds on post-operative days 1-12. Treatment with the isolated compound (single 100 µl dose per day of 100.0 µg/ml solution) improved the formation of new blood vessels, structuring the extracellular matrix for migration of keratinocytes and increasing tensile strength of the injury, thus leading to a faster and more effective closure (Table 1, entry 11). 3β,6β,16β-trihydroxylup-20(29)-ene seems to have a role in the cutaneous healing process, and the study provided evidence of the activity of C. *leprosum* on wound healing.

2.1.9 Anxiolytic activity

Leaves and bark from the genus *Souroubea* are used by healers of the Q'eqchi' Maya traditional healer's association in Belize as well as by Amazonian indigenous groups to make a tea for the treatment of witchcraft, fever, ulcer, and dysentery (Puniani et al., 2015). Betulinic acid was obtained from the leaves of *Souroubea sympetala* and *Souroubea gilgii* in a bioassay-guided isolation by Puniani et al., (2015). In the study, the isolated triterpene was submitted to *in vivo* evaluation, using the elevated plus-maze (EPM) test, a validated test to assess anxiety-like behavior in laboratory rodents (Table 1, entry 12). Oral administration of betulinic acid at 0.5 mg/kg daily, for 3 days prior the test, increased the time spent in the open arms of the elevated plus maze significantly, although the high level of activity of the crude extract and the presence of other triterpenes as well as flavonoids in the extract suggests a probable additive or synergistic effect.

2.1.10 Hepatomodulatory activity

Alstonia scholaris Linn. R. Br. has its therapeutic use described in India for the treatment of a series of illnesses including malarial fever, hepatitis, leprosy, cancer, among others. Singh et al., (2015) isolated α-amyrin as the major constituent of *A. scholaris* stem bark ethanol extract, and assessed its hepatomodulatory activity *in vivo*, through a series of biochemical parameters (Table 1, entry 14). Rats were hepato-oxidatively stressed by administration of tetrachloromethane while concurrently receiving α-amyrin at a dose of 20.0 mg/kg body weight/day, orally. Hepatomodulatory potential was assessed by the serum-markers γ-glutamyl transpeptidase (GGT), aspartate and alanine transaminases (AST, ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), acid phosphatase (ACP), sorbitol dehydrogenase (SDH), glutamate dehydrogenase (GDH), and total bilirubin, total protein, glutathione reduced (GSH), ceruloplasmin, β-carotene, vitamin C and vitamin E in serum as well as the hepatic-antioxidants like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-s-transferase (GST), and 5´-nucleotidase, acid ribonuclease, glucose-6-phosphatase, succinic dehydrogenase and cytochrome-P-450 in liver tissue, whereas lipid peroxidation (LPO) was estimated in both serum and liver contents. The assessment of all biochemical parameters registered a significant hepatic oxidative stress in rats that received tetrachloromethane, which was considerably recovered to almost normal level in rats co-administered with α-amyrin. The histoarchitectural examination of liver sections from treated groups further corroborated with the hepatomodulatory potential of α-amyrin and compared with standard drug silymarin. In the *in vivo* 30-days chronic toxicity study, authors report that the median lethal dose (LD_{50}) value for α-amyrin was found to be higher than 100.0 mg/kg body weight/day in the experimental animals.

2.1.11 Antiplasmodial activity

Uapaca paludosa Aubrév. & Landri is commonly used in Central Africa as a remedy against malaria and associated symptoms. Banzouzi et al., (2015) isolated a series of triterpenes from the trunk bark dichloromethane extract of *U. paludosa,* and assessed their activity *in vitro* against *Plasmodium falciparum* FcM29-Cameroon, a chloroquine (CQ) resistant strain. Squalene, samvisterin, betulin and betulinic acid showed IC_{50} of 1.7, 10.4, 6.8 and 3.7 µM, respectively (Table 1, entry 15). The compounds also had their cytotoxicity assessed on african green monkey kidney (VERO) and human epidermoid carcinoma (KB) cells. Although the compounds showed good antiplasmodial results, their cytotoxicity on

VERO and KB cells resulted in poor selectivity index (SI) values, except for samvisterin, which showed a SI higher than 13.

Namukobe et al., (2015) isolated the triterpene 3-O-acetyloleuritolic acid from the ethyl acetate extract of *Neoboutonia macrocalyx* stem bark. *Neoboutonia* plants have been reported in Uganda, Kenya and Cameroon for their traditional use as an antimalarial remedy. 3-O-acetyloleuritolic acid had its antiplasmodial activity assessed *in vitro* against *Plasmodium falciparum* D6 (CQ-sensitive) and W2 (CQ-resistant) strains. The IC_{50} results were 30.9 and 9.0 µM for D6 and W2 strains, respectively (Table 1, entry 16). A cytotoxicity study was performed using human diploid embryonic lung (MRC-5) cells, and the compound showed to be highly cytotoxic, with an IC_{50} of 6.7 µM, demonstrating that the activity of the compound may be related to its cytotoxic nature.

2.1.12 Anti-inflammatory activity

In Ghana, the stem bark decoction of *Anopyxis klaineana* is used for the treatment of arthritis, sexually transmitted infections, skin infections, bronchitis, malaria and as a poultice to heal wounds. Mireku et al., (2015) isolated bioactive compounds from the stem bark of *A. klaineana* after several extractions using organic solvents. The compounds anti-inflammatory potential was assessed using a prostaglandin E_2 (PGE_2) competitive enzyme immunoassay. 3,23-dioxotirucalla-7,24-dien-21-oic acid, a new tirucallane triterpene, showed an IC_{50} of 3.6 µM in the PGE_2 immunoassay, presenting remarkable anti-inflammatory potential (Table 1, entry 19). Cytotoxicity studies were not performed by the research group.

2.1.13 Inhibition of triglyceride accumulation

The leaves of *Ilex latifolia* have been widely used in China as a traditional beverage and in the adjuvant treatment of hyperlipidemia and hypertension. Wang et al., (2015) submitted the leaves of *I. latifolia* to successive extractions and isolated four compounds, among them, the triterpene β-kudinlactone. The effect of these compounds on decreasing intracellular lipid accumulation in human liver cancer (HepG2) cell line was evaluated, through the oleic acid/palmitic acid (OA/PA) induced triglyceride accumulation (OA/PA 0.350:0.175 mM), as well as the cytotoxic activity of compounds on HepG2 cells through MTT assay (Table 1, entry 20). At a concentration of 40.0 µM, β-kudinlactone showed weak cytotoxicity and potent inhibitory activity against triglyceride accumulation in HepG2 cells.

2.1.14 Anti-hyperglycemic activity

Parkia biglobosa (Jacq.) G. Don is commonly used in traditional medicine for the treatment of diabetes mellitus in Nigeria and Togo. Ibrahim et al., (2016) evaluated the activity of the butanolic fraction of *P. biglobosa in vivo,* in a type 2 diabetes (T2D) model of rats. Since the treatment (150 mg/ kg weight, 5 days a week, 4 weeks treatment) significantly decreased the blood glucose levels and improved the glucose tolerance ability of diabetic rats, fractionation of the extract was done, leading to the isolation of the triterpene lupeol. The α-glucosidase and α-amylase inhibitory activity of the pure compound was assessed *in vitro* (Table 1, entry 7), since these carbohydrate-hydrolyzing enzymes are a common approach in the search of new molecules for the treatment of T2D. Lupeol was able to inhibit α-glucosidase (IC_{50} = 105.7 µM) and α-amylase (IC_{50} = 601.0 µM), showing the compound could be involved in decreasing post prandial hyperglycemia through α-glucosidase and α-amylase inhibitions.

Table 2. Reported activities of extracts and their probable active triterpenes.

	Natural source species	Medicinal part	Traditional medicine	Extract (triterpene fraction)	Activity	Model	Probable active triterpenes	Reference
1	*Poikilacanthus glandulosus* (Nees) Ariza	Leaves and branches	Insect bites, cicatrization and inflammation	Ethanol (70%)	Anti-inflammatory	*in vivo* - croton-oil induced ear edema	Maslinic acid Uvaol	de Brum et al., (2016)
2	*Alisma orientale* Juzepzuk	Rhizomes	Dysuria, edema, diabetes, hyperlipidemia and hypertension	Ethanol (75%)	Anti-hyperlipidemic	*in vivo* - high-fat diet induced hyperlipidemia	Alisol derivatives	Li *et al.* (2016)
3	*Schleichera oleosa* (Lour.) Oken.	Stem bark	Rheumatic pain, topical itching, acne, burns, hair dressing and for promoting hair growth	Ethanol (95%)	Anti-inflammatory; Analgesic	*in vivo* - carrageenan-induced paw edema, formalin induced pain response, TPA-induced ear edema	Betulin Betulinic acid Lupeol Lupeol acetate	Khan et al., (2016)
4	*Pimenta pseudocaryophyllus* (Gomes) Landrum	Leaves and branches	Diuretic, sedative and aphrodisiac actions; Predisposition to arthritical and gouty affections of the joints, fever and other diseases	Ethyl acetate	Anti-hyperuricemic; Anti-inflammatory	*in vivo* - liver xanthine-oxidase residual activity; hyperuricemia induced by potassium oxonate and uric acid, MSU crystal-induced paw edema model	Lupeol α-amyrin β-amyrin	Ferrari et al., (2016)
5	*Zizyphus jujuba* Mill.	Fruits	Anticonvulsant, hypnotic-sedative, anxiolytic, tranquilizer, antioxidant and anti-inflammatory	Methanol (50%)	Neuroprotective, through anti-inflammatory and antioxidant activities	*in vivo* - middle cerebral artery occlusion (MCAo) model of focal cerebral ischemia	Betulinic acid	Gupta and Gupta, (2016)
6	*Cobretum leprosum* Mart.	Flowers	Treatment of bleeding, inflammation and as a natural sedative	Ethanolic	Neuroprotective; prevention of Parkinsons Disease	*in vivo* - Parkinson's disease induced by MPTP	3β, 6β, 16β-trihydroxilup-20(29)-ene (TTHL)	Moraes et al., (2016)
7	*Centella asiatica*	Leaves	Diabetes	Ethanol (70%)	Anti-hyperglycemic and anti-hyperlipidemic, increases insulin secretion	*in vivo* - obese diabetic (T2DM) animal model, induced by low dose of streptozotocin	Asiatic acid	Maulidiani et al., (2016)

8	*Terminalia arjuna* (Roxb. ex DC.) Wight & Arn.	Barks	Cardioprotectant, acute and chronic renal diseases	Ethanol (70%)	Diuretic potential - attenuates acute hypobaric hypoxia induced cerebral vascular leakage	*in vivo* - hypobaric hypoxia simulated to an altitude of 27,000 ft. in a decompression chamber	Arjunolic acid	Kumar et al., (2016)
9	*Maytenus guyanensis* Klotzsch ex Reissek	Barks	Analgesic, muscle relaxant, wound healing, insecticide, immunosuppressive, anti-inflammatory, anti-ulcerogenic, anti-rheumatism, anti-diarrheal, antibacterial, antifungal, anti-helminthic, antiprotozoal, antitumor, aphrodisiac and gynecologically active	Aqueous	Anti-genotoxic	*in vivo* - comet assay in peripheral blood, PCE/NCE correlation and occurrence of micronuclei in the bone marrow	Quinone-methide triterpenes: Tingenone 22-hydroxy-tingenone 22-hydroxy-pristimerine	Meneguetti et al., (2015)
10	*Lafoensia pacari* A.St.-Hil.	Stem bark	Gastric ulcers, wounds, pain, local and systemic inflammation, cancer, itch, diarrhea, kidney problems, antipyretic, slimming, healing and tonic for the loss of energy	Chloroform fraction of the ethanol (70%) extract	Anti-depressant effects	*in vivo* - forced swimming test	Lupeol	Galdino et al., (2015)
11	*Vitellaria paradoxa* C. F. Gaertn	Stem bark	Inflammation and fever	Ethyl acetate	Anti-inflammatory and anti-arthritic	*in vivo* - carrageenan-induced paw edema and FCA-induced rheumatoid arthritis models	1α,2β,3β,19α-tetrahydroxyurs-12-en-28-oic acid 5,6-dihydrobassic acid 16α-hydroxybassic acid 5,6-dihydro-16α-hydroxybassic acid 16α-hydroxyprotobassic acid	Eyong et al., (2015)

12	*Kalimeris indica* (L.) Sch.-Bip.	Whole plant	Fever, influenza and cold	95% ethanol fraction of 50% ethanol extract	Anti-inflammatory	*in vitro* - NO and TNF-α production in murine macrophage RAW264.7 cells after exposure to LPS	(3α)-12-oleanen-3-yl acetate (+)-3-oxo-urs-12-en-24-oic acid methyl ester	Zhong et al., (2015)

2.2 Reported activities of plant extracts

2.2.1 Anti-inflammatory

Poikilacanthus glandulosus (Nees) Ariza is widely used in traditional medicine as a healing aid for insect bites, cicatrization and inflammation by population in the City of Santiago, southern Brazil. The plant extract is traditionally prepared by maceration of leaves and branches in alcohol or sugarcane liquor, for topical application. However, according to de Brum et al. (2016), this plant does not have any validation regarding its medicinal effects or toxicity. The anti-inflammatory activity of *P. glandulosus* 70% ethanol extract of leaves was assessed by de Brum et al. (2016) using an *in vivo* croton-oil induced ear edema model (Table 2, entry 1). The topical application of crude extract of *P. glandulosus* reduced in a dose-dependent manner the croton oil-induced ear edema and myeloperoxidase activity, a neutrophil infiltration marker, with maximum inhibition of 87 ± 2% and 64 ± 12%, respectively, at a dose of 1000 mg/ear, thus proving the traditional use of the plant as an anti-inflammatory. High-performance liquid chromatography (HPLC) analysis of the extract revealed the presence of maslinic acid, uvaol and sitosterol. As the anti-inflammatory activity of triterpenes is discussed in previous studies (Herrera et al., 2006; Medeiros et al., 2007; Ríos, 2010; Yap and Lim, 2015), the triterpenes maslinic acid and uvaol found in the extract might be responsible for the activity of *P. glandulosus.*

Schleichera oleosa (Lour.) Oken. is traditionally used in different parts of India for the treatment of pain and rheumatism. The lack of scientific claims to justify the use of this plant in traditional medicine led to the investigation of the anti-inflammatory effects of the alcoholic extract of *S. oleosa* by Khan et al., (2016). In the study, *in vivo* carrageenan-induced paw edema, formalin induced pain response and 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced ear edema models were used in order to assess the anti-inflammatory and analgesic potential of 95% ethanol extract of *S. oleosa* stem bark (Table 2, entry 3). The extract administered orally (400 mg/kg) significantly inhibited the increase in paw edema, with more prominent inhibition of edema in the later stages of inflammation. In the ear edema model, the extract significantly inhibited the increase in ear weight after four hours at moderate (200 mg/kg) and higher (400 mg/kg) doses, exhibiting a dose-dependent inhibition. In the formalin induced pain response, the results demonstrated considerable decrease in paw licking time by *S. oleosa* extract at a dose of 400 mg/kg in the first phase (5 min after formalin injection) as well as the second phase (15–30 min after injection) of the study. An

acute toxicity study was also held and extract was found to be non-toxic up to the dose of 2000 mg/kg body weight. The triterpenes betulin, betulinic acid, lupeol and lupeol acetate were identified in the extract by HPLC analysis. Based on previous studies (Gallo et al., 2009; Lin et al., 2009), betulin seems to be the major analgesic compound present in the extract and the anti-inflammatory response can be attributed to lupeol and betulin as well as other biologically active related triterpenes present in the extract.

Kalimeris indica (L.) Sch.-Bip. has been used in China's traditional medicine as one of the main ingredients in Chinese medicinal preparations, for the treatment of inflammation-related diseases such as fever, influenza and cold. Zhong et al., (2015) evaluated the anti-inflammatory potential of *K. indica in vitro*, using murine macrophage (RAW264.7) cells after exposure to lipopolysaccharide (LPS) (Table 2, entry 12). The 50% ethanol extract was fractioned with solvents of different polarities and the fractions potential to inhibit the production of two important pro-inflammatory mediators, tumor necrosis factor alpha (TNF-α) and nitric oxide (NO), that can trigger a cascade of inflammatory response, was evaluated. The 95% ethanol fraction showed to inhibit NO production significantly, with potency comparable to that of the glucocorticoid dexamethasone (50 μg/ml) at the concentrations of 60 μg/ml. This fraction also significantly inhibited the TNF-α production at all tested concentrations (30, 60 and 120 μg/ml) in a concentration-dependent manner. Four constituents were detected by gas chromatography–mass spectrometry (GC-MS) in active 95% ethanol fraction, among them two triterpenes, (3α)-12-oleanen-3-yl acetate, known for its anti-inflammatory activity (Ding et al., 2010) and (+)-3-oxo-urs-12-en-24-oic acid methyl ester, which lack studies on its anti-inflammatory potential. These triterpenes could be involved in the activity shown by the 95% ethanol fraction of the extract, although, since only volatile compounds could be detected by GC-MS technique, authors do not discard the presence of unknown compounds, which might play a role in the activity of the fraction as well.

2.2.2 Anti-hyperlipidemic activity

The dried rhizome of *Alisma orientale* Juzepzuk is used in traditional medicine in China for the treatment of dysuria, edema, diabetes, hyperlipidemia and hypertension, being the extract prepared by boiling water. In the *in vivo* high-fat diet induced hyperlipidemia model, Li et al., (2016) assessed the lipid-lowering effects of dried rhizomes triterpene fraction (75% ethanol) as well as the non-triterpene fraction (30% ethanol) of the aqueous

extract of *A. Orientale* (Table 2, entry 2). Total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and atherogenic index (AI) in mice serum were evaluated. Results demonstrate that the hyperlipidemic mice treated with the triterpene fraction (180, 360 and 720 mg/kg body weight/day) had a significant decrease in serum TC and AI after continuous consumption of high-fat diet for four weeks. LDL-C also decreased, dose-dependently, after treatment with the triterpene fraction at 360 and 720 mg/kg body weight/day. The HPLC analysis of the fraction show the presence of 18 alisol derivatives, which are believed to be responsible for the activity, since the non-triterpene fraction did not present any lipid-lowering activity. Moreover, previous studies have shown the natural occurring triterpenes potential on lowering serum cholesterol by inhibiting 3-hidroxi-3-methyl-glutaril-CoA (HMG-CoA) reductase activity as well as increasing expression of LDL-receptors (Mäkynen et al., 2012; Rajendran et al., 1996). In toxicity study, the triterpene and non-triterpene fractions were found to be non-toxic up to a dose of 720 mg/kg body weight/day for four weeks and did not cause any death of the animals, which were in good health throughout the experiments.

2.2.3 Anti-hyperuricemic

In folk medicine from the state of Paraná, in Brazil, *Pimenta pseudocaryophyllus* (Gomes) Landrum leaves infusion is used to treat the predisposition to arthritical and gouty affections of the joints, fever and other diseases. Ferrari et al., (2016) investigated the mechanisms of anti-hyperuricemic effect and anti-inflammatory activity of *P. pseudocaryophyllus* ethyl acetate, ethanolic and aqueous extracts of leaves and branches (Table 2, entry 4). *In vivo* liver xanthine-oxidase assay and potassium oxonate and uric acid induced hyperuricemia as well as monosodium urate (MSU) crystal-induced paw edema models were used to evaluate the activity of extracts. The ethyl acetate extract of leaves, at the concentrations of 125 and 250 mg/kg, was able to increase the urinary excretion of uric acid, but was not able to inhibit the residual activity of liver xathine oxidase. The extract also showed significant reduction in paw edema induced by MSU crystals, at 125 and 250 mg/kg. Triterpenes were detected in the ethyl acetate extract of leaves, and previous phytochemical analysis of *P. pseudocaryophyllus* led to the isolation of lupeol, α-amyrin and β-amyrin (Paula et al., 2012). Lupeol has reports on reducing uric acid levels in hyperuricemic mice caused by oxonate (De Souza et al., 2012), as well as anti-inflammatory properties, inhibiting the production of inflammatory cytokines (Fernández et al., 2001). α-amyrin and β-amyrin

also have reported anti-inflammatory activities in mouse TPA-induced ear edema and carrageenan induced paw edema models, respectively (Krishnan et al., 2014; Medeiros et al., 2007). Thus, anti-hyperuricemic and anti-inflammatory activity of *P. pseudocaryophyllus* ethyl acetate extract can be partially attributed to these compounds.

2.2.4 Neuroprotective activity

Zizyphus jujuba Mill. is traditionally used for its anticonvulsant, hypnotic-sedative, anxiolytic, tranquilizer, antioxidant and anti-inflammatory properties in Asian countries. Gupta and Gupta, (2016) evaluated the activity of 50% methanol extract of *Z. jujuba* in *in vivo* middle cerebral artery occlusion (MCAo) model of focal cerebral ischemia in rats (Table 2, entry 5). Rats were pre-treated with the extract alone and in combination with silymarin, three days prior to induction of MCAo. Silymarin is a flavonoid isolated from dried seeds of *Silybum marianum* L. Gaertn., known for its neuroprotective potential (Hirayama et al., 2016). Neurological deficit score, motor impairment and cerebral infarction were assessed 24h following MCAo. A significant reduction in the neurological deficit score was observed with the extract (500 mg/kg), and the least effective dose (250 mg/kg) was able to further decrease neurological deficit when co-administered with silymarin (250mg/kg), in comparison with each of the treatments alone. The motor performance, assessed through rota rod test, showed significant improvement after administration of the extract (250 and 500 mg/kg), and the improvement was further enhanced when the extract (250 mg/kg) was co-administered with silymarin (250 mg/kg). The infarct volume was decreased dose-dependently in 100, 250 and 500 mg/kg, with a decrease of 38.1 ± 5.6% at 500mg/kg in rats pre-treated with the extract. The extract (250 mg/kg) in combination with silymarin (250 mg/kg) had a potentiated effect, with 57.5 ± 10.4% reduction in infarct volume. Authors claim the neuroprotective effects of *Z. jujuba* may occur through anti-inflammatory and antioxidant activities, in which case the triterpene betulinic acid, a major constituent of the plant (Hong et al., 2015) among other compounds present, could be responsible for the anti-inflammatory activity, since previous studies have discussed its action as an anti-inflammatory agent (Hong et al., 2015).

Cobretum leprosum Mart. is distributed in north and northeastern regions of Brazil and is used traditionally for the treatment of bleeding, inflammation and as a natural sedative. Moraes et al. (2016) evaluated the neuroprotective potential of *C. leprosum* ethanolic extract *in vivo* in a Parkinson's disease (PD) model induced by the toxin 1-methyl-4-phenyl-1,2,3,6-

tetrahydropyridine (MPTP), since the neurodegeneration that occurs in PD is associated with inflammatory toxic factors (Table 2, entry 6). The extract was orally administered in doses of 100 mg/kg over a 14 days period, starting three days before the first MPTP injection. Mice were assessed for behavioral impairments (amphetamine-induced locomotor activity and muscle strength), and mice treated with MPTP that received the extract exhibited attenuation of the hyperlocomotion and the muscular deficits. Striatum dopamine content was measured using HPLC, and mice treated only with MPTP presented a dopamine reduction of 80%, whereas the group treated with MPTP and the extract exhibited approximately a 3-fold increase in dopamine and its metabolites contents. Tyrosine hydroxylase (TH) and dopamine transporter (DAT) gene expressions were assessed using qPCR, and mice treated with MPTP had a 60% decrease in the expression of TH and DAT genes, whereas *C. leprosum* ethanolic extract concomitant treatment was able to increase the mRNA levels of TH and DAT genes in approximately 2-fold compared to the MPTP group. Authors discuss that the mechanism involved in the neuroprotective effect of *C. leprosum* ethanolic extract could be related to increase in dopamine availability and its metabolites. Previous studies have shown the neuroprotective, antinociceptive and anti-inflammatory properties of the triterpene 3β, 6β, 16β-trihydroxilup-20(29)-ene (TTHL) (Della-Pace et al., 2013; Longhi-Balbinot et al., 2012, 2009; Pietrovski et al., 2006), a major component of *C. leprosum*. Findings suggest that the extract might have exerted a protective action in the dopamine levels through its anti-inflammatory action, which may be related to the presence of TTHL, preventing the occurrence of MPTP-induced neuron death.

2.2.5 Anti-hyperglicemic

Centella asiatica has been used among the Malaysian population for the treatment of diabetes mellitus, thus, Maulidiani et al., (2016) aimed to validate this traditional claim using an obese diabetic (T2DM) animal model (Table 2, entry 7). Rats were fed with a high-fat diet and induced into diabetic condition by the treatment of a low dose of streptozotocin, while the treated group administered *C. asiatica* leaves 70% ethanol extract in a dose of 300 mg/kg, daily, over a period of four weeks. Biochemical analysis of serum samples was determined spectrometrically, with appropriate kits for the measurement of the typical serum parameters including glucose, cholesterol, LDL, and HDL. Plasma insulin level was determined using a radioimmunoassay kit. Parameters were also analyzed by a principal component analysis (PCA)-model fitted to the binned nuclear magnetic resonance (NMR) spectra of the urine and

serum of experimental rats. Results showed that long-term treatment of obese diabetic rats with *C. asiatica* extract decreased the glucose level significantly and increased level of serum insulin, attaining similar level as that of the normal group. As for the cholesterol, LDL and HDL levels were generally lower in the *C. asiatica*-treated group. Moreover, the treatment restored the tricarboxylic acid cycle and amino acid metabolic disorders back towards normal states. Based on previous studies (Jang et al., 2010), authors suggest that the anti-diabetic properties shown by *C. asiatica* could be related to enhancement of insulin secretion by triterpenes in the extract. Asiatic acid is a triterpene which is present in the extract and has been found to possess anti-diabetic potential (Liu et al., 2010; Ramachandran et al., 2014). Thus, the *C. asiatica* activity found in this study is probably due to β-cell inducement by asiatic acid, and results confirmed the traditional claim that *C. asiatica* extract has anti-hyperglycemic and anti-hyperlipidemic effects.

2.2.6 Diuretic activity, prevention of hypobaric hypoxia–induced vascular leakage

Terminalia arjuna (Roxb. ex DC.) Wight & Arn. has been used to treat angina pectoris, as a cardioprotectant and for acute and chronic renal diseases in traditional system of Ayurvedic medicine. Kumar et al., (2016) aimed at evaluating the diuretic action of the 70% ethanol bark extract of *T. arjuna* and validate its possible use in preventing vascular leakage during acute mountain sickness at high altitudes (Table 2, entry 8). Using an *in vivo* model of hypobaric hypoxia simulated to an altitude of 27,000 ft. in a decompression chamber, they measured total urine volume during exposure, and investigated the animals for cerebral vascular leakage and serum concentration of sodium, potassium, renin, angiotensin-II, aldosterone and atrial natriuretic peptide (ANP). *T. arjuna* bark extract was administered at a single dose of 150 mg/kg, 30 minutes prior exposure. Results show that *T. arjuna* administration ameliorated acute hypobaric hypoxia, prevented cerebral vascular leakage, maintained urine volume close to normoxic rats and also ameliorated hypobaric hypoxia induced decrease in serum potassium and increase in serum sodium. Increase in glomerular filtration and decrease in serum levels of renin and angiotensin-II and aldosterone, directly related to an increase of ANP were also observed. Moreover, free radical scavenging activity was observed, along with attenuation of hypobaric hypoxia induced oxidative stress. The group also performed acute and sub-chronic toxicity studies, and no mortality was observed over the period of 28 days after administration of 2000 mg/kg body weight/day of the extract. The triterpene arjulonic acid is considered an important bioactive constituent of *T. arjuna*

bark extract, and previous reports have demonstrated its anti-oxidative efficacy (Elsherbiny et al., 2016; Pal et al., 2015). Thus, arjulonic acid could be involved in *T. arjuna* renal protective effects. The study provides evidence for the use of *T. arjuna* as a prophylactic to prevent vascular leakage during acute mountain sickness at high altitude.

2.2.7 Antigenotoxic activity

Meneguetti et al., (2015) performed an analysis of the acute genotoxicity *in vivo* of the aqueous extract of *Maytenus guyanensis* Klotzsch ex Reissek, which is widely used in popular medicine in Brazil for a variety of illnesses, claiming that few studies have demonstrated the genotoxic safety of the popular use of this species (Table 2, entry 9). Performance of comet assay in peripheral blood, polychromatic erythrocytes/red normochromatic erythrocytes (PCE/NCE) correlation and occurrence of micronuclei in the bone marrow was assessed after oral administration (0.1 ml/10g of the animal) of *M. guyanensis* bark aqueous extracts, 48 and 24h before the tests began. In the comet assay, animals who received the extract at 3.85 mg/ml, 38.5 mg/ml and 77.0 mg/ml concentrations did not present genotoxic effects, unlike the 192 mg/ml, that presented significant genotoxicity. In the analysis of the antigenotoxic potential, 3.85 mg/ml and 38.5 mg/ml concentrations demonstrated significant decrease in both the damage index and the damage frequency. In the PCE/NCE correlation study, the 77.0 mg/ml and 192 mg/ml concentrations showed occurrence of cytotoxicity, whereas the 3.85 mg/ml and 38.5 mg/ml concentrations did not present cytotoxicity and demonstrated anticytotoxic action. The occurrence of micronuclei in bone marrow test showed that the 3.85 mg/ml, 38.5 mg/ml and 77.0 mg/ml concentrations did not present mutagenic effects, moreover, the 3.85 mg/ml and 38.5 mg/ml concentrations demonstrated antigenotoxic effects again, acting as antimutagenic. On the other hand, the 77.0 mg/ml and 192 mg/ml concentrations showed to be mutagenic. Besides the results obtained for the 77.0 mg/ml and 192 mg/ml concentrations, it is important to notice that these are respectively twenty- and fifty-fold higher than the concentration normally used by the population. Overall, it was found that the aqueous extract of *M. guyanensis*, with ten times higher concentration than those used in ethnopharmacology, did not present genotoxic effect and, moreover, it has antigenotoxic action in mice treated acutely. Quinone-methide triterpenes have been previously identified in the species (Facundo et al., 2015), and since studies have demonstrated the effects of these triterpenes isolated from other species of the genus *Maytenus* acting *in vitro* against tumor cells (Santos-Oliveira

et al., 2009), the antigenotoxic effects of *M. guyanensis* are probably due to the presence of these compounds. The study concludes that the aqueous extract of *M. guyanensis*, with ten-fold higher concentration than those used in ethnopharmacology, has antigenotoxic action in mice treated acutely.

2.2.8 Anti-depressant activity

The stem barks and leaves of *Lafoensia pacari* A.St.-Hil. are popularly used for cancer, gastric disorders, inflammation and as a tonic to treat loss of enthusiasm in the state of Goiás, Brazil (Table 2, entry 10). Traditional use involves decoction in water or maceration in alcoholic drinks. Based on previous studies (Galdino et al., 2010) on the anxiolytic-like activity of *L. pacari*, Galdino et al., (2015) performed a bioguided fractionation of the hydroethanolic extract in order to identify the active fraction, using an *in vivo* forced swimming test. Animals were treated orally with the fractions, 24, 5 and 1h before the test. Chloroform fraction (70.0 mg/kg) was the most active, reducing the immobility time by 22%. Action mechanism studies, were animals were pre-treated with two drugs that deplete monoamines, p-chlorophenylalanine (PCPA), an inhibitor of the enzyme tryptophan hydroxylase, at 100 mg/kg for 4 days, and α-methyl-p-tyrosine (AMPT), an inhibitor of tyrosine hydroxylase, at 100 mg/kg 4h before the tests, suggested that antidepressant-like effect is dependent on serotonergic and catecholaminergic systems, since the chloroform fraction did not exert the same effect when these treatments were applied. Phytochemical analysis of the chloroform fraction showed it is rich in triterpenes and also identified the presence of steroids. Analysis by HPLC showed the presence of steroid β-sitosterol and the triterpene lupeol, although further studies are needed in order to determine whether these compounds have a role in the activity of the chloroform fraction.

2.2.9 Anti-arthritic / Anti-inflammatory

Vitellaria paradoxa C. F. Gaertn is used among the population of Cameroon for the treatment of various ailments, including inflammation and fever. Eyong et al., (2015) evaluated the anti-inflammatory and anti-arthritic effects of the ethyl acetate extract of *V. paradoxa* stem barks *in vivo*, using carrageenan-induced paw edema and Freund's Completed Adjuvant (FCA)-induced rheumatoid arthritis model (Table 2, entry 11). In the carrageenan induced edema model, the extract at a dose of 150 mg/kg, orally, significantly inhibited the

first (after 1h) and the second phase (4–6 h) of edema formation. The same dose of extract was used for FCA-induced rheumathoid arthritis model, and it significantly reduced the score of arthritis, with maximum reduction on day 24th of the experimentation. Histological evaluation also revealed that treatment with the extract prevented cartilage destruction on the arthritic rats. Phytochemical evaluation of the extract and identification of the triterpenes 5,6-dihydrobassic acid, 16α-hydroxybassic acid, 5,6-dihydro-16α-hydroxybassic acid, 16α-hydroxyprotobassic acid and 1α,2β,3β,19α-tetrahydroxyurs-12-en-28-oic acid was performed, being the last one a novel ursane triterpenoic acid derivative, and one of the principal compounds present. Since anti-inflammatory activity of triterpenes has been demonstrated before (Suh et al., 1998), the presence of these compounds might play a role in the activity of the extract.

3. Discussion

Ethnopharmacological studies are often driven by the purpose of preserving traditional knowledge and understanding the pharmacological basis of the traditional use of some natural sources, as well of the discovery and development of new drugs. A diversity of activities were found for the triterpenes class of secondary metabolites in this review. Twenty studies on the activity of isolated triterpenes are reported, and 12 studies on which the activity found could be related to the presence of triterpenes.

Antibacterial activity *in vitro* shown by the triterpenes friedelin, friedelane-3-one-28-al, 3-O-acetyl-aleuritolic acid and 3-O-acetyl-erythrodiol isolated from the methanolic extract of *A. cordifolia* (Table 1, entry 1) provide pharmacological basis for the use of its decoctions in palm wine for infectious diseases in African countries. Full characterization of this extract should be done, in order to find the concentrations on which the compounds are present in the extract and whether effective concentrations of these compounds are reached at the doses used in ethnopharmacology. In a study, *C. kirkii* (Table 1, entry 8) also had its traditional use for wound infections confirmed to be related to its biological activity. As a few constituents isolated from the extract presented antibacterial activity, the triterpene polycarpol was only active in synergism, showing the ability to restore antibiotics efficacy against *S. aureus* resistant strains.

Antimycobacterial activities shown by isolated triterpenes from *S. purpurea* and *A. incana* (Table 1, entries 2, 10) might confirm the traditional use of these plants in the treatment of tuberculosis by communities in Canada. Betulinic acid was found to be a

constituent of both species, although, cytotoxicity studies revealed it has a poor selectivity index, being almost eight-fold more toxic to human cells than the mycobacterial cells, demonstrating the importance of evaluating the compounds selectivity index.

Arthritis is one of the many conditions related to excess of inflammation. The *in vivo* evaluation of taraxasterol (Table 1, entry 3) in an arthritis model lead to believe this triterpene extracted from Taraxacum (dandelion), widely used in the traditional medicine for treatment of inflammatory diseases, might be useful in the treatment of rheumatoid arthritis. On that account, the pharmacological basis for one of the medicinal uses of this herb has been shown. *V. paradoxa* (Table 2, entry 11), which is traditionally used for inflammation and fever also had its anti-arthritic and anti-inflammatory activities confirmed through *in vivo* evaluation. Although its traditional use involves maceration of barks in alcoholic beverages, maximum activity was obtained with the ethyl acetate extract, which is relatively less polar, meaning there could be a difference in the constitution of the extract used in ethnopharmacology. Nevertheless, five triterpenes seem to be involved in the activity shown by the extract, among them 1α,2β,3β,19α-tetrahydroxyurs-12-en-28-oic acid, a novel ursane triterpenoic acid derivative. The inhibition of induced hypersensitivity by two triterpenes isolated from the leaves of *C. cainito* (Table 1, entry 4) was also shown, through *in vivo* models. The plant is traditionally used for inflammatory diseases as rheumatoid arthritis, and chronic pain is one of the most difficult symptoms to be relieved. The study relates the ethnopharmacological use of the plant with the biological activity presented, nevertheless, more studies would be needed in order to evaluate the activity of the plant extract or isolated compounds in other aspects of rheumathoid arthritis.

Four studies shown in this review evaluated the antitumoral activity of 8 triterpenes from 3 different natural sources. Katonic acid, betulinic acid and koetjapic acid were isolated from *D. suffruticosa* (Table 1, entries 5, 13), commonly used in Malaysia for cancerous growth. Although betulinic acid showed to be the most active component against two human breast cancer cells lines, authors do not discard synergistic effects among compounds present in the extract. Since carcinogenesis may arise from damage to DNA or enzymes by reactive oxygen species, approaches other than direct cytotoxicity could be added to evaluate the anticancer potential of molecules *in vitro*, as antioxidant effects, stimulation of immune system, inhibition of angiogenesis, and upregulation of enzymes like glutathione-S-transferase (GST) (Houghton et al., 2007). In another study, the aerial parts of *S. urmiensis* (Table 1, entry 17), popularly used as a medicinal plant, spice and in perfumery, yielded two active triterpenes. Urmiensolide B and urmiensic acid showed outstanding activities against

MCF-7 and A549 cell lines. Three isomalabaricane-type triterpenes were also isolated from the marine sponge *S. tenius* (Table 1, entry 18). Marine sponges are known producers of secondary metabolites of biological interest. Interestingly, *S. tenius* had been previously investigated (Lin et al., 2007), and eleven isomalabaricane-type triterpenes had been identified, but in recent re-testing of a newly collected voucher from Sanya, the triterpenes stelletin N, O and P were isolated for the first time, showing there might occur variations in the composition depending on the period and/or site of collection. The isolated triterpenes most prominent activities were against human gastric cancer (AGS) cell line.

Another study supports the popular use of *S. buxifolia* (Table 1, entry 6) as a cardiotonic. Na^+, K^+-ATPase is an important target in the treatment of heart failure. The crude extract (70% ethanol), ethyl acetate and dichloromethane fractions as well as isolated compound ursolic acid, showed Na^+, K^+-ATPase inhibitory activity *in vitro*. The traditional use of the plant involves aqueous infusion of the stem bark, and the group found out that in addition to the stem bark extract, the leaf extract and fractions of *S. buxifolia* also present activity. It should be noticed that there might be variations in the composition of traditionally used extract and the extract studied, due to different polarities of the solvents used.

C. quadrangularis (Table 1 entry 9), has been used in traditional medicine for the treatment of bone fractures. Squalene, isolated from *C. quadrangularis* was able to stimulate ALP in osteoblastic cells. Nevertheless, due to high activity of the hexane extract itself, there is a probable synergistic effect among constituents of the extract. The potential activity of hexane extract and squalene on the treatment of bone fracture was shown, and the study supports the ethnopharmacological use of the plant in the treatment of bone fractures. Nevertheless, the application of the extract such as used in traditional medicine would require further studies.

C. leprosum (Table 1, entry 11), traditionally used for the healing of cutaneous wounds, had its healing potential evaluated *in vivo*. Both ethanolic extract as well as isolated triterpene 3β,6β,16β-trihydroxylup-20(29)-ene lead to a more effective closure of wounds. The study supports the traditional use of the plant, although further studies are needed to determine the mechanism of action of the isolated triterpene. Moreover, many anti-inflammatory drugs delay the process of wound healing, and this problem could be overcome with the use of 3β,6β,16β-trihydroxylup-20(29)-ene for healing of cutaneous wounds. As for the activity presented by the extract, a series of molecules could be acting in different aspects of the pathology and contributing to the overall clinical effect, as seen on traditional medicine.

The anxiolytic activity of the extract of *S. gilgii* and *S. sympetala* (Table 1, entry 12), as well as the triterpene betulinic acid, isolated from the two species was demonstrated. The plants have been used in traditional medicine for people affected by, in the traditional concept, "witchcraft". The symptoms include "becoming withdrawn", loss of interest for daily life, and reduced verbal communication. Q'eqchi' Maya healers from Belize suggested a decoction of the plants, 20-30 g of the leaf/stem in 1 L of boiling water, taken as 1 cup twice a day, for 3 days, restores patient mood and normal behavior. Interestingly, leaves and barks from the genus *Souroubea* have been used for Amazonian indigenous groups in similar means, and the two ethnic groups had never been in contact with each other. The experimentally validated activity corresponds to the traditional use, although the anxiolytic activity resided in the ethyl acetate fraction of the crude ethanolic extract (95%), whilst the traditional use involves decoction with water. This implies differences in the constitution of the extract used in traditional medicine versus the one used in the study, and the active compound could be present in the aqueous extract at a very low concentration.

L. pacari (Table 2, entry 10) is traditionally used, among other ailments, for symptoms like loss of enthusiasm. In traditional medicine, the plant is macerated in alcoholic drinks such as "cachaça", and in an *in vivo* evaluation, the chloroform fraction of the hydroethanolic extract has shown the most prominent antidepressant-like effect. The activity matches the ethnopharmacological use of the plant, and the triterpene-rich chloroform fraction calls for complementary studies in order to find the responsible active compounds.

The hepatomodulatory potential of α-amyrin isolated from the stem bark ethanol extract of *A. scholaris* (Table 1, entry 14) has been demonstrated *in vivo*, through oral treatment with the triterpene in rats. In traditional medicine, the plant is used for hepatitis, among other ailments, as it is believed to exert hepatoprotective effects. The active triterpene α-amyrin is one of the major constituents of the plant, which connects the popular use with the experimentally observed activity, although, it should be explored the activity of the extract, comparing it with the activity of the isolated compound, in order to investigate possible synergistic effects among compounds present in the extract used in traditional medicine.

U. paludosa and *N. macrocalyx* have been used in Africa for the treatment of malaria and related symptoms. In two studies (Table 1, entries 15 and 16) showing the antiplasmodial activity of isolated triterpenes from the two plant species, the isolated compounds showed good activity but poor selectivity indexes, being cytotoxic in the cell lines tested. Their antiplasmodial activities are probably related to their cytotoxic nature. Samvisterin, isolated

from *U. paludosa*, is the only compound which had a good selectivity index (SI= 13), being selectively toxic against *P. falciparum* FcM29-Cameroon strain. More studies should be performed in order to evaluate samvisterin activity on the symptoms related to malaria, along with its antiplasmodial activity.

One isolated compound and a range of plant extracts shown in this review presented anti-inflammatory activities. 3,23-dioxotirucalla-7,24-dien-21-oic acid isolated from the stem bark of *A. klaineana* (Table 1, entry 19) has been shown to present anti-inflammatory activity *in vitro*, which could validate its use in traditional medicine for a range of inflammatory-related diseases, as arthritis, skin infections, bronchitis. The research group also evaluated the anti-bacterial activity of the triterpene, which would be related to some of the diseases the plant is traditionally used for, eg. skin infections, but it did not show any activity. The effects seen in traditional medicine might result from several compounds present in the extract, each presenting one or more activities contributing to the overall healing potential. In another study, the 95% ethanol fraction of *K. indica* (Table 2, entry 12) extract has shown potent anti-inflammatory activity *in vitro*, which can be related to its traditional use in several traditional medicine preparations for inflammatory diseases. Moreover, two triterpenes were found to be present in this active fraction, and might play a role in the activity. Further studies need to be done aiming to isolate the active compounds, in order to find a marker for quality control of this widely used plant and its preparations. The anti-inflammatory potential of plant extracts was also evaluated *in vivo*, in two studies shown in this review. *P. glandulosus* (Table 2, entry 1) ethanol (70%) extract showed dose-dependent anti-inflammatory activity *in vivo*, by topical application. This validates the traditional use of the plant, as the extract is usually prepared by maceration with alcohol or sugarcane liquor, for topical application as a healing aid for inflammation. *S. oleosa* (Table 2, entry 3), traditionally used for pain and rheumatism was also proven to exert anti-inflammatory and analgesic activities *in vivo*. Moreover, acute toxicity of the 95% ethanol extract was performed, and the extract was not toxic up to 2 g/kg b.w. in rats, which may give an idea of the safe dose to be used in ethnopharmacology. Betulin and lupeol were identified in the extract and might play a role on the activity, nevertheless, further studies should be performed in order to define the extract and isolate active compounds.

β-kudinlactone, isolated from the leaves of *I. latifolia* (Table 1, entry 20), showed potent inhibitory potential against triglyceride accumulation and weak cytotoxicity in HepG2 cells. *I. latifolia* is widely used in China as adjuvant on the treatment of hyperlipidemia, and the study shows correlation between plant traditional use and the biological activity found *in*

vitro. Further studies *in vivo* should be done in order to confirm this activity, since variations can occur due to adsorption and metabolism. The anti-hyperlipidemic activity found *in vivo* for *A. orientale* (Table 2, entry 2) extract is also in agreement with this plant ethnopharmacological use. In traditional medicine the extract is prepared using boiling water and used in hyperlipidemia among other ailments, whilst in the study, the triterpene fraction (75% ethanol) of the aqueous extract comprised of alisol derivatives was shown to present anti-hyperlipidemic activity.

The anti-hyperglycemic activity of *P. biglobosa* (Table 1, entry 7) butanolic extract was assessed *in vivo*, and after the activity was confirmed, fractionations of the extract lead to the isolation of triterpene lupeol. Lupeol was able to inhibit two carbohydrate-hydrolyzing enzymes, showing play a role in decreasing post-prandial hyperglycemia. *P. biglobosa* has been used for diabetes mellitus in the traditional medicine from Nigeria and Togo, thus, it is important to validate its activity. *C. asiatica* (Table 2, entry 7) is also used in traditional medicine for the treatment of diabetes mellitus. In an assessment of *C. asiatica* activity *in vivo*, the leaf ethanol (70%) extract presented anti-diabetic properties, which are believed to be due to the presence of asiatic acid. The activity relates to the traditional use of the plant.

A study confirmed the anti-inflammatory and anti-hyperuricemic activities of a traditionally used plant, *Pimenta pseudocaryophyllus* (Table 2, entry 4). The leaves infusion is prepared in traditional medicine for the treatment of predisposition to arthritical and gouty affections of the joints. To some extent, all evaluated extracts (ethyl acetate, ethanol and water) reduced the serum levels of uric acid in the *in vivo* evaluation, although the ethyl acetate extract has shown the most prominent activity. Previous studies on the activity of triterpenes lupeol, α-amyrin, and β-amyrin, present in the ethyl acetate extract, lead to believe these compounds are involved in the activity. Nevertheless, more compounds might be involved, e.g. flavonoids, which were detected in all evaluated extracts. Full investigation on the chemical profile of the species is needed in order to find the compounds responsible for the activity. Moreover, toxicity studies should be done for to find the safe dose to be used in ethnopharmacology.

Neuroprotective activity was found in two *in vivo* studies. *Z. jujuba* (Table 2, entry 5), used in traditional medicine for its anticonvulsant, anxiolytic and tranquilizer, among other properties, was found to exert neuroprotective activity by oral treatment of 50% methanol extract *in vivo*, which was further improved when administered in combination with silymarin, a natural product known for its neuroprotective potential. Betulinic acid, which is a major constituent of the extract, seems to have a role in the activity through its previously

reported anti-inflammatory properties. The ethnopharmacological use is in agreement with the results found. *C. leprosum* (Table 2, entry 6) also presented neuroprotective properties, through anti-inflammatory activity of the ethanolic extract administered orally in an *in vivo* model of PD. The results are in agreement with the traditional use as an anti-inflammatory, and activity might be related to the triterpene TTHL, previously shown to exert neuroprotective, anti-inflammatory and antinociceptive activities. Further studies should be done looking for isolate the active compounds and characterize the extract, as well as studies of toxicity, to find the safe dose to be used in ethnopharmacology.

The ethnopharmacological uses of *T. arjuna* (Table 2, entry 8) include renal diseases, cardioprotectant and for treatment of angina pectoris. *T. arjuna* 70% ethanol bark extract, administered orally, presented *in vivo* diuretic action and was able to prevent vascular leakage during acute mountain sickness at high altitudes. Sub-chronic toxicity study was also performed, and the extract was non-toxic in a 28 days study, at the dose of 2 g/kg b.w per day in rats. This can be useful in order to determine the safe doses of the plant extract to be used as a prophylactic for acute mountain sickness in traditional medicine.

M. guyanensis (Table 2, entry 9), used in traditional medicine in Brazil for a variety of illnesses, had its genotoxic safety assessed *in vivo*. Very few studies to assess the toxicity of plant extracts used in traditional medicine, to an even less extent, the genotoxicity of these extracts, which is alarming. The extract not only did not shows genotoxic effects, it was shown to be anti-genotoxic at a concentration ten-fold higher than the used in ethnopharmacology. The anti-genotoxic activity might be related to the presence of quinine-methide triterpenes, previously identified in the species.

According to Cordell and Colvard, (2012) there is a disconnection between global drug needs and application of discovery resources, as the biggest pharmaceutical companies continue focusing on developing lifestyle or “me-too” drugs, while traditional plant-based medicines are typically the only treatments available for the major occurring diseases in middle and low-income countries. Moreover, these medicines usually lack the quality and the scientific evidence for safety and efficacy, hence the importance of studies addressing the plants used in traditional medicine.

Particular care must be taken when assessing the activity of a plant extract and/or its isolated compound in a laboratory environment and extrapolating the activity to validate the ethnopharmacological studies. Many studies do not specify exactly how the plant is used in traditional medicine e.g. whether it is decoction with water, extraction with alcoholic drinks, and so on, and most studies also lack information on daily dosages and duration of treatment

in traditional medicine. The “ethno” part of ethnopharmacology needs to be given more attention. In the majority of studies, isolation of many prospective active compounds involves extraction with low polarity organic solvents. The low polarity organic solvent extracts commonly used for pharmacology research could not reflect the real characteristic of traditionally used plant extracts, since traditional healers commonly use water as a solvent (Brusotti et al., 2014). As example, in Traditional Chinese Medicine, where remedies are usually prepared in the form of decoctions and administered orally (Song et al., 2012). The active compound, which has been isolated using organic solvent, will probably be present in the aqueous extract at very low concentration, not confirming that the compound is the responsible for the ethnopharmacological use.

Plant extracts may present variations in their contents, depending on the site of collection, period of the year, watering pattern and so on, meaning the concentration of active constituents is typically not known. Biological outcomes associated with a plant extract may vary in an unpredictable way. Therefore, it is scientifically important to define these extracts, chemically and botanically, prior to perform *in vitro*, *in vivo*, pharmacokinetic and other studies, and so, use the same criteria of investigation and assure the consistency and efficacy of these extracts (Cordell and Colvard, 2012). Before assuming if an isolated compound is responsible for most of the activity of an extract, it should be investigated the concentration in which each constituents are present, as sometimes the most active isolated compound can be present in very low concentration, thus cannot account for the activity of the extract (Houghton et al., 2007).

Isolation of active compounds, in theory, should result in aliquots having a higher activity than the extract itself. However, sometimes the original extract presents greater activity than any of the fractions or isolated compounds, as can be seen in the study by Carvalho et al., (2016), (Table 1, entry 6), on which the fractions of the extract had improved activity compared to the most active triterpene isolated, ursolic acid. The authors suggested that the extract activity may result from a synergistic effect between ursolic acid and a compound yet to be identified. Betulinic acid was also identified as the major anxiolytic compound in the study by Puniani et al., (2015) (Table 1, entry 12), but the high level of activity of crude extracts suggests a synergistic effect with related triterpenes and/or flavonoids. In some cases, secondary metabolites contained in complex plant extracts may be working synergistically, and a single metabolite might not be the only responsible for the biological activity (Brusotti et al., 2014; Cordell and Colvard, 2012). On the other hand, there

can be constituents in a plant extract that act in an antagonistic manner or present toxic effects.

Besides proving the efficacy of plants traditionally used, it is also important to address their toxicity. A more critical insight on the studies reviewed here would reveal that many lack toxicity studies for the extracts and/or isolated active compounds. Regarding *in vitro* tests for antitumoral activity, Houghton et al., (2007) claims that ideally several different cancer cell lines, as well as normal cell lines should be used, in order to assess selectivity. In this review, four studies reported the *in vitro* antitumoral activity of a total of eight triterpenes, none of which performed cytotoxicity studies on normal cells.

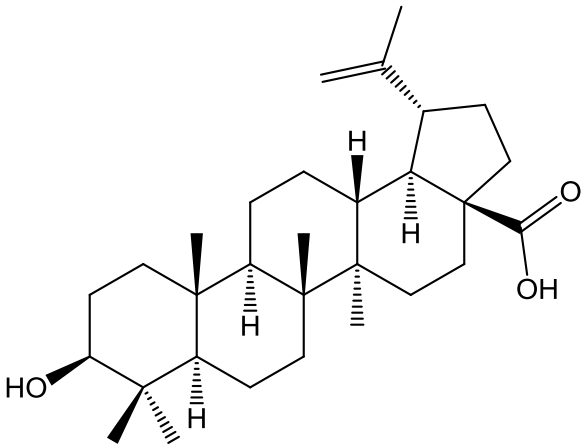

Figure 2. Structure of betulinic acid.

The most cited triterpene, betulinic acid (Figure 2), was found to have anxiolytic activity *in vivo* and antimycobacterial, antitumoral, and antiplasmodial activities *in vitro* (Table 1), although it was shown to be potentially toxic to normal cells in cytotoxicity studies using KB, VERO and HEK293 cell lines (Banzouzi et al., 2015; Li et al., 2016). Betulinic acid might also be related to anti-inflammatory activity, since it was one of the major compounds present in *Z. jujuba* and *S. oleosa* extracts, which presented neuroprotective effect through anti-inflammatory and antioxidant activities, and anti-inflammatory and analgesic potential, respectively.

In vitro bioassays, account for most part of ethnopharmacological studies in the last few years, mostly because they are practical and useful in screenings for active compounds. Moreover, although *in vivo* models give more representative results on the activities of isolated compounds and extracts, in many countries they are restricted due to ethical and financial concerns (Houghton et al., 2007). Care must be taken when making assumptions on

the activity presented by an extract based on *in vitro* studies, as sometimes the activity cannot be extrapolated to *in vivo* or clinical trials. The first problem lays on the dose, which is often too elevated to be extrapolated, secondly, problems with adsorption and metabolism, which can occur as well with *in vivo* studies, yielding differences when using intraperitoneal injection or oral administration (Gertsch, 2009; Houghton et al., 2007).

As discussed by Houghton et al., (2007), a disease is usually a result of a range of symptoms, and when using *in vitro* studies, it is important to design the experiments to approximate as closely as possible to the disease. A battery of tests related to the disease state should be done, keeping in mind that a molecule or a variety of components in an extract may act in one or more of the disease related assays, a phenomenon known as polivalency, working on the symptoms or in the causes of the disease and contributing to the overall clinical effect.

Natural resources come across as a low-cost alternative for use by traditional communities as well as for the discovery of new drugs. Moreover, it involves a "greener" process as opposed to the total synthesis of compounds, which frequently applies toxic reagents or solvents on their reactions. Many traditional communities around the world rely on plant-based treatments as primary health care. However, the majority of medicinal plants sold are collected indiscriminately from the field. Thus, we need to turn our attention to the fact that this is not a sustainable practice, and many essential medicinal plants are under threat or no longer available. The future availability of these resources is concerning, and strategies towards medicinal plants sustainability are required, for the benefit of future generations (Cordell, 2011; Cordell and Colvard, 2012).

Over the years, research on plant-derived active molecules has grown almost exponentially, although on the last few years, few significant discoveries have been made, partly due to the fact that most of the relevant plant constituents have already been found (Gertsch, 2009). Nevertheless, natural products continue to play an featured role in the discovery and development processes of drugs for the treatment of human diseases (Newman and Cragg, 2016).

4. Conclusion

In this review article, the relationship between traditional medicine and biological activity is presented. Ethnopharmacological studies call for attention regarding methods used and what results are considered relevant. It is important to define the extracts chemically and

botanically, proving or disproving their efficacy through relevant assays. Moreover, it is necessary to define the effective dose of extracts and isolated active compounds with potential to be novel drugs, and to perform toxicological studies in order to find the safe dose of extracts to be used in traditional medicine. Therefore, future studies are necessary focusing on understanding the pharmacological basis of the traditional use of some natural sources, as well as on generating conclusive data on their efficacy and safety, in order to validate their use in traditional medicine.

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7. References

Banzouzi, J.T., Soh, P.N., Ramos, S., Toto, P., Cavé, A., Hemez, J., Benoit-Vical, F., 2015. Samvisterin, a new natural antiplasmodial betulin derivative from Uapaca paludosa (Euphorbiaceae). J. Ethnopharmacol. 173, 100–104. doi:10.1016/j.jep.2015.07.023

Brusotti, G., Cesari, I., Dentamaro, A., Caccialanza, G., Massolini, G., 2014. Isolation and characterization of bioactive compounds from plant resources: The role of analysis in the ethnopharmacological approach. J. Pharm. Biomed. Anal. doi:10.1016/j.jpba.2013.03.007

Carvalho, F.B., Boligon, A.A., Athayde, M.L., Rubin, M.A., Ferreira, J., Trevisan, G., 2016. Inhibitory effect of Scutia buxifolia extracts, fractions, and ursolic acid on Na+, K+-ATPase activity in vitro in membranes purified from rat hearts. J. Ethnopharmacol. 179, 45–54. doi:10.1016/j.jep.2015.12.035

Cordell, G.A., 2011. Sustainable medicines and global health care. Planta Med. 77, 1129–1138. doi:10.1055/s-0030-1270731

Cordell, G.A., Colvard, M.D., 2012. Natural products and traditional medicine: Turning on a

paradigm. J. Nat. Prod. doi:10.1021/np200803m

de Brum, T.F., Camponogara, C., da Silva Jesus, R., Belke, B.V., Piana, M., Boligon, A.A., Pires, F.B., Oliveira, S.M., da Rosa, M.B., de Freitas Bauermann, L., 2016. Ethnopharmacological study and topical anti-inflammatory activity of crude extract from Poikilacanthus glandulosus (Nees) Ariza leaves. J. Ethnopharmacol. doi:10.1016/j.jep.2016.07.075

De Souza, M.R., De Paula, C.A., Pereira De Resende, M.L., Grabe-Guimarães, A., De Souza Filho, J.D., Saúde-Guimarães, D.A., 2012. Pharmacological basis for use of Lychnophora trichocarpha in gouty arthritis: Anti-hyperuricemic and anti-inflammatory effects of its extract, fraction and constituents. J. Ethnopharmacol. 142, 845–850. doi:10.1016/j.jep.2012.06.012

Della-Pace, I.D., Rambo, L.M., Ribeiro, L.R., Saraiva, A.L.L., De Oliveira, S.M., Silva, C.R., Villarinho, J.G., Rossato, M.F., Ferreira, J., De Carvalho, L.M., De Oliveira Lima, F., Furian, A.F., Oliveria, M.S., Santos, A.R.S., Facundo, V.A., Fighera, M.R., Royes, L.F.F., 2013. Triterpene 3β, 6β, 16β trihidroxilup-20(29)-ene protects against excitability and oxidative damage induced by pentylenetetrazol: The role of Na+,K+-ATPase activity. Neuropharmacology 67, 455–464. doi:10.1016/j.neuropharm.2012.10.022

Ding, Y., Liang, C., Kim, J.H., Lee, Y.M., Hyun, J.H., Kang, H.K., Kim, J.A., Min, B.S., Kim, Y.H., 2010. Triterpene compounds isolated from Acer mandshuricum and their anti-inflammatory activity. Bioorganic Med. Chem. Lett. 20, 1528–1531. doi:10.1016/j.bmcl.2010.01.096

Do Nascimento-Neto, L.G., Evaristo, F.F.V., De Alencar Alves, M.F., Albuquerque, M.R.J.R., Dos Santos, H.S., Bandeira, P.N., Arruda, F.V.S., Teixeira, E.H., 2015. Effect of the triterpene 3β, 6β, 16β-trihydroxylup-20(29)-ene isolated from the leaves of Combretum leprosum Mart. on cutaneous wounds in mice. J. Ethnopharmacol. 171, 116–120. doi:10.1016/j.jep.2015.05.037

Elsherbiny, N.M., Eladl, M.A., Al-Gayyar, M.M.H., 2016. Renal protective effects of arjunolic acid in a cisplatin-induced nephrotoxicity model. Cytokine 77, 26–34. doi:10.1016/j.cyto.2015.10.010

Etkin, N.L., 2001. Perspectives in ethnopharmacology: forging a closer link between bioscience and traditional empirical knowledge. J. Ethnopharmacol. 76, 177–182. doi:10.1016/S0378-8741(01)00232-X

Eyong, K.O., Foyet, H.S., Baïrys, G., Ngosong Folefoc, G., Acha Asongalem, E., Lagojda,

A., Lamshöft, M., 2015. A new ursane triterpenoic acid and other potential anti-inflammatory and anti-arthritic constituents from EtOAc extracts of Vitellaria paradoxa stem bark. J. Ethnopharmacol. 174, 277–286. doi:10.1016/j.jep.2015.08.014

Facundo, V.A., de Oliveira Meneguetti, D.U., Militão, J.S.L.T., Lima, R.A., Hurtado, F.B., Casseb, A.A., Teixeira, L.F., da Silva, I. do C., da Silva, G.V.J., Junior, V.L., 2015. Chemical constituents from Maytenus guianensis Klotzsch ex Reissek (Celastraceae) Amazon rainforest. Biochem. Syst. Ecol. 58, 270–273. doi:10.1016/j.bse.2014.11.010

Farimani, M.M., Bahadori, M.B., Koulaei, S.A., Salehi, P., Ebrahimi, S.N., Khavasi, H.R., Hamburger, M., 2015. New ursane triterpenoids from Salvia urmiensis Bunge: Absolute configuration and anti-proliferative activity. Fitoterapia 106, 1–6. doi:10.1016/j.fitote.2015.07.017

Fernández, M.A., de las Heras, B., García, M.D., Sáenz, M.T., Villar, A., 2001. New insights into the mechanism of action of the anti-inflammatory triterpene lupeol. J. Pharm. Pharmacol. 53, 1533–1539. doi:10.1211/0022357011777909

Ferrari, F.C., Lemos Lima, R.D.C., Schimith Ferraz Filha, Z., Barros, C.H., De Paula Michel Araújo, M.C., Antunes Saúde-Guimarães, D., 2016. Effects of Pimenta pseudocaryophyllus extracts on gout: Anti-inflammatory activity and anti-hyperuricemic effect through xantine oxidase and uricosuric action. J. Ethnopharmacol. 180, 37–42. doi:10.1016/j.jep.2016.01.007

Foo, J.B., Saiful Yazan, L., Tor, Y.S., Wibowo, A., Ismail, N., How, C.W., Armania, N., Loh, S.P., Ismail, I.S., Cheah, Y.K., Abdullah, R., 2015. Induction of cell cycle arrest and apoptosis by betulinic acid-rich fraction from Dillenia suffruticosa root in MCF-7 cells involved p53/p21 and mitochondrial signalling pathway. J. Ethnopharmacol. 166, 270–278. doi:10.1016/j.jep.2015.03.039

Foo, J.B., Yazan, L.S., Tor, Y.S., Wibowo, A., Ismail, N., Armania, N., Cheah, Y.K., Abdullah, R., 2016. Dillenia suffruticosa dichloromethane root extract induced apoptosis towards MDA-MB-231 triple-negative breast cancer cells. J. Ethnopharmacol. 187, 195–204. doi:10.1016/j.jep.2016.04.048

Galdino, P.M., Carvalho, A.A. V, Florentino, I.F., Martins, J.L.R., Gazola, A.C., de Paula, J.R., de Paula, J.A.M., Torres, L.M.B., Costa, E.A., de Lima, T.C.M., 2015. Involvement of monoaminergic systems in the antidepressant-like properties of Lafoensia pacari A. St. Hil. J. Ethnopharmacol. 170, 218–25. doi:10.1016/j.jep.2015.05.015

Galdino, P.M., Nascimento, M.V.M., de Sousa, F.B., Ferreira, R.N., de Paula, J.R., Costa,

E.A., 2010. Central activities of hydroalcoholic extract from Lafoensia pacari a. St.-Hil. stem bark. Brazilian J. Pharm. Sci. 46, 455–462. doi:Doi 10.1590/S1984-82502010000300008

Gallo, M.B.C., Sarachine, M.J., 2009. Biological activities of lupeol. Int. J. Biomed. Pharmaceut. Sci. 3, 46-66.

Gamo, F.-J., Sanz, L.M., Vidal, J., de Cozar, C., Alvarez, E., Lavandera, J.-L., Vanderwall, D.E., Green, D.V.S., Kumar, V., Hasan, S., Brown, J.R., Peishoff, C.E., Cardon, L.R., Garcia-Bustos, J.F., 2010. Thousands of chemical starting points for antimalarial lead identification. Nature 465, 305–310. doi:10.1038/nature09107

Gertsch, J., 2009. How scientific is the science in ethnopharmacology? Historical perspectives and epistemological problems. J. Ethnopharmacol. doi:10.1016/j.jep.2009.01.010

Gupta, S., Gupta, Y.K., 2016. Combination of Zizyphus jujuba and silymarin showed better neuroprotective effect as compared to single agent in MCAo-induced focal cerebral ischemia in rats. J. Ethnopharmacol. doi:10.1016/j.jep.2016.07.060

Herrera, M.D., Rodriguez-Rodriguez, R., Ruiz-Gutierrez, V., 2006. Functional Properties of Pentacyclic Triterpenes Contained in “Orujo” Olive Oil. Curr. Nutr. Food Sci. 2, 45–49. doi:10.2174/157340106775471976

Hirayama, K., Oshima, H., Yamashita, A., Sakatani, K., Yoshino, A., Katayama, Y., 2016. Neuroprotective effects of silymarin on ischemia-induced delayed neuronal cell death in rat hippocampus. Brain Res. 1646, 297–303. doi:10.1016/j.brainres.2016.06.018

Hong, E.H., Song, J.H., Kang, K. Bin, Sung, S.H., Ko, H.J., Yang, H., 2015. Anti-influenza activity of betulinic acid from Zizyphus Jujuba on influenza A/PR/8 virus. Biomol. Ther. 23, 345–349. doi:10.4062/biomolther.2015.019

Houghton, P.J., Howes, M.J., Lee, C.C., Steventon, G., 2007. Uses and abuses of in vitro tests in ethnopharmacology: Visualizing an elephant. J. Ethnopharmacol. doi:10.1016/j.jep.2007.01.032

Ibrahim, M.A., Habila, J.D., Koorbanally, N.A., Islam, M.S., 2016. Butanol fraction of Parkia biglobosa (Jacq.) G. Don leaves enhance pancreatic β-cell functions, stimulates insulin secretion and ameliorates other type 2 diabetes-associated complications in rats. J. Ethnopharmacol. 183, 103–111. doi:10.1016/j.jep.2016.02.018

Jang, S.M., Kim, M.J., Choi, M.S., Kwon, E.Y., Lee, M.K., 2010. Inhibitory effects of ursolic acid on hepatic polyol pathway and glucose production in streptozotocin-induced diabetic mice. Metabolism. 59, 512–519. doi:10.1016/j.metabol.2009.07.040

Khan, M. J., Saraf, S., Saraf, S., 2016. Anti-inflammatory and associated analgesic activities of HPLC standardized alcoholic extract of known ayurvedic plant Schleichera oleosa. J. Ethnopharmacol. doi:10.1016/j.jep.2016.08.021

Krishnan, K., Mathew, L.E., Vijayalakshmi, N.R., Helen, A., 2014. Anti-inflammatory potential of β-amyrin, a triterpenoid isolated from Costus igneus. Inflammopharmacology 22, 373–385. doi:10.1007/s10787-014-0218-8

Kumar, K., Sharma, S., Vashishtha, V., Bhardwaj, P., Kumar, A., Barhwal, K., Hota, S.K., Malairaman, U., Singh, B., 2016. Terminalia arjuna bark extract improves diuresis and attenuates acute hypobaric hypoxia induced cerebral vascular leakage. J. Ethnopharmacol. 180, 4–53. doi:10.1016/j.jep.2016.01.002

Li, H., Webster, D., Johnson, J.A., Gray, C.A., 2015. Anti-mycobacterial triterpenes from the Canadian medicinal plant Alnus incana. J. Ethnopharmacol. doi:10.1016/j.jep.2015.02.042

Li, S., Jin, S., Song, C., Chen, C., Zhang, Y., Xiang, Y., Xu, Y., Feng, Y., Wan, Q., Jiang, H., 2016. The metabolic change of serum lysophosphatidylcholines involved in the lipid lowering effect of triterpenes from Alismatis rhizoma on high-fat diet induced hyperlipidemia mice. J. Ethnopharmacol. doi:10.1016/j.jep.2015.11.017

Li, Y., Tang, H., Tian, X., Lin, H., Wang, M., Yao, M., 2015. Three new cytotoxic isomalabaricane triterpenes from the marine sponge Stelletta tenuis. Fitoterapia 106, 226–230. doi:10.1016/j.fitote.2015.09.012

Lin, H.W., Wang, Z.L., Wu, J.H., Shi, N., Zhang, H.J., Chen, W.S., Morris-Natschke, S.L., Lin, A.S., 2007. Stellettins L and M, cytotoxic isomalabaricane-type triterpenes, and sterols from the marine sponge Stelletta tenuis. J. Nat. Prod. 70, 1114–1117. doi:10.1021/np070069l

Lin, Y.-C., Cheng, H.-Y., Huang, T.-H., Huang, H.-W., Lee, Y.-H., Peng, W.-H., 2009. Analgesic and anti-inflammatory activities of Torenia concolor Lindley var. formosana Yamazaki and betulin in mice. Am. J. Chin. Med. 37, 97–111. doi:10.1142/S0192415X09006606

Liu, J., He, T., Lu, Q., Shang, J., Sun, H., Zhang, L., 2010. Asiatic acid preserves beta cell mass and mitigates hyperglycemia in streptozocin-induced diabetic rats. Diabetes. Metab. Res. Rev. 26, 448–454. doi:10.1002/dmrr.1101

Longhi-Balbinot, D.T., Lanznaster, D., Baggio, C.H., Silva, M.D., Cabrera, C.H., Facundo, V.A., Santos, A.R.S., 2012. Anti-inflammatory effect of triterpene 3β, 6β, 16β-trihydroxylup-20(29)-ene obtained from Combretum leprosum Mart & Eich in mice. J.

Ethnopharmacol. 142, 59–64. doi:10.1016/j.jep.2012.04.013

Longhi-Balbinot, D.T., Pietrovski, E.F., Gadotti, V.M., Martins, D.F., Facundo, V.A., Santos, A.R.S., 2009. Spinal antinociception evoked by the triterpene 3beta, 6beta, 16beta-trihydroxylup-20(29)-ene in mice: evidence for the involvement of the glutamatergic system via NMDA and metabotropic glutamate receptors. Eur. J. Pharmacol. 623, 30–6. doi:10.1016/j.ejphar.2009.09.004

Mäkynen, K., Chitchumroonchokchai, C., Adisakwattana, S., Failla, M.L., Ariyapitipun, T., 2012. Effect of gamma-oryzanol on the bioaccessibility and synthesis of cholesterol. Eur. Rev. Med. Pharmacol. Sci.

Maulidiani, Abas, F., Khatib, A., Perumal, V., Suppaiah, V., Ismail, A., Hamid, M., Shaari, K., Lajis, N.H., 2016. Metabolic alteration in obese diabetes rats upon treatment with Centella asiatica extract. J. Ethnopharmacol. 180, 60–69. doi:10.1016/j.jep.2016.01.001

McGonigle, I.V., 2016. Patenting nature or protecting culture? Ethnopharmacology and indigenous intellectual property rights. J. Law Biosci. lsw003. doi:10.1093/jlb/lsw003

Medeiros, R., Otuki, M.F., Avellar, M.C.W., Calixto, J.B., 2007. Mechanisms underlying the inhibitory actions of the pentacyclic triterpene α-amyrin in the mouse skin inflammation induced by phorbol ester 12-O-tetradecanoylphorbol-13-acetate. Eur. J. Pharmacol. 559, 227–235. doi:10.1016/j.ejphar.2006.12.005

Meira, N.A., Rocha, L.W., Da Silva, G.F., Quintal, Z.M., Delle Monache, F., Cechinel Filho, V., Quintão, N.L.M., 2016. Chrysophyllum cainito leaves are effective against pre-clinical chronic pain models: Analysis of crude extract, fraction and isolated compounds in mice. J. Ethnopharmacol. 184, 30–41. doi:10.1016/j.jep.2016.02.046

Meneguetti, D.U. de O., Lima, R.A., da Silva, F.C., Passarini, G.M., Facundo, J.B., Pagotto, R.C., Militão, J.S.L.T., Facundo, V.A., 2015. Acute genotoxicity analysis in vivo of the aqueous extract of maytenus guyanensis amazonian chichuá. Brazilian J. Pharmacogn. 25, 164–169. doi:10.1016/j.bjp.2015.03.003

Mireku, E.A., Kusari, S., Eckelmann, D., Mensah, A.Y., Talontsi, F.M., Spiteller, M., 2015. Anti-inflammatory tirucallane triterpenoids from Anopyxis klaineana Pierre (Engl.), (Rhizophoraceae). Fitoterapia 106, 84–91. doi:10.1016/j.fitote.2015.08.007

Moraes, L.S., Rohor, B.Z., Areal, L.B., Pereira, E. V., Santos, A.M.C., Facundo, V.A., Santos, A.R.S., Pires, R.G.W., Martins-Silva, C., 2016. Medicinal plant Combretum leprosum mart ameliorates motor, biochemical and molecular alterations in a Parkinson's disease model induced by MPTP. J. Ethnopharmacol. 185, 68–76. doi:10.1016/j.jep.2016.03.041

Morrison, S.A., Li, H., Webster, D., Johnson, J.A., Gray, C.A., 2016. Antimycobacterial triterpenes from the Canadian medicinal plant Sarracenia purpurea. J. Ethnopharmacol. 188, 200–203. doi:10.1016/j.jep.2016.04.052

Namukobe, J., Kiremire, B.T., Byamukama, R., Kasenene, J.M., Akala, H.M., Kamau, E., Dumontet, V., 2015. Antiplasmodial compounds from the stem bark of Neoboutonia macrocalyx pax. J. Ethnopharmacol. 162, 317–322. doi:10.1016/j.jep.2014.12.018

Newman, D.J., Cragg, G.M., 2016. Natural Products as Sources of New Drugs from 1981 to 2014. J. Nat. Prod. doi:10.1021/acs.jnatprod.5b01055

Noundou, X.S., Krause, R.W.M., Van Vuuren, S.F., Ndinteh, D.T., Olivier, D.K., 2016. Antibacterial effects of Alchornea cordifolia (Schumach. and Thonn.) Müll. Arg extracts and compounds on gastrointestinal, skin, respiratory and urinary tract pathogens. J. Ethnopharmacol. doi:10.1016/j.jep.2015.12.043

Pal, S., Sarkar, A., Pal, P.B., Sil, P.C., 2015. Protective effect of arjunolic acid against atorvastatin induced hepatic and renal pathophysiology via MAPK, mitochondria and ER dependent pathways. Biochimie 112, 20–34. doi:10.1016/j.biochi.2015.02.016

Pathomwichaiwat, T., Ochareon, P., Soonthornchareonnon, N., Ali, Z., Khan, I.A., Prathanturarug, S., 2015. Alkaline phosphatase activity-guided isolation of active compounds and new dammarane-type triterpenes from Cissus quadrangularis hexane extract. J. Ethnopharmacol. doi:10.1016/j.jep.2014.11.026

Paula, J.A.M. De, Silva, M.D.R.R., Costa, M.P., Diniz, D.G.A., Sá, F.A.S., Alves, S.F., Costa, É.A., Lino, R.C., Paula, J.R. De, 2012. Phytochemical analysis and antimicrobial, antinociceptive, and anti-inflammatory activities of two chemotypes of pimenta pseudocaryophyllus (Myrtaceae). Evidence-based Complement. Altern. Med. 2012. doi:10.1155/2012/420715

Pereira, F., Madureira, A.M., Sancha, S., Mulhovo, S., Luo, X., Duarte, A., Ferreira, M.J.U., 2016. Cleistochlamys kirkii chemical constituents: Antibacterial activity and synergistic effects against resistant Staphylococcus aureus strains. J. Ethnopharmacol. 178, 180–187. doi:10.1016/j.jep.2015.12.009

Pietrovski, E.F., Rosa, K.A., Facundo, V.A., Rios, K., Marques, M.C.A., Santos, A.R.S., 2006. Antinociceptive properties of the ethanolic extract and of the triterpene 3β,6β,16β-trihidroxilup-20(29)-ene obtained from the flowers of Combretum leprosum in mice. Pharmacol. Biochem. Behav. 83, 90–99. doi:10.1016/j.pbb.2005.12.010

Puniani, E., Cayer, C., Kent, P., Mullally, M., Sánchez-Vindas, P., Poveda Álvarez, L., Cal, V., Merali, Z., Arnason, J.T., Durst, T., 2015. Ethnopharmacology of Souroubea

sympetala and Souroubea gilgii (Marcgraviaceae) and identification of betulinic acid as an anxiolytic principle. Phytochemistry 113, 73–78. doi:10.1016/j.phytochem.2014.02.017

Rajendran, S., Deepalakshmi, P.D., Parasakthy, K., Devaraj, H., Devaraj, S.N., 1996. Effect of tincture of Crataegus on the LDL-receptor activity of hepatic plasma membrane of rats fed an atherogenic diet. Atherosclerosis 123, 235–241. doi:10.1016/0021-9150(96)05813-3

Ramachandran, V., Saravanan, R., Senthilraja, P., 2014. Antidiabetic and antihyperlipidemic activity of asiatic acid in diabetic rats, role of HMG CoA: In vivo and in silico approaches. Phytomedicine 21, 225–232. doi:10.1016/j.phymed.2013.08.027

Reyes-García, V., 2010. The relevance of traditional knowledge systems for ethnopharmacological research: theoretical and methodological contributions. J. Ethnobiol. Ethnomed. 6, 32. doi:10.1186/1746-4269-6-32

Ríos, J.L., 2010. Effects of triterpenes on the immune system. J. Ethnopharmacol. doi:10.1016/j.jep.2009.12.045

Sami, A., Mäkelä, T., Salme, K., Jari, Y., 2006. Pharmacological properties of the ubiquitous natural product betulin. Eur. J. Pharm. Sci. 29, 1–13. doi:10.1016/j.ejps.2006.04.006

Santos-Oliveira, R., Coulaud-Cunha, S., Colaço, W., 2009. Revisão da Maytenus ilicifolia Mart. ex Reissek, Celastraceae. Contribuição ao estudo das propriedades farmacológicas. Brazilian J. Pharmacogn. doi:10.1590/S0102-695X2009000400025

Schütz, K., Carle, R., Schieber, A., 2006. Taraxacum-A review on its phytochemical and pharmacological profile. J. Ethnopharmacol. doi:10.1016/j.jep.2006.07.021

Silvestre, A.J.D., Gandini, A., 2008. Terpenes: Major sources, properties and applications. Monomers, Polym. Compos. from Renew. Resour. 17–38. doi:10.1016/B978-0-08-045316-3.00002-8

Singh, D., Arya, P. V., Sharma, A., Dobhal, M.P., Gupta, R.S., 2015. Modulatory potential of α-amyrin against hepatic oxidative stress through antioxidant status in wistar albino rats. J. Ethnopharmacol. 161, 186–193. doi:10.1016/j.jep.2014.12.025

Song, C., Huang, L., Rong, L., Zhou, Z., Peng, X., Yu, S., Fang, N., 2012. Anti-hyperglycemic effect of Potentilla discolor decoction on obese-diabetic (Ob-db) mice and its chemical composition. Fitoterapia 83, 1474–1483. doi:10.1016/j.fitote.2012.08.013

Suh, N., Honda, T., Finlay, H.J., Barchowsky, A., Williams, C., Benoit, N.E., Xie, Q.W., Nathan, C., Gribble, G.W., Sporn, M.B., 1998. Novel triterpenoids suppress inducible

nitric oxide synthase (iNOS) and inducible cyclooxygenase (COX-2) in mouse macrophages. Cancer Res. 58, 717–723.

Sun, H., Fang, W., Wang, W., Hu, C., 2006. Structure-activity relationships of oleanane- and ursane- type triterpenoids. Bot. Stud. 47, 339–368.

Thimmappa, R., Geisler, K., Louveau, T., Maille, P.O., Osbourn, A., 2014. Triterpene Biosynthesis in Plants. Annu. Rev. Plant Biol. 65, 225–257. doi:10.1146/annurev-arplant-050312-120229

Wang, C.Q., Li, M.M., Zhang, W., Wang, L., Fan, C.L., Feng, R.B., Zhang, X.Q., Ye, W.C., 2015. Four new triterpenes and triterpene glycosides from the leaves of Ilex latifolia and their inhibitory activity on triglyceride accumulation. Fitoterapia 106, 141–146. doi:10.1016/j.fitote.2015.09.003

Wang, S., Wang, Y., Liu, X., Guan, L., Yu, L., Zhang, X., 2016. Anti-inflammatory and anti-arthritic effects of taraxasterol on adjuvant-induced arthritis in rats. J. Ethnopharmacol. 187, 42–48. doi:10.1016/j.jep.2016.04.031

Web of Science, 2016. URL http://apps.webofknowledge.com. Accessed November, 2016.

WHO, 2013. WHO Traditional Medicine Strategy. Altern. Integr. Med. 1, 1–78. doi:2013

WHO, 2000. General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine World Health Organization. World Heal. Organ. 1, 1–71. doi:WHO/EDM/TRM/2000.1

Yap, W.H., Lim, Y.M., 2015. Mechanistic Perspectives of Maslinic Acid in Targeting Inflammation. Biochem. Res. Int. doi:10.1155/2015/279356

Zhong, R.F., Xu, G.B., Wang, Z., Wang, A.M., Guan, H.Y., Li, J., He, X., Liu, J.H., Zhou, M., Li, Y.J., Wang, Y.L., Liao, S.G., 2015. Identification of anti-inflammatory constituents from Kalimeris indica with UHPLC-ESI-Q-TOF-MS/MS and GC-MS. J. Ethnopharmacol. 165, 39–45. doi:10.1016/j.jep.2015.02.034

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