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FULL COMMUNICATION

Consumption of Nopal Powder in Adult Women

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ABSTRACT Osteoporosis is a chronic disease in adult women caused by menopause and some other factors, which entails deficiency of calcium in diet. Natural products are the best source of nutriments to reduce the risk of chronic diseases. Nopal (*Opuntia ficus-indica*) is a plant characterized by its nutritional components and benefits to health. Its calcium content increases with maturation process that could be beneficial for consumers. Nopal powder (NP) was elaborated from nopal harvested within 16–24 weeks of maturation, and the nutritional content was determined. An experimental clinical trial was performed to evaluate the effect of NP. A total of 69 women between 40 and 60 years old participated in the study. During 24 weeks, experimental group ($n = 56$) consumed a daily dose of 5 g of NP and control group ($n = 13$) continue with habitual diet. Changes in bone mineral density (BMD), body mass index (BMI), body fat percentage and serum calcium were assessed. Between baseline and after 24 weeks of consumption, no significant changes were found in BMD $P = .885$ experimental group and $P = .970$ control group, BMI $P = .865$ experimental group and $P = .984$ control group, body fat $P = .744$ experimental group and $P = .740$ control group and serum calcium $P = .282$ experimental group and $P = .959$ control group. These results indicate that advanced maturation NP does not have influence in bone health, BMI, and body composition in adult women.

KEY WORDS: • *bone mineral density* • *calcium* • *nopal* • **Opuntia ficus indica** • *osteoporosis*

INTRODUCTION

Osteoporosis is a metabolic bone disease defined as significant reduction of bone mineral density (BMD) with T-score <2.5 standard deviation (SD), with continuous progress, is characterized by the accelerated loss of bone mass, some physiological changes, reduction of movements, fragility, weakness, and elevated risk of fractures.[1–3] The principal complication is hip fracture, which represent an important impact in quality of life.[4,5]

Deficient diets, hormonal changes, lifestyle, and menopause are some of the risk factors. The risk increases when diet is deficient in minerals, principally calcium from vegetable sources. Approximately, at 30 years-old, the maximum bone mass is reached. The loss of bone mass begins between 30 and 40 years, where a gradual decrease between 1% and 2% per year is seen during the next decade in men and women. However, the loss of bone mass accelerates to 2–3% per year in the following decades, especially in menopausal age women, due to estrogen deficiency, metabolism changes, and aging.[4,6,7] Thus calcium requirements in adult women increase to 1300 mg/day to prevent osteoporosis, this mineral is commonly found in vegetables and foods of animal origin, and in some cases is necessary to use supplements.

This disease is diagnosed through bone densitometry called dual X-ray densitometry known as DEXA is currently the standard technique for measuring body mass and bone density. The usual anatomical sites in adults for the measurement of BMD are lumbar spine, proximal femur, and distal forearm.[8–10]

Nowadays osteoporosis represents a public health problem around the world, the prevalence is between 35% and 40% in women over 50 years old and 52% in women older than 70 years of age.[11]

There are numerous medical treatments for menopause, such as hormone replacement therapies, medications to decrease bone resorption such as bisphosphonates, calcitonin, and parathyroid hormone, however, it has been proven that they cause side effects, contraindications or intolerance in the short or long term and they also usually are expensive.[12–14]

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Research Article

Safety of Aqueous Extract of *Calea ternifolia* Used in Mexican Traditional Medicine

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There is a trend to use medicinal plants for primary medical care or as dietary supplements; however, the safety of many of these plants has not been studied. The objective of this work was to determine the toxic effect of the aqueous extract of *Calea ternifolia* (*C. zacatechichi*), known popularly as "dream herb" *in vivo* and *in vitro* in order to validate its safety. *In vivo,* the extract had moderate toxicity on *A. salina*. *In vitro,* the extract induced eryptosis of 73% at a concentration of 100 $\mu g \cdot mL^{-1}$ and it inhibited CYP3A by 99% at a concentration of 375 $\mu g/mL$. After administering 8.5 mg/kg of *C. ternifolia* to rats, we found a reduction in platelets and leukocytes and an increase in urea and the liver enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). Histological analysis showed spongiform changes in the proximal tubules of renal tissue and a lymphoid infiltrate in liver tissue. This plant is used in the treatment of diabetes, and it is commercialized as a dietary supplement in several countries. Our results show renal and hepatic toxicity; therefore, more profound research on the toxicity of this plant is needed.

1. Introduction

Because of the current tendency to adopt a more "natural" lifestyle, there is currently great interest in herbal medicine, a medical system that searches for efficient and mildly or nontoxic therapeutic options. Medicinal plants have been used as a cultural inheritance in traditional medicine, but they require scientific validation [1], because they contain active substances that have biological activity and toxicity [2].

Calea ternifolia (syn. *C. zacatechichi*) (Astereaceae) is a plant native to Mexico and Central America, where it has a long tradition in indigenous culture [3]. It is also called the "dream herb" because it temporarily intensifies the clarity of dreams and significantly influences the central nervous system. For this, *C. ternifolia* is used in several countries, although it was placed on the prohibited plant list in Poland in 2013 [4]. A more recent study of the neuropharmacological action of the aqueous extract of *C. zacatechichi* indicates that it has insignificant neuropharmacological effects and also reduces the abdominal pain perception [5]. It is also traditionally used for the treatment of endocrine and gastrointestinal problems [6]. The plant is sold online as a dietary supplement for the treatment of diabetes due to its ability to cause hypoglycemia [7]. Although its mechanism of action is unknown, this activity can be due to compounds that have been isolated from the plant, such as flavonoids [8] and sesquiterpene lactones [9], which have some toxicity. Flavonoids cause deformation, osmotic fragility, and aggregation of erythrocytes [10]. Other flavonoids such as epigallocatechin produce toxicity in rat embryos [11], and quercetin, in addition to being able to capture free radicals and act as an antioxidant, can also carry out pro-oxidant reactions, exerting cytotoxic effects on cells and tissues [12].

marine drugs

MDPI

Article

Antitumoral Effect of Laurinterol on 3D Culture of Breast Cancer Explants

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Abstract: Macroalgae represent an important source of bioactive compounds with a wide range of biotechnological applications. Overall, the discovery of effective cytotoxic compounds with pharmaceutical potential is a significant challenge, mostly because they are scarce in nature or their total synthesis is not efficient, while the bioprospecting models currently used do not predict clinical responses. Given this context, we used three-dimensional (3D) cultures of human breast cancer explants to evaluate the antitumoral effect of laurinterol, the major compound of an ethanolic extract of *Laurencia johnstonii*. To this end, we evaluated the metabolic and histopathological effects of the crude extract of *L. johnstonii* and laurinterol on Vero and MCF-7 cells, in addition to breast cancer explants. We observed a dose-dependent inhibition of the metabolic activity, as well as morphologic and nuclear changes characteristic of apoptosis. On the other hand, a reduced metabolic viability and marked necrosis areas were observed in breast cancer explants incubated with the crude extract, while explants treated with laurinterol exhibited a heterogeneous response which was associated with the individual response of each human tumor sample. This study supports the cytotoxic and antitumoral effects of laurinterol in in vitro cell cultures and in ex vivo organotypic cultures of human breast cancer explants.

Keywords: laurinterol; *Laurencia*; antitumoral; breast cancer explants; organotypic culture; ex vivo

Application of a Multisystem Coating Based on Polymeric Nanocapsules Containing Essential Oil of *Thymus Vulgaris* L. to Increase the Shelf Life of Table Grapes (*Vitis Vinifera* L.)

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***Abstract*— In developing countries, the incidence of postharvest losses reduces the quantity and quality of food for human consumption and causes an economical damage along the food chain, especially, for primary producers. In this study, a multisystem coating (NC-EOt-C) based on pullulan and polymeric nanocapsules containing EO of *Thymus vulgaris* L. (EOt) was applied to increase the shelf life of table grapes (*Vitis vinifera* L.). The major components of EOt, chemically characterized by GC-MS, were *o-cymene* (32.68%), *thymol* (31.90%), and *γ -terpinene* (15.69%). The NC-EOt were prepared by nanoprecipitation and showed a particle mean size of 153.9 nm, a polydispersity index of 0.186, a zeta potential of −4.11 mV, and an encapsulation efficiency of 52.81%. The antioxidant capacity (DPPH and $ABTS^+$ methods) of EOt was maintained, or even improved, after its incorporation into NC. The shelf life study showed that grapes having the NC-EOt-C multisystem maintained their characteristics of color, firmness, TA, and SSC for longer time than those without the multisystem. NC-EOt-C multisystem acted as a barrier which reduced the metabolism of fruits. In addition, the compounds of EOt with antimicrobial activity avoided microorganism growth, while those with antioxidant activity reduced the oxidative stress induced during postharvest of grapes. Additionally, the polymeric structure of NC prevented the rapid evaporation of volatile compounds of EOt, increasing then their residence time on the fruit. Our study demonstrated that NC-EOt-C multisystem can be a viable alternative to preserve horticultural products for longer storage periods.**

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***Index Terms*— Edible coatings, essential oils, food preservation, polymeric nanoparticles, and *Thymus vulgaris* L.**

I. Introduction

FRUITS are perishable products because of their inherent tendency to deteriorate. During the postharvest period of fruit, it is necessary to guarantee a longer useful life of the vegetable. Reports from the Food and Agriculture Organization of the United Nations (FAO) mention that, in developing countries, there is a great deficiency in marketing infrastructure, therefore, postharvest losses of fresh products reach up to 50% of total production [1]. Losses of this magnitude trigger a considerable economic damage for food productive chain, especially, for primary producers. In addition, the presence of pests represents a serious health risk for the consumer. FAO, in collaboration with the Latin American Integration Association (ALADI) and the Economic Commission for Latin America and the Caribbean (ECLAC), prepared the Food Losses and Waste Plan (FLW) which promotes the development of innovative technologies that contribute to reduce food loss at all stages of the food production chain [2]. In recent years, different alternatives have been proposed in order to preserve the horticultural products, including the use of protective coatings. An edible coating is a thin layer of edible material formed as a coating on a food product. Using coatings modifies the interaction of the fruit with the environment due to their physicochemical properties, prolonging the shelf life of the treated fruits [3]. Different coating-forming compounds have been used, including chitosan, alginate, starch, and pullulan [4]–[7].

Pullulan is a polysaccharide produced by *Aureobasidium pullulans*; it can form edible coatings with several advantages over other polysaccharides. Concerning its properties, it has limited permeability to oxygen and carbon dioxide, has good adhesive properties, is colorless, and has no flavor [8]–[10]. The pullulan coating can influence on the physiology of fruit since it acts as a barrier between the environment and fruit. The protective effect of this coating can also be improved

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Protective effects of phenolic acids on mercury-induced DNA damage in precision-cut kidney slices

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ABSTRACT

Objective(s): Precision-cut tissue slices are considered an organotypic 3D model widely used in biomedical research. The comet assay is an important screening test for early genotoxicity risk assessment that is mainly applied on in vitro models. The aim of the present study was to provide a 3D organ system for determination of genotoxicity using a modified method of the comet assay since the stromal components from the original tissue make this technique complicated.

Materials and Methods: A modified comet assay technique was validated using precision-cut hamster kidney slices to analyze the antigenotoxic effect of the phenolic compounds caffeic acid, chlorogenic acid, and rosmarinic acid in tissue slices incubated with 15 µM $HgCl_2$. Cytotoxicity of the phenolic compounds was studied in Vero cells, and by morphologic analysis in tissue slices co-incubated with $HgCl_2$ and phenolic compounds.

Results: A modification of the comet assay allows obtaining better and clear comet profiles for analysis. Non-cytotoxic concentrations of phenolic acids protected kidney tissue slices against mercury-induced DNA damage, and at the same time, were not nephrotoxic. The highest protection was provided by 3 µg/ml caffeic acid, although 6 µg/ml rosmarinic and 9 µg/ml chlorogenic acids also exhibited protective effects.

Conclusion: This is the first time that a modification of the comet assay technique is reported as a tool to visualize the comets from kidney tissue slices in a clear and simple way. The phenolic compounds tested in this study provided protection against mercury-induced genotoxic damage in precision-cut kidney slices.

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Introduction

Precision-cut tissue slices represent a three-dimensional (3D) biological model closely resembling the organ from which they are prepared, where all cell types and tissue architecture are preserved, as well as metabolic activity and transport mechanisms (1-3). These characteristics are very treasured in the investigation of different biological activities. Several studies show that precision-cut kidney slices (PCKS) are a useful model for studying drug metabolism, nephrotoxicity, cryopreservation, and fibrosis (4-8) among other applications.

The comet assay is a quantitative technique through which visual evidence of DNA damage in individual eukaryotic cells can be measured. This method possesses a number of advantages when compared to other genotoxicity tests; one of them being that it´s readily modifiable for adaptation to a variety of experimental requirements. Over time, many improvements and variations of the method have been developed for a number of experimental purposes (9, 10); however, new applications for this method require standardization. A small number of DNA damage studies using the comet assay in cells from precision-cut tissue slices have been reported as tools for genotoxic compounds testing (1, 11-14). In these studies, the comet assay was used to study genotoxicity induced by several xenobiotics or nanomaterials in liver and lung slices (1, 12-14); and, to evaluate the antigenotoxic effects of flavonoids (11). Depending on the kind of tissue used, different modifications were made. For example, human or murine tissue slices were prepared for the assay by mincing the tissue (14), enzymatic digestion was applied to murine lung slices (12), and human and rat liver slices were directly gently squashed with a coverslip (1, 11, 13). These reports demonstrated the usefulness of 3D tissue culture models for genotoxicity studies given that the main advantage of the said models is that the results obtained are comparable to *in vivo* conditions.

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Research Article

Hypoglycemic Activity of *Tilia americana*, *Borago officinalis*, *Chenopodium nuttalliae*, and *Piper sanctum* on Wistar Rats

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Diabetes mellitus (DM) is considered the epidemic of the 21st century. Traditional medicine uses plants to treat DM; many of these have hypoglycemic effects in both animal models and diabetic patients. Our objective was to evaluate the hypoglycemic activity of *Tilia americana*, *Borago officinalis*, *Chenopodium nuttalliae*, and *Piper sanctum* on diabetic rats. The methanolic extracts of the plants under study were obtained by Soxhlet extraction. Toxicity was evaluated on *Artemia salina*; the antioxidant potential was evaluated using the DPPH technique. Hypoglycemic capacity at doses of 250 and 500 mg/kg was tested on Wistar rats with diabetes induced by alloxan (120 mg/kg). The toxicity on *A. salina* was null for the extracts of *B. officinalis* and *P. sanctum*, moderate for *T. americana*, and highly toxic for *C. nuttalliae*. The relevant extract of *T. americana* var. mexicana showed antioxidant activity. Three plants of the studied plants showed hypoglycemic activity: *Tilia Americana* ($p = 0.0142$), *Borago officinalis* ($p = 0.0112$), and *Piper sanctum* ($p = 0.0078$); *P. sanctum* was the one that showed the greatest reduction in glucose levels at a lower dose.

1. Introduction

Diabetes mellitus (DM) is a metabolic disease characterized by an elevated level of blood glucose that is associated in the long term with the dysfunction of different organs. Left untreated, it can cause blindness, renal insufficiency, myocardial infarction, cerebrovascular accidents, or amputation of the lower limbs [1].

DM is considered the epidemic of the 21st century, and its prevalence has increased in low- and middle-income countries. It is estimated that DM caused 1.6 million deaths in 2015 (WHO 2017) and is projected to be the seventh cause of mortality by 2030 [2, 3].

Most diabetic patients are included into two groups, type I (total deficiency of insulin secretion) and type II (insulin resistance). Treatment of type I diabetes is based on diet and the administration of insulin. Treatment of type II diabetes starts with diet and physical exercise; if the response is not adequate, then hypoglycemic drugs are administered orally (sulfonylureas and metformin).

Many plants used in traditional medicine (*Momordica charantia*, *Terminalia paniculata* Bar, *Scrophularia ningpoensis*, and *Anemarrhena asphodeloides*, among others) are used to treat patients with DM in various parts of the world, mainly in developing countries. The hypoglycemic effect of a large number of these plants or their preparations has been confirmed in animal models [4, 5], as well as in diabetic patients [6–8]. Among the active compounds with hypoglycemic effect that have been isolated are alkaloids, terpenoids [9], flavonoids [10], and saponins [11].

marine drugs

MDPI

Article

Anti-*Acanthamoeba* Activity of Brominated Sesquiterpenes from *Laurencia johnstonii*

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Abstract: Focused on our interest to develop novel antiparasistic agents, the present study was aimed to evaluate the biological activity of an extract of *Laurencia johnstonii* collected in Baja California Sur, Mexico, against an *Acantamoeba castellanii* Neff strain. Bioassay-guided fractionation allowed us to identify the amoebicidal diastereoisomers α-bromocuparane (**4**) and α-isobromocuparane (**5**). Furthermore, bromination of the inactive laurinterol (**1**) and isolaurinterol (**2**) yielded four halogenated derivatives, (**6**)–(**9**), which improved the activity of the natural sesquiterpenes. Among them, the most active compound was 3α-bromojohnstane (**7**), a sesquiterpene derivative which possesses a novel carbon skeleton johnstane.

Keywords: brominated sesquiterpene; marine natural products; *Laurencia johnstonii*; johnstane; 3-bromojohnstane; anti-amoeboid activity; *Acanthamoeba*

1. Introduction

Free-living amoeba (FLA) are widely distributed protozoa in the environment [1–3]. These parasites present a life cycle with two different stages: the trophozoite and the resistant phase, the cyst. Among FLA, *Acanthamoeba* genus [4] has been isolated from air, soil, water, contact lenses, air conditioning units, clinical samples, among others [5]. These parasites are able to cause pathologies in humans such as Granulomatous Amoebic Encephalitis (GAE) and Amoebic Keratitis (AK) [1–3,5]. Regarding *Acanthamoeba* infections, an early diagnosis is crucial to achieve a successful treatment [3,6]. Antimicrobial chemotherapy is the most widely used method for treating *Acanthamoeba*-caused infections. Pentamidine, azoles, sulfonamides, and possibly flucytosine, are among the most frequently used drugs in successfully treated cases of GAE, whereas topical chlorhexidine or polyhexamethylene biguanide appear to be the most effective option in cases of AK [7]. However, the existing therapies are not fully effective against these organisms mainly due to the existence of the cyst phase, and also due to the presence of strains that are resistant to the currently used anti-amoebic drugs [2,3,8].

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ORIGINAL ARTICLE

Headspace–Solid-Phase Microextraction Gas Chromatography Method to Quantify *Thymus vulgaris* Essential Oil in Polymeric Nanoparticles

Lorena Lugo-Estrada[1,2], Sergio Arturo Galindo-Rodríguez[2], Luis Alejandro Pérez-López[1], Noemí Waksman de Torres[1], Rocío Álvarez-Román[1]

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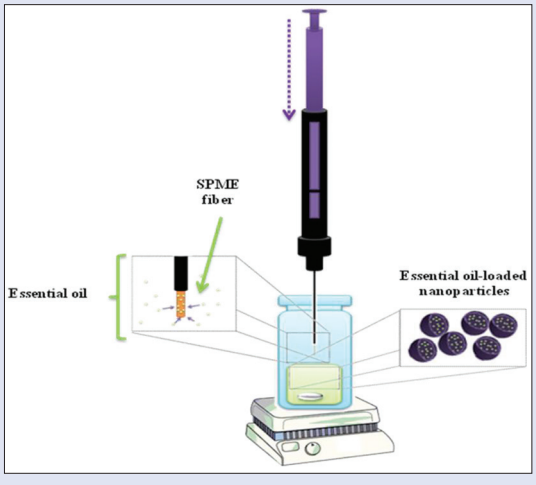
ABSTRACT

Background: *Thymus vulgaris* essential oil (*Tv*-EO) is known to have antibacterial, antifungal, and antioxidant activities. Encapsulation of *Tv*-EO in polymeric nanoparticles (NPs) can prevent volatilization of its components and can provide protection against external agents. Under these circumstances, it is crucial to assure the presence and quantity of the *Tv*-EO components (γ-terpinene, thymol, and carvacrol) in the NPs. **Objective:** To determine the chemical composition and physicochemical characterization of *Tv-EO* as well as develop and validate a HSPM-gas chromatography (GC) method for the analysis of *Tv*-EO components encapsulated in NPs. **Materials and Methods:** *Tv*-EO was characterized by physicochemical analysis for relative density, refractive index, and optical rotation and analyzed by GC flame ionization detector and GC-mass spectrometry. The headspace–solid-phase microextraction-gas chromatography (HS-SPME-CG) validation was assessed, *Tv-EO*-NPs were prepared by nanoprecipitation, and its properties were determined by photon correlation spectroscopy. **Results:** *Tv*-EO was characterized by physicochemical analysis for relative density (0.934 g/cm^3), refraction index (1.559), and optical rotation (−0.084°). Seventeen components were identified in *Tv-EO*; among these, the sesquiterpenes, thymol (34.28%), o-cymene (31.78%) and γ-terpinene (13.22%). The method was validated for linearity ($R^2 \geq 0.99$), precision (intraday 7.02, 10.33, and 8.60 and inter-day 10.60, 10.60, and 10.99), accuracy (99.35, 109.4, and 98.84%) and robustness for γ-terpinene, thymol and carvacrol, respectively. The limit of detection and limit of quantification were calculated as 0.69, 0.40, and 0.39 μg/mL and 2.11, 1.22, and 1.20 μg/mL for γ-terpinene, thymol, and carvacrol, respectively. An encapsulation percentage of 47.51% of total essential oil was obtained. **Conclusion:** The experimental data show that HS-SPME reduces interference of the NP-matrix and concentrates the *Tv*-EO components. HS-SPME-CG can be considered as a good alternative to the already existing methods for analysis of essential oil encapsulated in NPs.

Key words: Essential oil, headspace analysis, polymeric nanoparticles, solid-phase microextraction, *Thymus vulgaris*

SUMMARY

- The essential oil from leaves of *Thymus vulgaris* was extracted by hydrodistillation and characterized
- The headspace–solid-phase microextraction-gas chromatography (HS-SPME-CG) method was validated for linearity; intraday and interday precision; accuracy; robustness for γ-terpinene, thymol, and carvacrol; and the limits of detection and limits of quantification were calculated
- HS-SPME-CG can be considered as a good alternative to the already existing methods for the analysis of essential oil encapsulated in nanoparticles.



Abbreviations used: *Tv*-EO*: Thymus vulgaris* essential oil; NPs: Nanoparticles; HS-SPME: Headspace–solid-phase microextraction; GC: Gas chromatography; PI: Polydispersity index; ZP: Zeta potential; LOD: Limit of detection; LOQ: Limit of quantification; HPLC: High-performance liquid chromatography; *Tv*-EO-NP*: Thymus vulgaris* essential oil-loaded nanoparticles; NIST: National Institute of Standards and Technology; %E: encapsulation percentage; %EE: Encapsulation efficiency percentage.

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INTRODUCTION

Essential oils are secondary metabolites in plants that form part of the plant defense system against predators and infections. Such oils are composed of mixtures of volatile components such as monoterpenes, sesquiterpenes, and phenylpropanes. *Thymus vulgaris* essential oil *(Tv*-EO*)* is known to have antibacterial, antifungal, and antioxidant activities.[1-3] However, although this essential oil has good activities,

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Research Article

Simultaneous GC-FID Quantification of Main Components of *Rosmarinus officinalis* L. and *Lavandula dentata* Essential Oils in Polymeric Nanocapsules for Antioxidant Application

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The essential oils (EO) of *R. officinalis* and *L. dentata* have been widely used due to their antioxidant activity. However, due to their high volatility, the loading of EO into polymeric nanocapsules (NC) represents an efficient way of retaining their effect in future topical administration. In this way, the quantitative determination of EO incorporated into NC is necessary for simultaneous monitoring of the main components of the EO during the nanoencapsulation process as well as for precise and exact dosing of the components used during the performance of *in vitro* and *in vivo* biological tests. In this study, EO were isolated by hydrodistillation in a Clevenger-type apparatus and characterized by GC-MS and GC-FID analyses. The major constituents of EO-*R. officinalis* were camphor (39.46%) and 1,8-cineole (14.63%), and for EO-*L. dentata* were 1,8-cineole (68.59%) and β-pinene (11.53%). A new analytical method based on GC-FID for quantification of free and encapsulated EO was developed and validated according to ICH. Linearity, limit of detection and quantification, and intra- and interday precision parameters were determined. The methods were linear and precise for the quantification of the main components of EO. The EO were encapsulated by nanoprecipitation and were analyzed by the GC-FID method validated for their direct quantification. The NC size was 200 nm with homogeneous size distribution. The quantification of the incorporated EO within a NC is an important step in NC characterization. In this way, an encapsulation efficiency of at least 59.03% and 41.15% of total EO-*R. officinalis* and EO-*L. dentata*, respectively, was obtained. Simple, repeatable, and reproducible methods were developed as an analytical tool for the simultaneous quantification of the main components of EO loaded in polymeric nanocapsules as well as their monitoring in biological assays.

1. Introduction

Essential oils (EO) have been used for years in pharmaceuticals, cosmetics, and food products due to their health benefits [1], as antimicrobials [2], antidiabetics [3], analgesics [4], antioxidants [5], anti-inflammatories [6], and sedatives and anxiolytics [7]. EO are complex mixtures of volatile compounds that contain about 20 to 60 components at different concentrations. In general, it has been found that the main components could determine the biological properties of EO in living beings [1, 8]. In this sense, it has been reported that the EO of *Rosmarinus officinalis* (EO-*R. officinalis*) has antioxidant [9, 10], antimicrobial [11], anti-inflammatory, and antinociceptive [12, 13] properties, mainly due to the

marine drugs

MDPI

Article

Anti-*Acanthamoeba* Activity of Brominated Sesquiterpenes from *Laurencia johnstonii*

Sara García-Davis [1,2], Ines Sifaoui [3], María Reyes-Batlle [3], Ezequiel Viveros-Valdez [2], José E. Piñero [3], Jacob Lorenzo-Morales [3], José J. Fernández [1,4,*] and Ana R. Díaz-Marrero [1,*]

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Abstract: Focused on our interest to develop novel antiparasistic agents, the present study was aimed to evaluate the biological activity of an extract of *Laurencia johnstonii* collected in Baja California Sur, Mexico, against an *Acantamoeba castellanii* Neff strain. Bioassay-guided fractionation allowed us to identify the amoebicidal diastereoisomers α-bromocuparane (**4**) and α-isobromocuparane (**5**). Furthermore, bromination of the inactive laurinterol (**1**) and isolaurinterol (**2**) yielded four halogenated derivatives, (**6**)–(**9**), which improved the activity of the natural sesquiterpenes. Among them, the most active compound was 3α-bromojohnstane (**7**), a sesquiterpene derivative which possesses a novel carbon skeleton johnstane.

Keywords: brominated sesquiterpene; marine natural products; *Laurencia johnstonii*; johnstane; 3-bromojohnstane; anti-amoeboid activity; *Acanthamoeba*

1. Introduction

Free-living amoeba (FLA) are widely distributed protozoa in the environment [1–3]. These parasites present a life cycle with two different stages: the trophozoite and the resistant phase, the cyst. Among FLA, *Acanthamoeba* genus [4] has been isolated from air, soil, water, contact lenses, air conditioning units, clinical samples, among others [5]. These parasites are able to cause pathologies in humans such as Granulomatous Amoebic Encephalitis (GAE) and Amoebic Keratitis (AK) [1–3,5]. Regarding *Acanthamoeba* infections, an early diagnosis is crucial to achieve a successful treatment [3,6]. Antimicrobial chemotherapy is the most widely used method for treating *Acanthamoeba*-caused infections. Pentamidine, azoles, sulfonamides, and possibly flucytosine, are among the most frequently used drugs in successfully treated cases of GAE, whereas topical chlorhexidine or polyhexamethylene biguanide appear to be the most effective option in cases of AK [7]. However, the existing therapies are not fully effective against these organisms mainly due to the existence of the cyst phase, and also due to the presence of strains that are resistant to the currently used anti-amoebic drugs [2,3,8].

Breast Organotypic Cancer Models

Pilar Carranza-Rosales, Nancy Elena Guzmán-Delgado, Irma Edith Carranza-Torres, Ezequiel Viveros-Valdez and Javier Morán-Martínez

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Research Article
Bactericide, Antioxidant and Cytotoxic Activities from Marine Algae of Genus *Laurencia* Collected in Baja California Sur, México

[1]Sara García-Davis, [2]Iván Murillo-Álvarez, [2]Mauricio Muñoz-Ochoa, [1]Edith Carranza-Torres, [1]Ruth Garza-Padrón, [1]Eufemia Morales-Rubio and [1]Ezequiel Viveros-Valdez

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Abstract

Background and Objective: Marine environment represents countless and diverse resource for new drugs to combat major diseases. Extracts from four *Laurencia* species (*L. johnstonii, L. pacifica, L. gardneri* and *L. papillosa)* from Baja California Sur, México were evaluated for their antioxidant, antibacterial and cytotoxic activity. **Methodology:** The antioxidants activity of *Laurencia* sp. were evaluated using the radical scavenging activity in three *in vitro* radicals: 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2'-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and nitric oxide (NO). The antibacterial activity was evaluated by the broth microdilution method to determinate the Minimum Inhibitory Concentrations (MIC) against *Staphylococcus aureus, Bacillus subtilis, Enterococcus faecalis, Micrococcus luteus, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The cytotoxicity was analyzed on HeLa (cervix adenocarcinoma) and Vero (kidney epithelial) cells, using the reduction of tetrazolium salt WST-1. **Results:** The seaweed of genus *Laurencia* demonstrated an overall low activity, with half maximal effective concentration (EC_{50}) values >1.5 mg mL^{-1}. *Laurencia pacifica* showed the best biocide effects with MIC of 6.25 μg mL^{-1} against Gram positive bacterial and cytotoxic potential with half inhibitory concentration (IC_{50}) <30 μg mL^{-1} against Vero and HeLa cells. **Conclusion:** Some *Laurencia* species have a great antibacterial and cytotoxic activity which could be considered for future studies.

Key words: *Laurencia*, seaweeds, algal extract, marine antibacterial, antioxidant activity, cytotoxic activity, bioactive metabolites, bioprospection

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

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Research Article
Bactericidal Activity, Isolation and Identification of Most Active Compound from 20 Plants used in Traditional Mexican Medicine Against Multidrug-Resistant Bacteria

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Abstract

Background and Objective: Plants are used in Mexico as traditional medicine for the treatment of diverse illnesses such as stomach pain, fever, diarrhea, insomnia, flu and other respiratory diseases. Twenty were selected to determine their bactericidal activity. The aim of this study was the isolation of molecules from plants used in Mexican traditional medicine. **Materials and Methods:** Using chromatographic procedures, the responsible bactericidal molecules from rosemary was extracted and then identified by spectroscopic analysis IR, ^{1}H NMR, ^{13}C NMR, DEPT, HSQC and GC-MS. Measures of central tendency were determined by statistical analysis. **Results:** Ten of these plants showed bactericidal activity against multidrug-resistant bacteria. This biological activity was reported for *Carya illinoensis* against *Pseudomonas aeruginosa*, also for *Equisetum robustum*, *Stevia rebaudiana* and *Castela texana* against Methicillin resistant *Staphylococcus aureus* (MRSA). The methanolic extract of *Rosmarinus officinalis* (rosemary) showed important bactericidal activity against MRSA (ATCC BAA-44) and clinically isolated MRSA. **Conclusion:** Rosemary's bactericidal molecules were isolated and then identified as a mixture of betulinic, oleanolic and ursolic acid (MIC = 725 μg mL^{-1}).

Key words: *Rosmarinus officinalis*, multidrug-resistance, MRSA, thin layer chromatography, flash chromatography, ^{1}H NMR, ^{13}C NMR, triterpenic acids

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

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Current Proteomics, 2019, *16*, 000-000

RESEARCH ARTICLE

Proteomic Analysis of a Bioactive *Aloe vera* Extract

Ethel Daniela Cabello-Ruiz[1], Víctor Manuel Torres-de la Cruz[2,3,*], Catalina Rivas-Morales[1], Gloria María Molina-Salinas[4], María Adriana Núñez-González[1], María Julia Verde-Star[1] and Catalina Leos-Rivas[1]

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ARTICLE HISTORY

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Abstract: ***Background***: *Aloe vera*, a plant belonging to the family Xanthorrhoeaceae, has received special interest in recent years, not only for the commercial importance of its derivatives, but also because of the identification of new molecules from this plant. The latter may provide a scientific support for ethnobotany, which has been beneficial to mankind for centuries.

Objective: Recently, the pharmacological activity of proteins derived from natural sources, including plants, is being explored. We report on the extraction and identification of proteins from *A. vera* with antimicrobial activity.

Result: The protein extract (yield, 0.15%) contained 15 peptides or proteins, whose sequences were associated with membrane proteins, enzymes, and proteins involved in stress tolerance and defense against pathogens. The latter is consistent with the previously reported antimicrobial activity of an *A. vera* protein extract.

Keywords: *Aloe vera*, extraction, protein, antimicrobial.

1. INTRODUCTION

Pre-hispanic knowledge on the healing power of plants has been transmitted from generation to generation and forms the basis of traditional medicine, which is still widely practiced in Mexico. Whereas the history of traditional medicine dates back thousands of years, modern medicine has a relatively short existence of about a hundred years. However, the combination of traditional with modern medicine may provide more effective treatments than either one on itself [1]. Indeed, the search for active substances in plants used in traditional medicine has led to the discovery of new molecules. Some of these molecules have been included in the collection of modern pharmacological formulations and their working mechanisms have been elucidated [2].

Use of plants for nutritional and medicinal purposes is common among members of the large family of Liliaceae *sensu lato*, and *A. vera* is among its most well-known and well-studied species. In traditional Hindu medicine, *A. vera* is used in the treatment against gastrointestinal and dermatological problems. Furthermore, it provides a rapid relief in case of minor burns and wounds because of its anti-inflammatory and skin regenerating effects [3]. In Mexico, *A. vera* is also used to treat cough, diabetes, erysipelas, stomachache, skin inflammation and infections, and as an anti-helminthic [4]. These applications reflect the numerous pharmacological activities attributed to this plant, such as: anti-inflammatory, immunomodulatory, skin regenerative, antitumor, antimicrobial, antihelminthic, antidiabetic, antiulcer, and hepatoprotective, amongst others [5]. *A. vera* has also been considered a "natural" ingredient in the modern Food and Drugs Industry. Considering the wide variety of effects of *A. vera*, there has been an effort to identify the responsible bioactive substances. As a result, various secondary metabolites (flavonoids, terpenoids, cholesterol derivatives, anthraquinones, and saccharides) have been described [6]. A more recent tendency is the quest for plant-derived proteins, and *A. vera* has not been exempted. The contrary, three interesting lectins have been found: a 35-kDa

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Short Communication
Biological Activities from the Marine Sponge *Suberites aurantiacus*

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Abstract

Background and Objective: Invertebrates comprises more than fifty percent of marine animals. Among them, sponges are the most prolific source of bioactive compounds. *Suberites aurantiacus* (*S. aurantiacus*) is an abundant sponge in Mexican Pacific, which has been scarcely studied, for this reason authors screened it for its bioactivities. The current study proposed to determine the enzymatic inhibition and scavenger, toxic and antibacterial activities from *Suberites aurantiacus*. **Materials and Methods:** *S. aurantiacus* samples were collected in Magdalena Bay, Mexico. The ethanolic extract and its fractions were assayed for their antioxidant effect using DPPH, ABTS and NO assays, evaluated their toxicity against *Artemia salina* (*A. salina*) and their antibacterial activity against *Staphylococcus aureus* (*S. aureus*), *Bacillus subtilis* (*B. subtilis*), *Enterococcus faecalis* (*E. faecalis*), *Enterobacter aerogenes* (*E. aerogenes*), *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*) as well as their inhibitory effect on α-glucosidase and α-amylase. **Results:** The results showed median lethal doses (LD_{50}) against *A. salina* < 1 mg mL^{-1} for two fractions and a moderate antibacterial activity against Gram positive bacteria. **Conclusion:** In view of these results, *S. aurantiacus* could be considered a potential source of antibacterial compounds.

Key words: Sponge, bactericide, toxicity, antioxidant, enzyme inhibition

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

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ANTIUROLITHIC ACTIVITY OF *BERBERIS TRIFOLIATA* EXTRACT ON INDUCED UROLITHIASIS IN RATS BY ZINC DISC IMPLANTATION

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Abstract

Background: In clinical therapy, there is no satisfactory drug available for treatment of urolithiasis, especially for the prevention of their recurrence. The aim of this work was to evaluate *in vivo* antiurolithic activity of methanolic extract of *Berberis trifoliata* leaves.
Material and methods: Urolithiasis was induced in Wistar rats by zinc disc implantation in urinary bladder**.** Upon postsurgical recovery, different doses of the methanolic extract of *B. trifoliata* leaves (50, 100 and 150 mg/kg body weight) were administered orally to zinc disc implanted rats for a period of 20 days. Antiurolithiatic activity was evaluated by measuring the difference between the weight of the implanted zinc discs at the time of implantation and the final weight of the dried calculi taken out from the bladder at the end of the 20 days period of treatment.
Results: Extract of *B. trifoliata* significantly reduced calculi deposition around the implanted zinc disc at all doses (50, 100, and 150 mg/kg).
Conclusion: Treatment with methanolic extract of *B. trifoliata* is useful agent against the kidney stone formation.

Keywords: Antiurolithic, *Berberis trifoliata*, urolithiasis, zinc disc implantation.

Introduction

Urolithiasis is a condition in which urinary calculi are formed and located at any level of the urinary system (Tiwari et al., 2012; Rajeshwari et al., 2013). Formation of stones is the third most common problem of the human urinary system. (Bashir and Gilani, 2011; Khan et al., 2011). It is a worldwide problem; it is estimated that 12% of the world population experiences renal stone disease with a high recurrence rate (Khan et al., 2011; Narendra and Ameeta, 2013). Urinary stone formation is the result from several physicochemical events including nucleation, supersaturation and crystal growth. Calcium oxalates are the primary constituent of the majority of urinary tract stones (Bangash et al., 2011). Minimally invasive surgery including extracorporeal shock wave lithotripsy (ESWL), percutaneous nephrolithotomy (PCNL) or ureteroscopy (URS) are considered effective removal techniques, but they are costly, making them an limited option and data suggest that these techniques have some side effects (Khan et al., 2012). Complications include residual stone fragments as potential nidus for new stone formation, compromised renal function, acute renal injury and urinary tract infection. Despite of advancements in the pathophysiology and treatment modalities of urolithiasis, there is no satisfactory drug and medical treatment available, especially for the prevention of stones formation recurrence (Padma et al., 2016).

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Review

Nanoprecipitation process: From encapsulation to drug delivery

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ABSTRACT

Drugs encapsulation is a suitable strategy in order to cope with the limitations of conventional dosage forms such as unsuitable bioavailability, stability, taste, and odor. Nanoprecipitation technique has been used in the pharmaceutical and agricultural research as clean alternative for other drug carrier formulations. This technique is based on precipitation mechanism. Polymer precipitation occurs after the addition of a non-solvent to a polymer solution in four steps mechanism: supersaturation, nucleation, growth by condensation, and growth by coagulation that leads to the formation of polymer nanoparticles or aggregates. The scale-up of laboratory-based nanoprecipitation method shows a good reproducibility. In addition, flash nanoprecipitation is a good strategy for industrial scale production of nanoparticles. Nanoprecipitation is usually used for encapsulation of hydrophobic or hydrophilic compounds. Nanoprecipitation was also shown to be a good alternative for the encapsulation of natural compounds. As a whole, process and formulation related parameters in nanoprecipitation technique have critical effect on nanoparticles characteristics. Biodegradable or non-biodegradable polymers have been used for the preparation of nanoparticles intended to *in vivo* studies. Literature studies have demonstrated the biodistribution of the active loaded nanoparticles in different organs after administration *via* various routes. In general, *in vitro* drug release from nanoparticles prepared by nanoprecipitation includes two phases: a first phase of "burst release" which is followed by a second phase of prolonged release. Moreover, many encapsulated active molecules have been commercialized in the pharmaceutical market.

Contents

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In vitro and *in vivo* Methods for the Evaluation of Natural Products against Dermatophytes

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Dermatomycoses are infections caused by fungi called dermatophytes; these affect 20-25% of the world population and the incidence continues to grow each year. Recently, an alternative for the treatment of these diseases is the use of natural products, thanks to the fact that they possess great chemical diversity and thus biological activity. However, to understand the therapeutic potential of natural products, their microbiological assessment presents certain limitations. Currently, there is no established reference method to determine the antifungal capacity *in vitro* and *in vivo* of natural products (i.e., essential oils). This review focuses on describing the various microbiological methods as well as the many adaptations used to evaluate the antifungal activity of natural products both *in vitro* and *in vivo*. In addition, the antifungal evaluation of natural products formulated in creams, gels, nanoemulsions, nanocapsules and solid lipid nanoparticles is included.

Keywords: Natural products, Dermatophytes, Susceptibility methods, Formulations.

Introduction

Dermatomycoses or superficial mycoses are infections in which a fungus invades the outer layers of the skin, hair and nails [1]. Specifically, dermatomycoses are caused by fungi called dermatophytes, which have the ability to invade keratinized tissues producing lesions popularly known as "ringworm". It is estimated that this type of disease affects 20-25% of the world population [2-3]. Conventional treatment of superficial mycoses is specifically the use of azoles. In particular, this group of antifungal drugs has a broad spectrum of action; however, the most important limitation is its poor penetration of affected tissues, in addition to the possible emergence of resistance. Recently, an alternative used in the treatment of dermatomycosis is natural products (i.e., essential oils). This is because they have great chemical diversity, and therefore, biological activity, and they are even used directly as therapeutic agents [4].There are numerous reports that indicate the antifungal activity of essential oils against a variety of dermatophytes [5]. However, to be used as antimicrobials they still have certain limitations among which are: i) the high rate of degradation and chemical reactivity of the present compounds, ii) their low solubility in water which limits their biological application, and iii) the short time of bioactivity due to their volatile nature [6]. Therefore, it is necessary to use new alternative technologies that protect the essential oil and facilitate their proper administration without losing their antifungal properties.

At the same time, to define the therapeutic potential of natural products, their microbiological assessment also has certain limitations. Currently, there is no generally established procedure to evaluate the antifungal activity of natural products against strains of dermatophyte fungi. Despite various reports, modifications and adaptations for determining the antifungal activity of natural products, it is difficult to compare the techniques or results obtained in each because of the great methodological variability applied to evaluate this type of active molecules.

In vitro susceptibility methods

Susceptibility tests are performed in order to compare the activity of the active compounds against different strains of microorganisms and detect possible resistance to them.

Microdilution technique, M38-A2 protocol of the Institute of Clinical and Laboratory Standards: For the *in vitro* evaluation of antifungal drugs there is a method approved by the Institute of Clinical and Laboratory Standards (NCCLS), known as microdilution M38-A2 for molds and filamentous fungi [7]. To perform this method it is essential to respect the recommendations established by the CLSI, such as inoculum size 0.5 x 10^3-2.5 x 10^3 CFU/mL, the culture medium that should be used (RPMI 1640), the incubation temperature and time (35°C for 96 h) and finally, the definition and assessment criteria for determining antifungal activity (ie, filamentous fungi can only be classified as sensitive or with reduced sensitivity to the antifungal agent) [8]. There are several reports that use these methodologies as a reference to evaluate the susceptibility of antifungal drugs and also naturally occurring compounds such as essential oils and fractions from plant extracts.

Oliveira *et al.*, in 2008, employed the microdilution technique, CLSI M38-A2, to determine the antifungal activity of oils that had exuded directly from trunks of trees of different species of *Copaifera*. In this study, strains of *Thrichophyton rubrum* ATCC 28189 *T. metagrophytes* ATCC 4481, *Microsporum canis* ATCC 32903 and *M. gypseum* ATCC 14683, and the culture medium

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Research Article
Bactericide, Antioxidant and Cytotoxic Activities from Marine Algae of Genus *Laurencia* Collected in Baja California Sur, México

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Abstract

Background and Objective: Marine environment represents countless and diverse resource for new drugs to combat major diseases. Extracts from four *Laurencia* species (*L. johnstonii, L. pacifica, L. gardneri* and *L. papillosa)* from Baja California Sur, México were evaluated for their antioxidant, antibacterial and cytotoxic activity. **Methodology:** The antioxidants activity of *Laurencia* sp. were evaluated using the radical scavenging activity in three *in vitro* radicals: 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2'-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and nitric oxide (NO). The antibacterial activity was evaluated by the broth microdilution method to determinate the Minimum Inhibitory Concentrations (MIC) against *Staphylococcus aureus, Bacillus subtilis, Enterococcus faecalis, Micrococcus luteus, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The cytotoxicity was analyzed on HeLa (cervix adenocarcinoma) and Vero (kidney epithelial) cells, using the reduction of tetrazolium salt WST-1. **Results:** The seaweed of genus *Laurencia* demonstrated an overall low activity, with half maximal effective concentration (EC_{50}) values >1.5 mg mL^{-1}. *Laurencia pacifica* showed the best biocide effects with MIC of 6.25 μg mL^{-1} against Gram positive bacterial and cytotoxic potential with half inhibitory concentration (IC_{50}) <30 μg mL^{-1} against Vero and HeLa cells. **Conclusion:** Some *Laurencia* species have a great antibacterial and cytotoxic activity which could be considered for future studies.

Key words: *Laurencia*, seaweeds, algal extract, marine antibacterial, antioxidant activity, cytotoxic activity, bioactive metabolites, bioprospection

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Antiprotozoal Activity of a *Thymus vulgaris* Methanol Extract and Its Fractions

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Abstract

Introduction: *Thymus vulgaris* is used in traditional medicine to treat gastrointestinal diseases because of its antifungal, antibacterial, and antispasmodic activity. **Objective:** To verify whether *Thymus vulgaris* also has antiprotozoal activity against *Trichomonas vaginalis*, *Giardia lamblia* and *Entamoeba histolytica* trophozoites. **Materials and methods:** Conventional cultures of parasites were measured on the third day during the logarithmic growth phase. The antiprotozoal activity of the methanol extract and its fractions were evaluated comparing growth in cultures with and without extracts. Next, the extract was fractionated by polarity-based partitioning. Then, the purity of each fraction was determined by thin layer chromatography (TLC). The percentage of growth inhibition was calculated with respect to untreated controls. The 50% inhibitory concentration (IC_{50}) of each extract was calculated by PROBIT analysis. **Results:** We found that a methanol extract of the aerial parts of *Thymus vulgaris*, at 300 μg/mL, inhibited the *in vitro* growth of *G. lamblia* and *T. vaginalis*, while *E. histolytica* growth was poorly inhibited. The methanol extract was further separated into mixtures of ursolic, oleanolic, and betulinic acids. The IC_{50} values of ursolic acid against *G. lamblia* and *T. vaginalis* were 8.12 μg/mL and 5.51 μg/mL, respectively. **Conclusions:** The methanol extract fraction containing ursolic acid obtained from *Thymus vulgaris* has antiprotozoal activity against *G. lamblia* and *T. vaginalis* trophozoites.

Evaluation of hypocholesterolemic activity of extracts of *Bidens odorata* and *Brickellia eupatorioides*

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Abstract: We sought to evaluate the hypolipidemic activity of extracts of *Bidens odorata* and *Brickellia eupatorioides* using a model of hyperlipidemia induced in rats by Triton WR-1339 (300mg/kg intraperitoneally). The rats were divided into 5 groups of 3 rats each: normal control group, hyperlipidemic control group, hyperlipidemic with 20 mg/kg atorvastatin, hyperlipidemic with 300 mg/kg *B. odorata* extract, and hyperlipidemic with 300mg/kg *B. eupatorioides* extract, respectively. After 10 d of treatment by intragastric administration, the extract of *B. odorata* caused a significant decrease of serum total cholesterol and triglyceride levels without altering the liver enzymes aspartate transaminase and alanine aminotransferase. In addition, the extract had antioxidant potential as shown by the 2,2′-diphenyl-1-picrylhydrazyl technique. These findings indicate that *B. odorata* has potential as a hypolipidemic agent and might be beneficial in treatment of hyperlipidemia and atherosclerosis.

Keywords: Cholesterol, hypercholesterolemia, plant extracts, triglycerides.

INTRODUCTION

Obesity has reached proportions of being considered a pandemic (Kopelman 2000). In 2014, 1.9 billion people worldwide were estimated as being overweight and 600 million obese (Chestnov *et al*., 2014).

Obesity is associated with the appearance of other diseases, including cardiovascular diseases. At present, these diseases, according to reports of the World Health Organization (WHO), are the main cause of mortality in the world.

Hyperlipidemia is the main cause of atherosclerosis, a disease that begins with the deposition of low density lipids (LDLs) on the wall of the arteries. These lipids are attacked by reactive oxygen species (ROS) causing the release of chemokines and inflammatory cells, finally resulting in the formation of an atherosclerotic plaque (Weber, 2011).

There are several drugs for the treatment of hyperlipidemia such as fibrates, niacin, bile acid sequestrants, and inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) called statins, the latter being the most commonly used (Tiwari and Khokhar, 2014).

Despite the existence of various alternatives for the treatment of high blood lipid levels, a large percentage of patients with this disease do not receive proper treatment for the control of the levels of lipids in the blood, thus increasing the risk of cardiovascular problems such myocardial infarction and stroke (WHO, 2011).

In the search for new hypolipidemic agents, various plants have been evaluated. In particular, some plants in the family Asteraceae have demonstrated effects on reducing serum lipids (Wider *et al*., 2002; Bahar, 2016; Hong *et al*., 2012). In addition, various plants of this family have an important antioxidant activity (Kenny *et al*., 2014; Teugwa *et al*., 2013; Dewan *et al*., 2013), and other biological activities such as hypoglycemic (Palacios *et al*., 2008; Abdullahi *et al*., 2015) and hepatoprotective effects (Achika *et al*., 2014; Syed *et al*., 2014).

In Northeast Mexico there is a great diversity of plants in the family Asteraceae (Villaseñor, 2004). These include *Bidens odorata* and *Brickellia eupatorioides.*

B. odorata, popularly known as aceitilla, is used in Mexican folk medicine to treat gastrointestinal and kidney disorders, and for the treatment of diabetes (Astudillo-Vázquez *et al*., 2015). An aqueous extract has been assessed for diuretic activity (Camargo *et al*., 2004), and a chloroformic extract for antidiarrheal activity, where various fatty acids (oleic, palmitic, linoleic, and stearic acids) in the active fraction of the extract were identified. Kaempferol, quercetin, and flavonoids quercetin and luteolin have been isolated from *B. eupatorioides*, popularly known as the false eupatorium (Wollenweber *et al*., 1996). However, there are no reports of any pharmacological activity. Other species of the same genus of this plant, *Brickellia cavanillesii* and *Brickellia veronicaefolia*, have been shown to possess hypoglycemic activity (Escandon *et al*., 2012; Pérez *et al*., 2000).

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Formaldehyde Induces DNA Strand Breaks on Spermatozoa and Lymphocytes of Wistar Rats[1]

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Abstract—Formaldehyde (FA) interacts with biological molecules such as DNA and it induces DNA-protein cross-links (DPCs), oxidative stress, reactive oxygen species (ROS), methylation, chromosomal damage, fragmentation, and adducts of DNA, which are considered the most important genotoxic effects caused by exposure to FA. The purpose of this study was to evaluate the percentage of DNA fragmentation on lymphocytes and spermatozoa from Wistar rats exposed to different doses of FA. The results about the percentage of fragmentation of DNA in lymphocytes and spermatozoa, were statistical different from controlled group versus treated groups respectively to ($p < 0.05$). Pathological changes were observed in the seminiferous tubules, especially in rats exposed to 30 mg/kg of FA. This study provided additional evidence supporting that FA induces DNA strand breaks in both cells and therefore genotoxic damage in Wistar rats.

Keywords: Formaldehyde, Fragmentation, Spermatozoa, Lymphocytes, Rats

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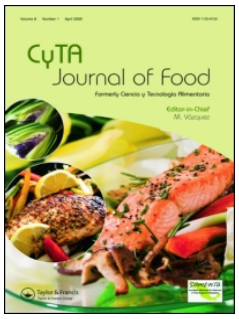
INTRODUCTION

Formaldehyde (FA) is a highly reactive chemical compound that may interact with macromolecules such as proteins and nucleic acids, or with low molecular weight molecules such as amino acids [1]. The main interactions attributed to the FA that are considered biologically significant are held in proteins and DNA [2]. In this manner, it induces damage to DNA, as well as the formation of DNA adducts, alteration of protein structure, and DNA-protein cross-links (DPCs) [3–5]. DPCs formation is considered the primary genotoxic effect induced by exposure to FA [2, 6].

It has been suggested two mechanisms in which FA induces DPCs. The first one is through the binding of FA in a nucleophilic site of a protein, followed by the crosslinking of the protein methylol adduct, and a nucleophilic site on DNA. The second one is the interaction of FA with a nucleophilic site in a DNA nucleotidic base, which is followed by the cross-linking between the DNA methylol adduct in DNA and a protein residue [2]. Additional mechanisms for the toxicity induced by FA include oxidative stress, ROS generation, and methylation of DNA, all of them lead to chromosomal damage, fragmentation, the formation of adducts, protein cross-linkings of the DNA, structural and functional alterations of enzymes, hormones, and proteins [7] as well. DNA fragmentation is an initial distinctive seal of apoptosis of any cell line, cells may die by programmed cellular death, or they can acquire chromosomal mutations, which are inherited in succeeding generations [8, 9]. In lymphocytes, the most important mechanism causing damage to DNA is the abnormal packaging of chromatin, while in sperm cells this is due to insufficient chromatin protamination triggered by ROS production [10].

Oxidative stress occurs when there is an excessive production of ROS by leucocytes [11]. Previous studies on humans occupationally exposed to FA showed

[1] The article is published in the original.



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Chemical composition, antimicrobial, and antioxidant activities of orange essential oil and its concentrated oils

C. Torres-Alvarez, A. Núñez González, J. Rodríguez, S. Castillo, C. Leos-Rivas & J. G. Báez-González

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REVIEW

Plant extracts: from encapsulation to application

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ABSTRACT

Introduction: Plants are a natural source of various products with diverse biological activities offering treatment for several diseases. Plant extract is a complex mixture of compounds, which can have antioxidant, antibiotic, antiviral, anticancer, antiparasitic, antifungal, hypoglycemic, anti-hypertensive and insecticide properties. The extraction of these extracts requires the use of organic solvents, which not only complicates the formulations but also makes it difficult to directly use the extracts for humans. To overcome these problems, recent research has been focused on developing new ways to formulate the plant extracts and delivering them safely with enhanced therapeutic efficacy.

Areas covered: This review focuses on the research done in the development and use of polymeric nanoparticles for the encapsulation and administration of plant extracts. It describes in detail, the different encapsulation techniques, main physicochemical characteristics of the nanoparticles, toxicity tests and results obtained from *in vivo* or *in vitro* assays.

Expert opinion: Major obstacles associated with the use of plant extracts for clinical applications include their complex composition, toxicity risks and extract instability. It is observed that encapsulation can be successfully used to decrease plant extracts toxicity, to provide targeted drug delivery and to solve stability related problems.

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Polymeric nanoparticles; plant extracts; biological activity; drug delivery; encapsulation techniques

1. Introduction

Since ancient times, it is known that plants are a natural source of various products with diverse biological activities. These products have been used for the treatment of different diseases.[1] Actually, about three quarters of the world's population rely on the use of particular plant extracts as a remedy for various afflictions.[2] Within the natural products, we can find essential oils, plant extracts, tea, salves, etc.

Natural extracts are complex mixtures of chemicals with biological properties derived mainly from the leaves, the stems, the fruits, or roots of medicinal plants. Among the biological activities presented by plant extracts, the most prominent ones include the antioxidant, antibiotic, anticancer, antifungal, antiparasitic, hypoglycemic, and antihypertensive properties.[3–9]

Even though, the plant extracts are suitable for treatment for various diseases, studies show that their therapeutic use is still limited because of their complex composition and toxicity when they are applied in organisms with more complex metabolic systems. Furthermore, for obtaining these extracts, generally organic solvents (e.g. methanol, ethanol, hexane, dichloromethane, ethyl acetate, etc.) are used. Hence, the final vehicle in which the extracts are found prevents their direct application in organisms. In addition to this, the protection, conservation, and targeted delivery (into organism) of plant extracts are another challenge to overcome their potential use as a treatment for diseases.[10]

At present, research is more focused on the composition of plant extracts, whereas, solutions that enable the efficient, safe, and direct application of these natural products need to be more focused on. One of the newest and most current ways for the application of the natural extracts, which also reduces the limitations outlined, is the use of polymeric nanoparticles (nanoparticles). Polymeric nanoparticle is a particle of polymer of any shape and an equivalent diameter from 1 to 100 nm and polymeric microparticle is a particle of polymer of any shape with an equivalent diameter from approximately 0.1–100 µm. Polymers can be of natural source, e.g. chitosan, albumin, gelatin, etc. or synthetic, e.g. methacrylates. Due to their size and unique physicochemical characteristics of nanoparticles, they generate formulations with several advantages, such as: (i) encapsulation of compounds of different chemical nature in the same formulation (mixture of compounds), (ii) targeting of specific organs (low toxicity), (iii) easy removal of organic solvent during the development of the nanoparticles (effective purification procedures), (iv) protection and conservation of the encapsulated active (enzymes damage, environment, etc.), and (v) controlled release of incorporated actives.[11–13]

In order to combine the diversity of biological activities of plant extracts and the advantages offered by the

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Research Article
Antimicrobial Effect of the Methanolic Extract *Psacalium decompositum* on Periodontopathogenic Bacteria

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Abstract

Background and Objective: Periodontal disease and dental caries are frequent oral illnesses. Both have an important impact on quality of life are of polymicrobial origin and progress slowly. Plants are a valuable resource in health systems in developing countries and a large part of traditional treatments involve the use of plant extracts or their active substances. The objective of this study is to determine the antimicrobial activity of the methanolic extract of *Psacalium decompositum* using the plate diffusion method *in vitro* and to determine the minimum inhibitory concentration of *Porphyromonas gingivalis*, *Prevotella intermedia* and *Streptococcus mutans*. **Materials and Methods:** The plant was collected and identified, *Psacalium decompositum* and a methanol extract was prepared by mashing. Phytochemical screening was also performed. The methanol extract was evaluted by the plate diffusion method using different microdilutions in broth against the ATCC strains *Porphyromonas gingivalis*, *Prevotella intermedia* and *Streptococcus mutans*. About 0.2% chlorhexidine was used as a positive control and 5% ethanol as a negative control. The nonparametric statistic, the Kruskal-Wallis test was used to compare the three treatments and the Mann-Whitney test for each pair of treatments to identify significant differences between them. **Results:** The minimum inhibitory concentration of the methanolic extract of *P. decompositum* was 500 μg mL^{-1} for *P. gingivalis* and *P. intermedia* and 700 μg mL^{-1} for *S. mutans* ($p<0.002$). The minimum inhibitory concentration for chlorhexidine (positive control) was 900 μg mL^{-1} for the three bacteria studied. **Conclusion:** The methanolic extract was active against cariogenic and periodontopathogenic bacteria and represents a natural alternative for the control of these microorganisms that produce oral diseases. The methanolic extract of *P. decompositum* has antimicrobial activity making it a natural alternative for the treatment of caries and periodontal disease.

Key words: *Psacalium decompositum*, *P. gingivalis*, *P. intermedia*, *S. mutans*, antimicrobial activity

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Research Article

Antibacterial and Antibiofilm Activity of Methanolic Plant Extracts against Nosocomial Microorganisms

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Biofilm is a complex microbial community highly resistant to antimicrobials. The formation of biofilms in biotic and abiotic surfaces is associated with high rates of morbidity and mortality in hospitalized patients. New alternatives for controlling infections have been proposed focusing on the therapeutic properties of medicinal plants and their antimicrobial effects. In the present study the antimicrobial and antibiofilm activities of 8 methanolic plant extracts were evaluated against clinical isolated microorganisms. Preliminary screening by diffusion well assay showed the antimicrobial activity of *Prosopis laevigata*, *Opuntia ficus-indica*, and *Gutierrezia microcephala*. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined ranging from 0.7 to >15 mg/mL. The specific biofilm formation index (SBF) was evaluated before and after the addition of plant extracts (MBC × 0.75). *Opuntia ficus-indica* caused the major reduction on SBF in dose-dependent manner. Cytotoxic activity of plant extracts was determined using brine shrimp lethality test (*Artemia salina* L.). Lethal Dose concentration (LD_{50} values) of the plant extracts was calculated. LD_{50} values for *P. laevigata* and *G. microcephala* were 141.6 and 323.3 μg/mL, respectively, while *O. ficus-indica* showed a slight lethality with 939.2 μg/mL. Phytochemical analyses reveal the presence of flavonoids, tannins, and coumarines.

1. Introduction

Microbial biofilms are communities of bacteria, embedded in a self-producing matrix, forming on living and nonliving solid surfaces [1]. Biofilm-associated cells have the ability to adhere irreversibly on a wide variety of surfaces, including living tissues and indwelling medical devices as catheters, valves, prosthesis, and so forth [2].

They are considered an important virulence factor that causes persistent chronic and recurrent infections; they are highly resistant to antibiotics and host immune defenses [3]. Bacteria protected within biofilm exopolysaccharides are up to 1,000 times more resistant to antibiotics than planktonic cells (free-floating) [4], which generates serious consequences for therapy and severely complicates treatment options [5]. An estimated 75% of bacterial infections involve biofilms that are protected by an extracellular matrix [6].

Biofilm resistance is due to several reasons, like restricted diffusion of antibiotics into biofilm matrix, expression of multidrug efflux pumps, type IV secretion systems, decreased permeability, and the action of antibiotic-modifying enzymes [7]. The increased biofilm resistance to conventional treatments enhances the need to develop new control strategies [8].

Biofilm inhibition is considered as major drug target for the treatment of various bacterial and fungal infections, and pharmacological development of this drugs is now extensively studied [9]. In recent years, several green nonlethal strategies for biofilm control have been developed, because the mode of action of these novel antibiofilm agents is

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Research Article

Miconia sp. Increases mRNA Levels of PPAR Gamma and Inhibits Alpha Amylase and Alpha Glucosidase

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Diabetes mellitus is a public health problem worldwide. For this reason, ethanolic extract of *Miconia* sp. from Oaxaca, Mexico, was selected in search of an alternative against this disease. The effect of *Miconia* sp. on mRNA expression of PPARγ on cell line 3T3-L1, its effect on alpha amylase and alpha glucosidase, lipid accumulation during adipogenesis, and cell viability on VERO cells were evaluated. The mRNA levels of PPARγ increased on 1.393 ± 0.008 folds, lipid accumulation was increased by 29.55% with *Miconia* sp. extract and 34.57% with rosiglitazone, and α-amylase and α-glycosidase were inhibited with IC_{50} values from 28.23 ± 2.15 μg/mL and 1.95 ± 0.15 μg/mL, respectively; the IC_{50} on antiproliferative activity on VERO cells was 314.54 ± 45.40 μg/mL. In case of α-amylase and α-glycosidase assays, IC_{50} (inhibitory concentration 50) refers to necessary extract amounts to inhibit 50% of enzymatic activity. On the other hand, on antiproliferative activity, IC_{50} (inhibitory concentration 50) refers to necessary extract amounts to inhibit 50% of cell proliferation. It was concluded that the compounds present in *Miconia* sp. ethanolic extract increase mRNA expression of PPARγ, inhibit α-amylase and α-glucosidase, and increase lipid accumulation. It constitutes an alternative as adjuvant in diabetes mellitus treatment; therefore, we recommend continuing identifying the compounds responsible for its promising in vivo antidiabetic activity.

1. Introduction

Diabetes mellitus is a chronic metabolic disease considered a serious global public health problem. In 2010, approximately 285 million people suffered from this disease and this amount is expected to double up within the next 20 years [1]. Diabetes mellitus type 2 (DM2) is the most common form of diabetes. It is a complex metabolic alteration characterized by an insulin combination resistance (IR, low sensitivity of one or multiple tissues to insulin) and insulin secretion alteration [2].

The search for new drugs that act against peroxisome proliferator-activated receptor gamma (PPARγ) is very important because ligands of these transcription factors exhibit multiple biological responses such as decreasing the IR and avoiding high levels of plasm glucose. It has been shown that the adipogenesis process is under the control of a complex cascade of transcriptional regulatory factors in which PPARγ and other transcriptional factors of C/EBP family play a fundamental role [3, 4].

Enzymes α-amylase and α-glucosidase found in saliva and the brush border of the small intestine, respectively, act on hydrolysis oligosaccharides and disaccharides to produce easy absorption monosaccharides such as glucose. For the above mentioned, delaying absorption of glucose through inhibition of enzymatic hydrolysis of carbohydrates, carried

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Analytical Methods

Antioxidant comparative effects of two grape pomace Mexican extracts from vineyards on erythrocytes

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ABSTRACT

Maceration and Soxhlet methods were used to obtain methanol extracts from a Mexican grape (Ruby Cabernet) pomace and the biological activity and phenolic profiles were compared. The antioxidant capacity was used to evaluate the mechanism of action, using a physiological model (erythrocytes) of damage induced by AAPH-generated free radicals. The extract obtained by maceration presented a total phenolic content twice the one obtained using the Soxhlet method. It also contained the most potent antioxidants, reducing anisotropy in the presence of AAPH to the levels of untreated cells, restoring membrane fluidity, preventing the morphological changes, as demonstrated by scanning electron microscopy (SEM), and providing protection against protein oxidation at the higher concentration. Our work showed that both extracts presented significant antioxidant activity through positive interactions with the lipid bilayer.

1. Introduction

Alterations in redox systems may cause the oxidative damage by free reactive oxygen species (ROS) and reactive nitrogen species (RNS) to trigger a variety of structural component alterations (Zhang et al., 2014). Free radicals are highly reactive due to the structural instability arising from unpaired electrons. ROS production can modify antigenic transport and mechanical cell characteristics through DNA damage, loss of enzyme function, an increase in cell permeability, protein oxidation, disruption of cell signaling, cell death by necrosis or apoptosis and lipid peroxidation (Kim et al., 2006; Mohandas & Gallagher, 2008).

In recent years, there has been a great deal of focus on the relationship between ROS generation and the pathophysiological mechanisms of chronic human illnesses such as inflammation and heart disease, hypertension, neurodegenerative disorders and cancer (Kim, Choi, Ham, Jeong, & Lee, 2012; Mitjans et al., 2011).

In order to prevent and protect cellular components from free radical damage, the endogenous defense system use enzymatic and non-enzymatic antioxidants. There are different mechanisms of action involved in antioxidant defense: prevention of ROS production; scavenging, quenching or removing ROS; repairing damage; and reconstituting membranes and tissues (Niki, 2014).

In addition to these systems, a balanced diet containing basic nutrients as well as antioxidant-rich foods may have an important role in maintaining lower levels of ROS (Touriño et al., 2008).

Increasing evidence suggests that the unique pharmacological properties of antioxidants from natural sources represent an alternative way of preventing the risk associated with many of the disorders associated with oxidative stress (Torres et al., 2002). In order to determine the effects of dietary antioxidants, their interaction with cell membranes has been studied using human erythrocytes, which provide readily accessible human cell membrane model. As oxygen carriers, erythrocytes are important potential targets for oxidation (van Zwieten, Verhoeven, & Roos, 2014).

In previous studies, polyphenolic compounds from several natural sources have been shown to exert potential antioxidant activity: white and red grape pomace (*Vitis vinifera*), bark from pine (*Pinus pinaster*) and bark from witch-hazel (*Hamamelis virginiana*)

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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and characterization of six nonsymmetric A_3B porphyrins with *p*-chlorophenyl as *meso*-substituent A or B and determination of their photodynamic activity

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Abstract Photodynamic therapy is a promising alternative for cancer treatment of solid tumors through generation of reactive oxygen species mediated by light-activated photosensitizing agents. One of the most promising types of photosensitizers is tetrapyrrolic macrocyclic compounds, such as *meso*-substituted porphyrins. In the present study, the synthesis of nonsymmetric porphyrins A_3B was accomplished, with *p*-chlorophenyl as B or A *meso*-substituent along with hydroxyl group, methoxyl group, or hydrogen atom as substituents of the phenyl in *meso* position and target molecules were designed in order to evaluate the synergistic effect of these hydrophobic and hydrophilic moieties unsymmetrically distributed. In vitro cytotoxic effect was demonstrated against human breast and cervix cancer cell lines (MCF-7 and HeLa cells, respectively). Terminal deoxynucleotidyl transferase dUTP nick end labeling assay and morphological studies were performed in order to elucidate part of the mechanism of cell damage for the most active compounds.

Keywords Nonsymmetric porphyrin · Photodynamic therapy · Photosensitizer · Cytotoxic effect

Introduction

The most common types of cancer therapies (surgery, radiotherapy and chemotherapy) are nonselective to cancer tissue (Nguyen *et al.*, 2010) and cause several undesirable side effects, such as nausea, alopecia, myalgias, myelosuppression, fatigue, etc. (Partridge *et al.*, 2001). To avoid such side effects, the development of new and effective treatments, capable of discriminating between normal and cancer tissue is imperative. Photodynamic therapy (PDT) is a binary therapy for the treatment of solid tumors, based on the selection by aggregation of a photosensitizing agent in tumoral tissue (Pushpan *et al.*, 2002; Sachs and Brenner, 2005). The irradiation of neoplasic regions with light at specific wavelength results in the activation of the photosensitizer (PS), which generates (in the presence of molecular oxygen) reactive oxygen species (ROS), such as superoxide and singlet oxygen, that destroys abnormal tissue without any significant damage to regular tissue. In several cases, amphiphilic porphyrin derivatives have shown their potential in the treatment of tumors by PDT (Boyle and Dolphin, 1996).

In the last years, the design of new photosensitizer molecules has been directed to attain several of the following desirable characteristics: (a) simple and high purity synthesis under laboratory conditions, (b) high solubility in biological fluids favoring localization at the cellular and subcellular level, (c) photoactivity at wavelengths higher than 630 nm, (d) minimal cytotoxicity in the absence of light and (e) optimal absorption, distribution, metabolism and excretion (ADME) properties (Postigo *et al.*, 2004;

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REVISTA MEXICANA DE CIENCIAS FARMACÉUTICAS

Trabajo Científico

Actividad fungicida, antioxidante e identificación de los compuestos más activos de 20 plantas utilizadas en la medicina tradicional mexicana

Fungicide, antioxidant activity and more active compounds identification of 20 plants used in the mexican traditional medicine.

David Gilberto García Hernández[1], Azucena Oranday Cárdenas[1], María Julia Verde Star[1], Ramiro Quintanilla Licea[1], Catalina Leos Rivas.[1] Elvira Garza González[2], Catalina Rivas Morales[1].
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Resumen:

En éste trabajo se evaluó la actividad fungicida sobre dermatofitos, antioxidante en función de la capacidad de atrapar el radical ABTS e identificación de compuestos activos de extractos metanólicos de 20 plantas medicinales; siendo los extractos más activos: el de semilla de apio, con una concentración mínima inhibitoria (CMI) de 1.4 mg/mL y una concentración mínima fungicida (CMF) de 2.8 mg/mL contra todas las especies de dermatofitos evaluados, excepto *T. rubrum*; y el de gobernadora como antioxidante con una CI_{50} = 1.67 µg/mL y una actividad equivalente de Trolox (TEAC) = 136.72 mg Trolox 100 g de planta. Se identificaron de la semilla de apio dos compuestos por cromatografía de gases acoplada a masas neocnidilido y 3-butilftalido.

Abstract

This work evaluated the fungicidal activity of dermatophytes, antioxidant depending on the capacity to catch the ABTS radical and identification of active compounds from methanolic extracts of 20 medicinal plants; being the most active extracts: that of celery seed, with a minimal inhibitory concentration (MIC) of 1.4 mg/mL and a minimal fungicide concentration (MFC) 2.8 mg/mL against all species of dermatophytes except *T. rubrum* and the Governor as an antioxidant with an IC_{50} 1.67 µg/mL. The activity equivalent of Trolox (TEAC) was 136.72 mg Trolox 100 g of plant. We identified two compounds in celery seed by gas chromatography coupled to mass neocnidilide and 3-butilphtalide.

Palabras clave: Dermatofitos, ABTS, TEAC, Fungicida, Antioxidante.

Keywords: Dermatophytes, ABTS, TEAC, fungicidal and antioxidant

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Trabajo científico

Contenido de fenoles totales y actividad anti-radical de extractos metanólicos de la planta silvestre y cultivada *in vitro* de *Leucophyllum frutescens*

Total phenols and anti-radical activity of methanolic extracts from wild and *in vitro* conditions of *Leucophyllum frutescens*

Claudia Espinosa-Leal, Jaime Francisco Treviño-Neávez, Ruth Amelia Garza-Padrón, María Julia Verde-Star, Catalina Rivas-Morales, María Eufemia Morales-Rubio

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Resumen

Leucophyllum frutescens es un arbusto conocido comúnmente como cenizo, utilizado en la medicina tradicional para tratar diversos padecimientos. El objetivo del trabajo fue cuantificar los fenoles totales y actividad anti-radical de los extractos metanólicos obtenidos de diferentes estructuras de planta silvestre (localidades: Sabinas Hidalgo y Apodaca) y tejido obtenido *in vitro*. El tamizaje fotoquímico de los extractos obtuvo resultados positivos para cumarinas, lactonas y flavonoides. Los extractos del tallo de las localidades de Sabinas Hidalgo y Apodaca mostraron actividad relevante para Fenoles Totales con 120.22 y 78.78 µg/mL de equivalentes de ácido gálico, DPPH (1,1-difenil-2-picrilhidrazil) con CE_{50} de 213.96 y 283.27 µg/mL y ABTS (2,2-azinobis-[3 etilbenzotiazolin-6-sulfónico]) con 97.58 y 108.27 µg/mL de equivalentes de Trolox. Estos estudios muestran que *L. frutescens* es una potencial fuente de metabolitos con actividad antioxidante.

Abstract

Leucophyllum frutescens is a shrub commonly known as cenizo, used in traditional medicine to treat various ailments. The objective of the study was to quantify total phenols and test anti-radical activity of the methanolic extracts obtained from different structures of wild plant (locations: Sabinas Hidalgo and Apodaca) and tissue obtained *in vitro*. The phytochemical screening of extracts obtained positive results for coumarins, flavonoids and lactones. Sabinas Hidalgo and Apodaca stem extracts showed relevant activity with Total Phenols: 120.22 and 78.78 µg/mL of Gallic acid equivalents, DPPH (1, 1-diphenyl-2-picrilhidrazil) with EC_{50} of 213.96 and 283.27 µg/mL and ABTS (2, 2-azinobis-[3 etilbenzotiazolin-6-sulfonic acid]) with 97.58 and 108.27 µg/mL of Trolox equivalents. These studies show that *L. frutescens* is a potential source of metabolites with antioxidant activity.

Palabras clave: *Leucophyllum frutescens*, cultivo *in vitro*, DPPH, ABTS.

Key Words: *Leucophyllum frutecens*, *in vitro* tissue culture, DPPH, ABTS.

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Trabajo Científico

Efecto *in vitro* en la inhibición del proceso de nucleación en litiasis renal, capacidad de captura de radicales libres, actividad antimicrobiana y tóxica del extracto metanólico de *Berberis trifoliata*

In vitro effect on the inhibition of the process of nucleation in renal lithiasis, ability to capture radical free, antimicrobial and toxic of methanol extract of *Berberis trifoliate*

Raymundo Alejandro Pérez Hernández, Catalina Leos Rivas, Azucena Oranday Cárdenas, Carlos Eduardo Hernández Luna, Eduardo Sánchez García, Catalina Rivas Morales

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Resumen

Se evaluaron mediante técnicas *in vitro* las actividades antiurolítica, antimicrobiana, capacidad de captura de radicales libres, toxicidad y composición fitoquímica del extracto metanólico de las partes aéreas de *Berberis trifoliata*. El extracto en estudio inhibió en un 93 ± 0.01 % la nucleación de cristales de oxalato de calcio a una concentración de 1000 µg/mL, exhibió capacidad de captura de radicales libres con una CE_{50} de 12.84 µg/mL. El extracto no presentó actividad antimicrobiana frente a aislados clínicos responsables de infecciones en tracto urinario y se obtuvo mediante el ensayo de *Artemia salina* una CL_{50} de 925 µg/mL. En cuanto a su composición fitoquímica se determinó la presencia de compuestos y grupos funcionales tales como: carbohidratos, cumarinas, dobles enlaces, flavonoides, grupos carboxilo, sesquiterpenlactonas, alcaloides y taninos.

Abstract

In vitro antiurolithic, antimicrobial and free radicals scavenger activities, toxicity, and phytochemical composition of methanolic extract of aerial parts of *Berberis trifoliata* was evaluated. The studied extract inhibits up to a 93 ± 0.01 % the nucleation of crystals of calcium oxalate at a concentration of 1000 µg/mL and have free radical scavenging activity with a CE_{50} = 12.84 µg/mL. The extract did not exhibit antimicrobial activity against isolated clinical bacteria that causes urinary infections. In terms of toxicity against *Artemia salina*, the extract showed a medium lethal concentration value of 925 µ g/mL. It has a diversity of compounds and functional groups like carbohydrates, coumarins, double bonds, flavonoids, carboxyl group, sesquiterpenlactones, alkaloids and tannins.

Palabras clave: *Berberis trifoliata*, antiurolítico, antimicrobiana, antirradical, toxicidad.

Key words: *Berberis trifoliata*, antiurolithic, antimicrobial, antiradical, toxicity.

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Trabajo Científico

Actividad antimicrobiana del extracto proteico de hojas de *Aloe vera*

Antimicrobial activity of protein extract of *Aloe vera* leaves

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Resumen

Las enfermedades infecciosas son un problema de Salud Pública que se agrava con microorganismos resistentes. Esto conduce al desarrollo de nuevos fármacos. Recientemente surgió el estudio de actividades farmacológicas de proteínas de fuentes naturales. En esta investigación se realizó la extracción de proteínas del gel de *Aloe vera* y su evaluación antimicrobiana. Se obtuvieron extractos proteicos de *A. vera* por dos métodos: Extracto 1 (EP1) precipitación con $(NH_4)_2SO_4$ y extracto 2 (EP2) extracción con fenol. El rendimiento para el método 2 (0.33 % recuperación). Análisis por espectrometría de masas EP1 permitió identificar un péptido y una proteína de 5 y 31 kDa, relacionada con glicoproteínas de *Arabidopsis thaliana*. EP2 mostró actividad contra *Staphyllococcus aureus* y *Candida albicans*. *A. vera* es una fuente prometedora de proteínas con actividad anti–*S. aureus* y –*C. albicans*.

Abstract

Infectious diseases remain a public health problem that is exacerbated by the presence of resistant organisms leading to development of novel drugs. Study of pharmacological proteins from natural sources recently emerged. In this research extraction of proteins from gel of *Aloe vera* and antimicrobial evaluation were done. Protein extracts from *Aloe vera* were obtained by two methods: extract 1(EP1) by precipitation with $(NH_4)_2SO_4$ and (EP2) by phenol extraction. The yield for method 2 (0.33 % recovery). Analysis by mass spectrometry of EP1 identified with a high degree of confidence, peptide 5 and 31-kDa protein, whose identity has been associated with the glycoproteins of Arabidopsis thaliana. EP2 exhibited inhibitory activity against *Staphylococcus aureus* and *Candida albicans*. *A. vera* extract comprises a promising source of protein with anti-*S. aureus* and anti-*C. albicans* activity.

Palabras clave: *Aloe vera*, proteínas, actividad antimicrobiana.

Key words: *Aloe vera*, protein, antimicrobial activity.

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Cnidoscolus chayamansa hidropónica orgánica y su capacidad hipoglucemiante, calidad nutraceutica y toxicidad*

Cnidoscolus chayamansa organic hydroponic and its hypoglycemic capacity, nutraceutical quality and toxicity

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Resumen

La chaya es una planta con calidad nutricional y posee un alto potencial en la salud pública en el tratamiento de diabetes mellitus. La diabetes *mellitus* es una de las enfermedades crónico-degenerativas con mayor prevalencia en México. Por otra parte, en los últimos años se ha incrementando el interés por la evaluación de los efectos del consumo de extractos de plantas como alternativa inocua para el tratamiento de diabetes. Estudios recientes han demostrado que extractos de chaya (*Cnidoscolus chayamansa*) tienen propiedades antioxidantes. Sin embargo, se desconoce si las infusiones (extractos acuosos) de dicha planta poseen propiedades hipoglicemiantes. La capacidad hipoglicemiante y toxicidad de una infusión de hojas de chaya hidropónica producida orgánicamente fueron evaluadas mediante modelos *in vivo*, usando ratas macho Wistar albinas (evaluación de capacidad hipoglicemiante), y larvas de *Artemia salina* (determinación de toxicidad). Asimismo se determinaron el contenido fenólico y la capacidad antioxidante de la infusión. El consumo de la infusión evaluada redujo los niveles de glucosa de las ratas diabéticas, teniendo un mayor efecto hipoglicemiante que la aplicación de glibenclamida. La

Abstract

Chaya is a plant nutritional quality and has high potential for public health in the treatment of diabetes *mellitus*. Diabetes *mellitus* is one of the most chronic degenerative diseases prevalent in Mexico. Moreover, in recent years has been increasing interest in assessing the effects of using plant extracts as safe alternative for the treatment of diabetes. Recent studies have shown that extracts of chaya (*Cnidoscolus chayamansa*) have antioxidant properties. However, it is unknown whether infusions (aqueous extracts) of the plant possess hypoglycemic properties. The hypoglycemic ability and toxicity of an infusion of leaves of hydroponic chaya organically produced were evaluated by *in vivo* models using male Wistar albino rats (evaluation of hypoglycemic capacity), and larvae of *Artemia salina* (determination of toxicity). The phenolic content and antioxidant capacity of infusion were also determined. The consumption of the evaluated infusion reduced glucose levels of diabetic rats, having a higher hypoglycemic effect that the application of glibenclamide. The toxicity assessment showed that infusion of organic hydroponic chaya leaf is not toxic and is safe for consumption as potential hypoglycemic agent.

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Sperm chromatin dispersion by formaldehyde in Wistar rats

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ABSTRACT. Formaldehyde (FA) is an environmental xenobiotic, which is genotoxic and carcinogenic to humans and animals; it induces DNA damage, mutations, and clastogenicity during critical cytogenetic events. FA-mediated oxidative stress is an important mechanism that has been associated with the induction of cytotoxic and genotoxic damage. Therefore, the objective of this study was to evaluate the dispersion of sperm chromatin and reproductive parameters induced by exposure to

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Research Article

Organotypic Culture of Breast Tumor Explants as a Multicellular System for the Screening of Natural Compounds with Antineoplastic Potential

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Breast cancer is the leading cause of death in women worldwide. The search for novel compounds with antitumor activity, with less adverse effects and higher efficacy, and the development of methods to evaluate their toxicity is an area of intense research. In this study we implemented the preparation and culture of breast tumor explants, which were obtained from precision-cut breast tumor slices. In order to validate the model we are proposing to screen antineoplastic effect of natural compounds, we selected caffeic acid, ursolic acid, and rosmarinic acid. Using the Krumdieck tissue slicer, precision-cut tissue slices were prepared from breast cancer samples; from these slices, 4 mm explants were obtained and incubated with the selected compounds. Viability was assessed by Alamar Blue assay, LDH release, and histopathological criteria. Results showed that the viability of the explants cultured in the presence of paclitaxel (positive control) decreased significantly ($P < 0.05$); however, tumor samples responded differently to each compound. When the explants were coincubated with paclitaxel and compounds, a synergic effect was observed. This study shows that *ex vivo* culture of breast cancer explants offers a suitable alternative model for evaluating natural or synthetic compounds with antitumor properties within the complex microenvironment of the tumor.

1. Introduction

Cancer is the leading cause of mortality worldwide, with 8.2 million deaths and 14.1 million new cases recorded during 2012 alone. According to the World Health Organization, the number of deaths will continue to rise across the globe, with the alarming prediction of 19.3 million new cases by 2025. Breast cancer is the most frequent cancer found in women; it possesses the most elevated morbidity and mortality. In 2012, approximately 1.7 million women were diagnosed with breast cancer in the world, and 522,000 died as a direct result of this disease [1].

Conventional cancer therapies include surgery, radiation, and chemotherapy. Although the latter is widely used, in most cases it produces undesirable side effects. Chemoresistance and/or recurrence of cancer after chemotherapy are frequent events seen with treatment of this disease [2]. Thus, different research groups are now focused on finding novel drugs or anticancer compounds [3, 4] while others are developing methodologies for the evaluation of these drugs [5–7].

One of the current approaches for investigating novel antineoplastic or chemopreventive compounds is based on natural products research. This is because some of these compounds inhibit cell proliferation and promote apoptosis