Journal of
Medicinal Plants Research

About JMPR

The Journal of Medicinal Plants Research (JMPR) provides researchers, students and academicians an avenue to present their findings on the value of medicinal plants, indigenous medications, ethnobotany and ethnomedicine, herbal medicines and the cultivation of aromatic and medicinal plants.

The journal will consider for publication original research, reviews and meta-reviews, and short communication on areas covering nutraceuticals, drug discovery and development, pharmacopoeia, traditional medicine, monographs, and natural products research.

The Journal of Medicinal Plants Research is indexed in:

CAB Abstracts CABI's Global Health Database Chemical Abstracts (CAS Source Index) China National Knowledge Infrastructure (CNKI) Google Scholar Matrix of Information for The Analysis of Journals (MIAR) ResearchGate

Open Access Policy

Open Access is a publication model that enables the dissemination of research articles to the global community without restriction through the internet. All articles published under open access can be accessed by anyone with internet connection.

The African Journals of Biotechnology is an Open Access journal. Abstracts and full texts of all articles published in this journal are freely accessible to everyone immediately after publication without any form of restriction.

Article License

Self-Archiving Policy

The Journal of Medicinal Plants Research is a RoMEO green journal. This permits authors to archive any version of their article they find most suitable, including the published version on their institutional repository and any other suitable website.

Please see http://www.sherpa.ac.uk/romeo/search.php?id=213&fIDnum=|&mode=simple&la=en

Digital Archiving Policy

The Journal of Medicinal Plants Research is committed to the long-term preservation of its content. All articles published by the journal are preserved by Portico. In addition, the journal encourages authors to archive the published version of their articles on their institutional repositories and as well as other appropriate websites..

https://www.portico.org/publishers/ajournals/

Metadata Harvesting

The Journal of Medicinal Plants Research encourages metadata harvesting of all its content. The journal fully supports and implements the OAI version 2.0, which comes in a standard XML format. See Harvesting Parameter

Memberships and Standards

Academic Journals strongly supports the Open Access initiative. Abstracts and full texts of all articles published by Academic Journals are freely accessible to everyone immediately after publication.

All articles published by Academic Journals are licensed under the Creative Commons Attribution 4.0 International License (CC BY 4.0). This permits anyone to copy, redistribute, remix, transmit and adapt the work provided the original work and source is appropriately cited.

Crossref is an association of scholarly publishers that developed Digital Object Identification (DOI) system for the unique identification published materials. Academic Journals is a member of Crossref and uses the DOI system. All articles published by Academic Journals are issued DOI.

Similarity Check powered by iThenticate is an initiative started by CrossRef to help its members actively engage in efforts to prevent scholarly and professional plagiarism. Academic Journals is a member of Similarity Check.

CrossRef Cited-by Linking (formerly Forward Linking) is a service that allows you to discover how your publications are being cited and to incorporate that information into your online publication platform. Academic Journals is a member of CrossRef Cited-by.

Academic Journals is a member of the International Digital Publishing Forum (IDPF). The IDPF is the global trade and standards organization dedicated to the development and promotion of electronic publishing and content consumption.

COUNTER (Counting Online Usage of Networked Electronic Resources) is an international initiative

serving librarians, publishers and intermediaries by setting standards that facilitate the recording and reporting of online usage statistics in a consistent, credible and compatible way. Academic Journals is a member of COUNTER

Archiving In

Portico is a digital preservation service provided by ITHAKA, a not-for-profit organization with a mission to help the academic community use digital technologies to preserve the scholarly record and to advance research and teaching in sustainable ways.

Academic Journals is committed to the long-term preservation of its content and uses Portico

Academic Journals provides an OAI-PMH(Open Archives Initiatives Protocol for Metadata Harvesting) interface for metadata harvesting.

Contact

Editor-in-chief

Prof. Akah Peter Achunike
Department of Pharmacology & Toxicology
University of Nigeria
Nsukka,

Nigeria.

Dr. Ravichandran Veerasamy
Faculty of Pharmacy
AIMST University
Semeling,
Malaysia.

Dr. Bellamkonda Ramesh
Department of Food Technology,
Vikrama Simhapuri University,
India

Table of Content

ACADEMIC JOURNALS
expand your knowledge

Journal of Medicinal Plants Research

Full Length Research Paper

A review of Ginseng species in different regions as a multipurpose herb in traditional Chinese medicine, modern herbology and pharmacological science

Mohamad Hesam Shahrajabian[1,2], Wenli Sun[1,2] and Qi Cheng[1,2]*

[1]Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, China.
[2]Nitrogen Fixation Laboratory, Qi Institute, Building C4, No.555 Chuangye, Jiaxing 314000, Zhejiang, China.

Ginseng is the most famous of the Chinese herbs throughout the world, and has been one of the most valued herb in China. Traditional Chinese medicine as an essential element of alternative and complementary medicine, advanced over thousands of years with its own distinctive arrangement of therapies, diagnostics theories and in Asian countries, particularly China. In most parts of the world, especially western countries, Ginseng has been largely employed in recent decades and has become renowned for its important function in treating and preventing so many diseases. *Panax ginseng* consisted of a number of active constituents, like ginsenosides, nitrogenous substances, carbohydrates, phytosterol, organic acids, essential oils, amino acids, peptidoglycans, it's repeated, nitrogen-containing compounds, fatty acids, vitamins, minerals and other phenolic compounds. Ginsenosides are classified into two main groups known as protopanaxadiol (PPD) and protopanaxatriol (PPT). Pharmacological activities of ginseng extracts are effects on the central nervous system, antipsychotic action, tranquilizing effects, protection from stress ulcers, increase of gastrointestinal motility, anti-fatigue action, endocrinological effects, enhancement of sexual behaviour, acceleration of metabolism, or synthesis of carbohydrates, lipids, RNA, and proteins. More clinical studies are necessary to uncover the numerous substances and their effects in ginseng that contribute to public health.

Key words: Ginseng, traditional Chinese medicine, herbology, pharmacological science.

INTRODUCTION

The ancient Chinese have identified 11,146 medicinal species from 383 families, and more than 400 of which are widely used throughout the world (Drasar and Moravcova, 2004; Soleymani and Shahrajabian, 2012; Ogbaji et al., 2018; Shahrajabian et al., 2018; Soleymani et al., 2018; Shahrajabian et al., 2019a, 2019b). *Panax ginseng* (Giseng) is a well-known herb in traditional Chinese medicine (TCM) (Hsu et al., 2013; Li et al., 2017).

Panax means cure for all disease, as it combines the

Greek words pan meaning all and zxos meaning medicine (Jeong et al., 2012). In TCM, food and medicine are understood to share similar origin but with diverse applications and uses (Chan et al., 2010). Thus, the Chinese commonly incorporates variety of TCM herbs into their diet to make a number of healthy food recipes that are more appealing of better taste, improved texture, and will most importantly improve one's health (Guo et al., 2008). TCM originates in ancient China with a 5000-year history. Rooted in ancient eastern philosophies such as Taoism, TCM focuses on a holistic view between humans and nature. Through the observations of universal principles within nature, TCM inquire from a macro level into the microcosm of human physiology and the mutual relationships between our body's internal workings and the external environment (Cheung et al., 2017).

Traditional Chinese medicine is still commonly used in China. More than half of the population regularly uses traditional remedies, with the highest prevalence of use in rural areas. About 5000 traditional remedies are available in China; they account for approximately one fifth of the entire Chinese pharmaceutical market. *P. ginseng* is often described as the lord or king of herbs (Wen and Zimmer, 1996), which occupies an esteemed spot in TCM and traditional oriental medicine in most countries (Xie et al., 2005).

Panax quinquefolius is employed in TCM to treat cases of deficiency connected with symptoms like irritability, thirst, dryness of the mouth, fatigue, and respiratory tract (Chen et al., 2004). The most important common names of ginseng in different parts of the world are American ginseng, sang, give finger root, redberry, tartar root, man's health, dwarf groundnut, root of life, garantogen, ninsin, jinshard, garent-oquen, and little man. The name ginseng comes from the Chinese words, Jen Sheng stands for man herb due to the rhizome of the plant or human-like shape of the root. The word *Panax* implies cure all and refer to the traditional belief that ginseng has healing properties for all bodily disease (Kim et al., 2018).

Till date, fourteen (14) plants, which include 12 species and two infra specific taxa, have been grouped under the genus *Panax* (Shin et al., 2015). The three main types of commercial ginseng are the Chinese ginseng (*Panax notoginseng* (*Burk*.). *F. H.*), the American ginseng (*P. quinquefolius L.*), and the Korean ginseng (*P. ginseng* Meyer), and have been used all over the world as herbal medicines for thousands of years (Kim et al., 2012). Ginseng is also part of Sasang Constitution Medicine (SCM) and Korean Oriental Medicine (KOM) (Choi et al. 2006).

Recent researches have revealed that processing of ginseng modifies its chemical profile and may alter its pharmacological activities and properties (Xie et al., 2012; Wan et al., 2015). The origin of ginseng dates back to prehistory. In China, Shennong (Divine Peasant) also known as Emperor Yan, the Yellow Emperor, one of the three Emperors, the Emperor who is said to have commenced herbal medicine about 5500 years ago, is reported to have tasted hundreds of plants to as certain number of medicinal herbs (Zheng, 1985). According to Yun (2001), three hundred and sixty-five kinds of herbs are listed and are separated into three groups based on their toxicity level. The much better ones are non-toxic and serve to strengthen vibrant energy, and can be taken on a regular basis.

GINSENG TAXONOMY, PLANTATION AND DIFFERENT SPECIES IN VARIOUS PARTS OF THE WORLD

This is a perennial plant with fleshy roots, grows slowly, and identifies with the *Panax* genus in the Araliaceae family. It is grown in cooler climatic regions of the Northern Hemisphere, majorly in eastern Siberia, Korea, and northern China (Komatsu et al., 2005; Chhotaram et al., 2010; Park et al., 2012; Kim and Yang, 2018). Ginseng faces an array of stressful conditions, including biotic attack by bacteria, fungi, and nematodes. Fungi are the main causative agents of ginseng root rot disease, among which *Cylindrocarpon destructans* is the most culpable pathogen, other important pathogens include *Alternaria panax* (spotting disease, *Botrytis cinerea* (blight), *Rhizoctonia solani* (damping off), and *Pythium* species (root rot) (Kim et al., 2019).

Plant taxonomy

Kingdom: Plantae
Division= Angiosperms
Sub division= Eudicots
Class= Asterids
Order=Apiales
Family= Araliaceae
Subfamily= Aralioideae
Genus= *Panax*
Species= *ginseng*

Ginseng is cultivated naturally between 33°N and 48°N, which corresponds to the subarctic and temperate climate regions in Korea (between 33°7$'$N and 43°1$'$N), Manchuria (between 43°N and 47°N), and the Maritime province of Siberia (Choi et al., 2007; Ryu et al., 2012). Different environmental factors like soil and climatic such as hydrogen ion, nutrients, microbial populations and moisture content affect plants. Normally, precipitation, amount of sunshine and air temperature are included among climatic factors (Ryu et al., 2012).

The physiological characteristics of *P. ginseng* in relation to air temperature have been reviewed comprehensively in the literature (Mahfuzur and Zamir, 2005). Park (1979) studies show that ginseng does not

favour high temperature; hence it was necessary to develop culture methods that will help avoid the result of large increases in the air temperature of the arable land. In deep mountains, wild ginseng is cultivated under trees and prefers an exceedingly cold climate. The optimal temperature ranges between 10 and 20°C during the leafing phase and between 21 and 25°C during the flowering and fruiting phases (Mork et al., 1981).

High temperature adversely affects ginseng by initiating photosynthesis cessation, drying of leaves, and early defoliation (Ohh, 1981). Besides this, root rot, leaf spot disease, and anthracnose are also consequences that emanates from high temperature (Mahfuzur and Zamir, 2005). In the case of a temperature above 21°C, there will be great increase in leaf spot disease incidence (Ohh and Park, 1980). Among the ginseng species, Korean ginseng (*P. ginseng*), Chinese ginseng (*P. notoginseng*), and American ginseng (*P. quinquefolius*) are the most common throughout the world (Lee and Kim, 2014).

Liu et al. (2008) reported that based on the grown environment and the cultivated method, the commercial trade ginseng is classified into three grades of ginseng, Cultivated Ginseng (CG), Mountain Cultivated Ginseng (MCG), and Mountain Wild Ginseng (MWG), and CG is cultivated artificially in forms and contributes the major quantity of ginseng in the current market. There are two species of ginseng in Canada, the American ginseng (*P. quinquefolius*) and the Dwarf ginseng (*Panax trifolius*). The Dwarf ginseng does not have economic value since it does not possess any medicinal qualities. Among the other species of ginseng, the *P. ginseng* is also valuable, but cannot be cultivated in our climates.

Szymanska et al. (2013) reported that as a perennial herb, American ginseng is native to Eastern North American, and grows in deciduous and mixed forests in the northeast of the United States of America and Canadian provinces of Quebec and Ontario. With wild ginseng population decreasing, and *P. quinquefolius* a slow-growing plant, ginseng is grown in many regions and countries: in Wisconsin, Michigan, North Carolina, and a number of other states in the USA, in Ontario and British Columbia in Canada (Punja, 2011), and near Lublin in plant (Kochan et al., 2008).

Siberian ginseng, *Eleutherococcus senticosus*, is the most commonly used ginseng in the United States. While not considered to be a true ginseng, it belongs to the ginseng family and is native to Siberia, Korea, Japan and China. Siberian ginseng has been shown to have many properties comparable to those of true ginseng and some studies indicate that it may improve physical and mental performance. Wild ginseng is ginseng that has not been planted and cultivated domestically, rather it is that which grows naturally and is harvested from wherever it is found to be growing; wild ginseng is relatively rare and even increasingly endangered, due in large part to high demand for the product in recent years, which has led to the wild plants being sought out and harvested faster so

that new ones can grow. Wild ginseng can be either Asian or American and can be processed to be red ginseng (Seervi et al., 2010).

Asian and American ginseng shows different properties and medicinal values in pharmacology, even though the major bioactive ingredients of Asian ginseng and American ginseng are ginsenosides. In the ginseng market, American ginseng is more expensive than Asian ginseng (Li et al., 2010). Optimal light required for growing Asian and American ginseng (*P. ginseng* Meye*r* and *P. quinquefolius* L., respectively) is characterized as follows: too little light, which reduces root yield; and too much light which leads to photo inhibition of photosynthesis, photo bleaching and leaf death; generally, optimal light intensity for Asian ginseng ranged from 5 to 20% (Proctor and Palmer, 2017). Brazilian ginseng (*Pfaffia glomerata* (Spreng.) Pedersen, Amaranthaceae), is a medicinal plant that is largely used as adaptogenic herb. It commonly grows in Africa and Americas and is highly considered both pharmaceutically and commercially, largely due to β-ecdysone accumulation in its roots.

Brazil remains the greatest supplier of *P. glomerata* in the world. Due to the similarity in morphology of its root to those of *P. ginseng* (Korean ginseng), the species came to be known as the Brazilian ginseng (Neves et al., 2016). In *P. glomerata*, different substances have been reported: triterpenoid (glomeric acid), nortriterpenoid (pfameric acid), ecdysterone, subrosterone, oleanolic acid and glucopyranosiloleanolate (Shiobara et al., 1993). Some species have different TCM natures. *P. ginseng* is hot while, *P. quinquefolius* is cool (Schlag and McIntosh, 2013). Modern biochemical and molecular studies have proved the TCM belief that there exist conflicting effects between American and Asian ginsengs (Sievenpiper et al., 2004).

Sengupta et al. (2004) observed that Asian ginseng roots extracts had higher Rg1:Rb1 ratios compared to American ginseng and showed that while angiogenesis results from Rg1 dominance, the opposite effect of limiting growth of cancer cells is promoted by Rb1 dominance. Brazilian ginseng (*P. glomerata*) is a plant native from the countries of South America, particularly of some states of Brazil, like Sao Paulo, Parana, Mato Grosso and Goias. Due to the similarity in their pharmacological effects, it is employed commercially as an alternative for Asian ginseng (*Panax* species). The Brazilian ginseng roots (BGR) are traditionally used in folk medicine as analgesic, anti-inflammatory, tonic, anti-diabetic, aphrodisiac, and antiulcer-gastric, with several researches describing its efficacy (Neto et al., 2005; Vardanega et al., 2017).

The ginseng products distributed on the market can be largely classified as fresh ginseng and its primary processing products in its original shape, red ginseng, and dried ginseng. In herbal market, ginseng is commercially obtainable in fresh, red, white and other

Table 1. Species of Ginseng (Yun, 2001).

Panax ginseng C. A. Meyer (Korean ginseng)
Panax japonicas C. A. Meyer (Japanese ginseng)
Panax major Ting
Panax notoginseng (*Burkill*) *F. H. Chen* (Sanchi ginseng)
Panax omeiensis J. Wen
Panax pseudoginsengWallich
Panax quinquefolius L. (American ginseng)
Panax sinensis J. Wen
Panax stipuleanatus H. T. Tsai&K. M. Feng
Panax trifolius L. (Dwarf ginseng)
Panax wangianus Sun
Panax zingiberensis C.Y. Wu&K.M. Feng
Panax vietnamensis Ha et Grushv. (Vietnamese ginseng)

processed products (Sun et al., 2009, 2011). Zhao et al. (2015) reported that in the market, there is a huge price variation among the different grades of ginseng; the price trend is usually as follows: wild American ginseng (WAG) > cultivated American ginseng (CAG) > Asian ginseng (ASG). Dried ginseng product is not cooked but dried by sunlight, hot wind, or other methods (Cho et al., 2014). The white ginseng is usually prepared by air-drying, the fresh ginseng is prepared by simple washing, the black ginseng is generated by an intensive and long steaming process, the stoved ginseng is prepared by a stoving process, the frozen ginseng is produced by a freezing process, and the red ginseng is commonly made by a moderate steaming or heating process (Kim et al., 2000; Wang et al., 2006) (Table 1).

BIOACTIVE PHYTOCHEMICALS OF GINSENG AND THEIR THERAPEUTIC ROLES

P. ginseng comprises 80 to 90% organic, approximately 10% inorganic substances, including several active constituents like ginsenosides orsaponins, nitrogenous substances, carbohydrates, essential oils, phytosterol, fatty acids, organic acids, amino acids, peptidoglycans, carbohydrate, compounds containing nitrogen, vitamins, minerals and other phenolic compounds (Attele et al., 1999; Gillis, 1997; Xie et al., 2005; Guo et al., 2015; Lu et al., 2017; Beccaria et al., 2018).

Lakshmi et al. (2011) mentioned that more often than ever, medicinal plants are being used as drugs in treatment of humans either singly or in combination. Also, previously unknown vital chemical substances with potential therapeutic effect can be found among medicinal plants. It has been shown that the key active components of *P. ginseng* are ginsenosides which boast a number of beneficial effects. Ginsenosides are grouped into two major groups known as protopanaxatriol (PPT)

and protopanaxadiol (PPD), due to the hydroxylation pattern at C6 and sugar moieties attachment (Pengelly and Bennett, 2011; Pace et al., 2015) (Tables 2, 3, 4 and 5).

Patel and Rauf (2017) also mentioned antioxidant, anti-inflammation, anti-fatigue, antidiabetic, antitumor, immunomodulation, anti-obesity, cardioprotective, antimicrobial, neuroprotective and aphrodisiac properties. They have presented the potential of ginseng as a complementary and alternative medicine (CAM). Ginseng polysaccharides comprised starch-like glucan and pectin with pectin accounting for around 20% of water-soluble polysaccharides (Zhang et al., 2009; Sun et al., 2019). Ginsenosides are distributed in many parts of the ginseng plant including the root, leaf and berry (Kim et al., 2014).

Different parts of the plant contain distinct ginsenoside profiles (Attele et al., 1999), which may exhibit different pharmacological activities (Kim et al., 2014). Shi et al. (2007) revealed that the leaf and root hair contain higher ginsenoside levels than the root. Wan et al. (2015) concluded that the contents of malonyl ginsenosides, amino acids, and polysaccharides, based on decreasing order, ranked as follows: fresh ginseng >frozen ginseng >white ginseng >stoved ginseng >red ginseng >black ginseng. They have also mentioned that processing should be paid more attention for the quality control of ginseng products. A lot of studies have been conducted on the pharmacological properties of Ginseng extract such as lipid-lowering, anti-allergic, antidiabetic, anti-inflammatory, hypoglycaemia and anti-stress, anti-aging, is repeated, anticarcinogenic, anti-fatigue, anti-adhesive, antidepressive, hypocholesterolemic and hypolipidemic, hepatoprotective activities, immune-modulatory activities, improving working memory and perceptual systems, stimulation and inhibition of central nervous system, and inhibiting the growth of tumor cells, especially in female reproductive system (Kim et al., 2013; Cho et al., 2014; Sun et al., 2015; Uluisik and Keskin, 2016; Silvestrini et

Table 2. *Panax* bioactive phytochemicals and their proven therapeutic roles (Patel and Rauf, 2017).

P. ginseng (Chinese ginseng)
P. quinquefolius (American ginseng)
Ginsenoside (Rb, Rc, Rd, re, Rf, Rg, Rh)
Polysaccharides
Oligosaccharides
Saponins
Anticancer effect
Protection against diabetic retinopathy and cardiomyopathy
Neural stem cell proliferation
Attenuation of β-amyloid generation
Protection from ischemia-induced oxidative stress and apoptosis
Protection from impairment of hippocampal neurons
Attenuation of pathogen virulence factors production
Treatment of erectile dysfunction
Fatigue alleviation in multiple sclerosis
Prevention of atopic dermatitis and rheumatoid arthritis
Amelioration of high fat diet-induced obesity

Table 3. Ginsenosides classification in *Panax* spp. (Leung and Wong, 2010).

Protopanaxadiol group (PPD)	Protopanaxatriol group (PPT)	Others
Rb1, Rb2, Rb3	Re	F11 ocotillo saponin (*P. quinquefolius* only)
Rc	Rf (*P. ginseng* only)	Oleanane saponins
Rd	Rg1, Rg2	Quinquenosides
Rg3	Rh1	-
Rh2	-	-
Rs1	-	-

al., 2017; Ardalanian and Fadaei, 2018; Zhang et al., 2018; Balusamy et al., 2019).

Kim et al. (2011) confirmed the use of Ginseng as an antioxidant supplement. Kim et al. (2018) also found that *P. ginseng* might be a potential alternative medicine for the prevention and treatment of natural aging-induced osteoporosis in human. Kuo et al. (2003) reported that glutamine and arginine were the two major free proteinogenic amino acids in the ginseng plants and together they constituted over 50% of all the free amino acids detected in the root. Uluisik and Keskin (2016) *P. ginseng* root powder may be useful for hepatic damage and fibrosis associated with high cholesterol diet. These beneficial effects of ginseng on liver enzymes is attributed to its active components knows as ginsenosides. Lee and Rhee (2017) reported that the potential use of ginseng in the prevention and treatment of chronic inflammatory diseases such as diabetes, rheumatoid arthritis, and allergic asthma. Qi et al. (2015) found that ginseng appears to be a prospective radio-protector that can potentially attenuate the deleterious effects of radiation on normal human tissue, and mostly for cancer patients going through radiotherapy which might be related to its immunomodulation and antioxidative properties (Tables 6, 7 and 8)..

RED AND WHITE GINSENG

When fresh ginseng is skinned, and then sun-dried or hot air-dried without application of steam, white ginseng is obtained. White ginsengs are separated based on their final shapes after the drying process into curved, half-curved, and straight ginseng (Song et al., 2014). While curved ginseng is obtained by rolling the whole length of the ginseng root into a round shape prior to drying; and half-curved ginseng is obtained by folding the roots upward to condense the entire length to about half the original length; straight ginseng is skinned and maintains the original shape from the field.

Table 4. Different concepts of the Ginseng products between countries.

Country (Region)	Type	Type
Korea	Root	Processed product
	Food	Food
China	Drug	Health food/New resource for food
Hong Kong	Food	Food
Taiwan	Drug	Food
Japan	Food	Food
Vietnam	Drug	Food
USA	Food	Dietary supplement
Canada	Food	Food/Health Food
France	Drug	Food supplement
Russia	Drug	Drug/Food
Thailand	Drug	Food
Spain	Drug	Food

Table 5. Some pharmacological effects of ginsenosides (Pengelly and Bennett, 2011).

Compound	Pharmacological action
Rb1	Estrogen-like activity
	Antidiabetic, insulin sensitizing
	Anti-obesity
	Angiogenesis inhibitor
	Neurotropic, neuroprotective
Rc	Inhibit proliferation of breast cancer cells
Re	Antidiabetic
	Antioxidant, cardioprotective
Rg1	Neurotropic, neuroprotective
	Ligand for glucocorticoid
	Receptor
	Suppresses oxidative stress
	Promotes angiogenesis
Rg2	Neuronal Ach inhibitor
Rg3	Inhibits proliferation of prostate cancer cells
Rh1	Activates estrogen receptor
Rh2	Cytotoxic, inhibits breast cancer cell proliferation
	Inhibits proliferation of prostate cancer cells
F11	Assists memory improvement neuroprotective

The one that is not skinned before being steamed or otherwise heated to be finally dried is red ginseng. Korean red ginsengs are classified into Yang-sam, Chun-sam and Ji-samon the basis of their rhizome firmness, characteristics of body tissues, colors, proportion of main roots to lateral roots, etc. During the steaming process, there is gelatinization of ginseng starch, giving rise to activation of effective ingredients and an upsurge in saponin. Even though colors and shapes of Korean ginsengs differ based on processing type, with minimal variation in ingredients, in the world, their collective efficacy and advanced properties of each remains the

Table 6. Essential ginseng effects and their likely actions on different body systems (Radad et al., 2004).

Subject	Ginseng's effect	Possible action
Whole body	General tonic and adaptogen	Resistance against adverse conditions (Physical, chemical and biological factors). Restores body 's homeostasis Anti-aging effects
Central nervous system	Neuroprotection either *in vivo* or *in vitro*	Potentiates nerve growth factor Antioxidative and anti-apoptotic mechanisms Reduces lipid peroxidation Inhibits excitotoxicity and Ca^{2+} over-influx into neurons Maintains cellular ATP levels Preserves structural integrity of neurons
	Glial cells	Prevents astroglial swelling Inhibits microglial respiratory burst activity and NO production by activated microglia
	Increasing cognitive performance (learning and memory)	Modulates neurotransmission Direct effect on hippocampal neurons
Cardiovascular system	Antihypertensive	Relax vascular smooth muscle cells through NO and Ca^{2+} medicated mechanisms Inhibits production of endothelin which plays a role in blood vessel constriction
	Anti-atherosclerotic effect	Prevents platelet aggregation Shows antagonistic action for platelet activity factor Suppresses thrombin formation
	Acceleration of wound healing	Promotes functional neovascularisation through endothelial proliferation
Inflammation and allergy	Anti-inflammatory and anti-allergic effects	Inhibits cytokine production such as IL-1β, IL-6 and TNF-α Abrogates cycooxygenase-2 gene expression Suppresses histamine and leukotrienes release from mast cells Stabilizes inflammatory cells such as neutronphils and lymphocytes Antifibroblastic activity
Immune system	Immunostimulant	Enhances interferon induction, phagocytosis, natural killer cells, and B and T cells
Carcinogenesis	Anticarcinogenic effect	Suppresses malignant transformation

Table 6. Cont.

		Inhibits proliferation of tumor cells
		Inhibits tumor invasiveness, metastasis and angiogenesis
Aphrodisiac effect	Enhancement of male copulatory behaviour	Relaxes corpus cavernosum smooth muscles via NO mediated processes
		Increases serum testosterone levels and reduces plasma levels of prolactin hormone
		Direct effects on anterior pituitary and hypothalamic dopaminergic mechanisms
Hyperglycemia	Antihyperglycemic activity	Increases plasma insulin levels, the number of insulin receptors and insulin sensitivity

Table 7. Key points about *Panax ginseng* (Kiefer and Pantuso, 2003).

Efficacy	Psychologic functioning: effective; conflicting evidence
	Physical performance: ineffective
	Immune system: effective
	Diabetes: modest effect; evidence limited
Adverse effects	Nausea, diarrhea, euphoria, insomnia, headaches, hypertension, hypotension, mastalgia, vaginal bleeding, blood pressure abnormalities
Interactions	Caution advised about concomitant use with phenelzie (Nardil), warfain (Coumadin), oral hypoglycemics, insulin, or caffeine, and about use in patients with hypertension or bleeding
Bottom line	A safe, well-tolerated herbal medicine that may be used for a variety of medical conditions

Table 8. Some information of clinical literature regarding interactions.

Drug	Interaction with ginseng
Alcohol	*P. ginseng* increases the clearance of alcohol (Lee et al., 1987)
Anti-platelet agents, Anti-coagulants, Pentazocine	*P. ginseng* potentiates the effects of various drugs including anticoagulants such as warfarin (Lee et al., 2008), the antiplatelet activity of NSAIDs such as aspirin, and pentazocine (Mitra et al., 1996)
Antidiabetic agents	Ginseng can reduce blood glucose levels (Reay et al., 2005; Sotaniemi et al., 1995) and therefore the use of both in combination use may lead to additive effects.
Phenelzine	*P. ginseng* should not be combined with monoamine oxidase inhibitors such as phenelzine, as it may lead to headache, tremor and mania (Jones and Runikis, 1987)

best (Gui and Ryu, 2014).

In TCM practice, White ginseng and red ginseng are used for different purposes; white ginseng is used to supply qi and promote the production fluids of body fluids as well as enhance physical fitness and disease resistance, while red ginseng has a warming effect and is used for boosting yang and replenishing vital essence (Zhang et al., 2012, 2019).

Xu et al. (2018) reported that both white and red ginseng is the most widely used in clinical applications because of their considerable pharmacological activity. But, red ginseng exhibits more potential anticancer activity than white ginseng likely because of the abundant amount of rare ginsenosides generated from processing such as ginsenosides Rg3 and Rh2 (Li et al., 2011; Kim et al., 2014). It is believed that various processing techniques modify the therapeutic effects of *P. ginseng* (Keum et al., 2000). For boosting fluids, white ginseng is better and is regarded as warmer and stronger for supplementing Qi. It has been demonstrated that in terms of chemical compositions, red and white ginseng are different, hence their different biological effects (Park et al., 2001). It has been anecdotally considered that white ginseng, which has a low PPD/PPT ratio, increases body temperature, whereas red ginseng, which has a high PPD/PPT ratio, does not (Cho et al., 2017). As white ginseng and red ginseng possess different bioactivities and clinical purposes, discrimination of the white one and the red one are very significant for quality control, standardizing the processing procedures, as well as the effective and safe usage of ginseng (Zhou et al., 2018).

Horacek et al. (2010) explained that red ginseng is steam-cured after harvesting, thus generating a glossy reddish-brown color, and thereafter dried. It is believed that to modify its biochemical composition and prevent the bioactive ingredients from possible breakdown, the root needs to undergo steaming; hence it remains the preferred ginseng product. After harvest, white ginseng is peeled and dried. It is assumed that during drying, bioactive constituents are broken down by enzymes in the ginseng root, making white ginseng to contain fewer bioactive components compared to red ginseng (Horacek et al., 2010).

In the Chinese pharmacopoeia, ginsenosides Rg1, Rb, and Re (the main components of Red ginseng and White ginseng) are still used as chemical markers for quality control (Zhao et al., 2019). Like Asian ginseng, white American ginseng (WAG) is prepared by air-drying; if fresh American ginseng is processed by steaming, from white color to red, the steamed product is called red American ginseng (RAG) (Wan et al., 2018).

During the steaming process, extensive conversion of original ginsenosides in white ginseng to degradation compounds in red ginseng was observed, leading to different ginsenoside profiles (Sun et al., 2011). Akhter et al. (2018) also indicated that polysaccharides are major active component of American ginseng root which

showing various biological activities including anticarcinogenic, anti-aging, immunostimulatory and antioxidant effects. Chung et al. (2014) reported that of the two kinds of ginseng, white ginseng is air-dried, and red ginseng is produced by steaming raw ginseng at 98 to 100°C for 2 to 3 h. Korean ginseng contains saponin, an element of glycosides; nitrogenous compounds such as protein, amino acid, nucleic acid and alkaloid; fat-soluble ingredients such as fatty acid, ethereal oil, polyacetylene, phenolic compound, phytosterol and terpenoid; saccharides such as monose, oligosaccharide, polysaccharide and pectin; vitamins and inorganic substances; and many other useful ingredients. Thus, ginseng contains an abundance of diversified chemical elements hardly found in other medicinal herbs (Proctor et al., 1990; Vinh et al., 2017) (Tables 9, 10 and 11).

GINSENOSIDES AND PHENOLICS OF GINSENG

Ginsenosides and phenolics in ginsengs are among the most important health-beneficial compounds in Asian ginseng (Chung et al., 2012). More than 25 ginsenosides including Rb, Rg, Rc and Ro, as well as more than 10 phenolics such as ferulic, gentistic, cinnamic, syringic, and p-hydrobenzoic acids, have been reported so far, their amounts differ among cultivars, cultivation conditions and processing (Shibata, 2001; Choi et al., 2006; Fishbein et al., 2009; Chung et al., 2012).

Ginsenosides Rb_1, Rb_2, RC, Rg_2, etc., are the major extract constituents at normal temperature (<100°C), while less polar ginsenosides such as Rg_3. Rg_6, F_4, Rs_5, Rs_4, Rg_5, and Rk_1 are the unique extract constituents at higher temperatures (>120°C) (Zhang et al., 2017). Wu et al. (2018) also reported that ginsenosides are usually divided into three groups: (1) the protopanaxadiol ginsenosides (PPD), (2) the protopanaxatriol ginsenosides (PPT), and (3) the oleanonic acid-type saponins; five major ginsenosides, Rb1, Rb2, Rc, Re, and Rg1, belong to the PPD and PPT types, constituting more than 80% of all ginsenosides. Others, such as Rg3, Rg2, F1, Rh2 and Rh4 are minor or rare ginsenosides which were found to have special physiological activities (Wei et al., 2011).

Some studies have demonstrated that many ginsenosides only exist in red ginseng such as ginsenosides-Rg_3, -Rg_5, -Rg_6, -Rh_1, -Rh_2, -Rk_1 -Rk_3 and -Rs_3-Rs_7, and fortunately, some of them have remarkable biological activities (Zhou and Yang, 2015). Kim et al. (1987) noted that the main ginsenosides are glycosides that contain an aglycone with a dammarane skeleton, and include protopanaxadiol-type saponins such as ginsenosides Rb1, Rb2, Rc, and Rd, as well as protopanaxatriol-type saponins such as ginsenosides Re and Rg1, constituting more than 80% of the total ginsenosides. Black ginseng contains some new ginsenosides (Rg3, Rg5, F4, Rg6, Rk3, Rs3, Rs4, etc.)

Table 9. Comparison of protein and free amino acid contents between Korean Ginseng and ginsengs of other countries.

Classification	Korean Ginseng			American Ginseng	Chinese Ginseng
	Main root	Lateral roots	Fine roots		
Soluble protein (mg/g dry weight)	38.0	29.5	19.6	11.4	17.0
Thermostable protein (mg/g dry weight)	28.1	22.3	12.8	10.1	7.3
Free amino acid (mg/g dry weight)	73.7	54.9	14.8	32.8	24.7

Table 10. Comparison of typical ginsenoside composition of American ginseng (*P. quinquefolius L.*) and Asian ginseng (*P. ginseng C. A. Meer*) (Schlag and McIntosh, 2006).

Chemical composition	American ginseng	Asian ginseng
Total ginsenosides	40-60 g/kg	20-40 g/kg
Major ginsenosides	Rb1, Re, Rd	Rb1, Rg1, Rb2
Pseudoginsenoside F11	1.0-2.0 g/kg	0
Ginsenoside Rf	0	1.0-2.0 g/kg
PPD-group to PPT-group	>2.0	<2.0
Rb1: Rg1	>5.0	<5.0
Rg1: Re	<1.0	>1.0
Rb2: Rc	<0.4	>0.4

Table 11. Concentration of medical ingredients (Comparison of saponin in ginsengs of various sources).

Ingredient	Korean Ginseng Red Ginseng	Korean Ginseng White Ginseng	Hwagi-sam (American ginseng)	Sanchi-sam (Chinese ginseng)	Bamboo-sam (Japanese ginseng)
Total kinds of saponin	30	23	14	15	8
Panaxadiols	18	15	9	6	6
Panaxatriols	11	7	4	9	4
Oleananes	1	1	1	-	1

which are not present in white ginseng, and exhibits more potent biological activities than white and red ginseng (Sun et al., 2009).

Qi et al. (2011) found that ginsenosides are dammarane-type saponins that naturally occur in many forms. Rg1, Rb1, Rc, Rd and Re (5, 1, 2, 3 and 4) are the major ginsenosides that commonly occur in both American and Asian ginseng. Schlag and McIntosh (2013) explained that the major ginsenosides are classified by structural type as protopanaxatriol (PPT) ginsenosides and have 20(S)-protopanaxatriol (20[S]-dammar-24-ane-3β,12β,20-triol) glycosides or as protopanaxadiol (PPD) ginsenosides and have 20(S)-protopanaxadiol (6α-hyroxy-20[S]-protopanaxadiol) glycosides. Rg1 (5) and Re (4) are PPT ginsenosides, whereas Rb1 (1), Rc (2), and Rd (3) are PPD ginsenosides.

At room temperature, as fresh ginseng appears to be easily degraded, it has traditionally undergone processing into red ginseng through root steaming followed by drying or into white ginseng through drying of the root (Lee et al., 2015; Park et al., 2016). In Korea, red ginseng and other several ginseng products are popularly used as either nutritional supplements or functional foods. Recent researches have shown that compared to fresh and white ginseng, red ginseng has biological benefits while inducing fewer side effects (Babiker et al., 2014; Lee et al., 2015). Korean

Red Ginseng is known to havea number of biological activities which include memoryenhancement, improving the blood circulation, boosting the immune system, antioxidant effects, positive effects on menopausal disorder, and antifatigue effects (Babiker et al., 2014). Olgun et al. (2016) indicated that Korean red Ginseng (KRG) has been extracted from the roots of *P. ginseng*. KRG has beneficial effects on learning and memory impairment. KRG has been found to be effective in various problems that cause hearing loss such as gentamycin toxicity, age-related hearing loss, or 3-nitropropionic acid-induced cochlear damage. Ginseng effectively prevents liver injury, mainly through down regulation of oxidative stress and inflammatory response

(Youssef, 2016).

Oh et al. (2015) reported the influence of ginseng in enhancing cognitive performance in Alzheimer's disease (AD), and improves movement's deficit in Parkinson's disease. Fatmawati et al. (2014) also reported that *P. ginseng* might be an important herbal medicine in preventing diabetic complications. Van Kampen et al. (2014) discovered that ginseng extract maybe a potential neuroprotective therapy for the treatment of Parkinson. Choi et al. (2006) reported that Korean and Chinese ginseng reduced systolic and diastolic BP, and red ginseng reduced headache symptoms. American ginseng showed antihypertensive effect on diastolic BP and reduced headache symptom.

However, there was no statistical significance in the between-group analysis. Lee et al. (2013) demonstrated that ginseng effectively reduces adipose tissue and prevents obesity in diet-induced obese mice that this process may be mediated in part through the anti-angiogenic actions of ginseng. Rocha et al. (2018) found that *P. ginseng* is effective in the control of abdominal pain in irritable bowel syndrome patients, analogous to trimebutin. Wang and Ng (2004) reported that the ribonuclease isolated from Chinese ginseng flowers; the root ribonuclease exhibits antifungal and inhibitory activities toward HIV-1 reverse transcriptase. Shin and Yoon (2018) demonstrated that ginseng may be able to prevent obesity, hyperlipidemia, and hepatic steatosis in men with testosterone deficiency.

Gray et al. (2016) found that ginseng protects against chromatin damage and thus maybe beneficial to reproductive fitness. Lee and Oh (2015) revealed that when red ginseng is administered over long periods, age-related decline of learning and memory is ameliorated through anti-inflammatory activity. Sharma and Goyal (2015) also insist on potential role of *P. ginseng* to become a pivotal chemo-preventive agent that can reduce cancer in mammals. Hwang et al. (2017) concluded that *P. ginseng* can prevent aging by inhibiting wrinkle formation and increasing moisture in the human skin. Park et al. (2017) reported that Korean Red Ginseng has beneficial effects on chronic liver disease, a condition encompassing non-alcoholic fatty liver disease, alcoholic liver disease, chronic viral hepatitis, and hepatocellular carcinoma. Lee and Son (2011) found the strong positive potential for glucose metabolism, psychomotor function, and pulmonary disease, but not for physical performance enhancement.

CONCLUSION

In order for Chinese medicine, and in particular, TCM, to become more integrated into medical practice in the West, there is a need to bridge the many conceptual and practical differences between western medicine and Chinese medicine. Among the ginseng species, Korean

ginseng (*P. ginseng*), Chinese ginseng (*P. notoginseng*), and American ginseng (*P. quinquefolius*) are the most common through the world. Cultivated Ginseng (CG), Mountain Cultivated Ginseng (MCG), and Mountain Wild Ginseng (MWG) are three categories of ginseng. Dwarf ginseng (*P. trifolius*) is another type of ginseng in Canada. Siberian ginseng, *E. senticosus* is also another common ginseng in the United States. Brazilian ginseng (*P. glomerata* Spreng. Pedersen, Amaranthaceae), is a medicinal plant largely used as adaptogenic herb. Although, field cultivation of ginseng is occurring in Asia and Europe, these endeavours are small in scale and have not made any significant impact on the supply structure of the market. This versatile medicinal plant is the unique source of various types of chemical compounds, which are responsible of the various activities of the plant. As the public scenario is changing towards the use of non-toxic plant products having Traditional Medicinal Asian Crops, development of modern drugs from *P. ginseng* should be emphasized for the control of various diseases.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Akhter KF, Mumin MA, Lui EMK, Charpentier PA (2018). Fabrication of fluorescent labeled ginseng polysaccharide nanoparticles for bioimaging and their immunomodulatory activity on macrophage cell lines. International Journal of Biological Macromolecules 109:254-262.

Attele AS, Wu JA, Yuan CS (1999). Ginseng pharmacology: multiple constituents and multiple actions. Biochemical Pharmacology 58(11):1685-1693.

Babiker LB, Gadkariem EA, Alashban RM, Aljohar HI (2014). Investigation of stability of Korean ginseng in herbal drug product. American Journal of Applied Sciences 11(1):160.

Balusamy SR, Rahimi S, Yang DC (2019). Characterization of squalene-induced PgCYP736B involved in salt tolerance by modulating key genes of abscisic acid biosynthesis. International journal of Biological Macromolecules 121:796-805.

Beccaria C, Silvestrini P, Renna MS, Ortega HH, Calvinho LF, Dallard BE, Baravalle C (2018). Panax ginseng extract reduces Staphylococcus aureus internalization into bovine mammary epithelial cells but does not affect macrophages phagocytic activity. Microbial Pathogenesis 122:63-72.

Chan E, Wong CYK, Wan CW, Kwok CY, Wu JH, Ng KM, So CH, Au ALS, Poon CCW, Seto SW, Kwan YW, Yu PHF, Chan SW (2010). Evaluation of anti-Oxidant capacity of root of *Scutellariabaicalensis*Georgi, in comparison with roots of *Polygonum multiflorumThunb* and *Panax ginseng CA Meyer*. The American Journal of Chinese Medicine 38(04):815-827.

Chen JK, Chen TT, Crampton L (2004). Chinese medical herbology and pharmacology (Vol. 1267). City of Industry, CA: Art of Medicine Press.

Cheung KLC, Buckley ER, Watanabe K (2017). Traditional Chinese medicine market in Hong Kong. Journal of Alternative and Complementary Medicine 4(1):555-630.

Chhotaram S, Rupali K, Pandurang D, Pallavi S (2010). Ginseng multipurpose. Journal of Biomedical Sciences and Research 2(1):6-17.

Cho CW, Kim YC, Rhee YK, Lee YC, Kim KT, Hong HD (2014).

Chemical composition characteristics of Korean straight ginseng products. Journal of Ethnic Foods 1(1):24-28.

Cho HT, Kim JH, Lee JH, Kim YJ (2017). Effects of *Panax ginseng* extracts prepared at different steaming times on thermogenesis in rats. Journal of Ginseng Research 41(3):347-352.

Choi DJ, Cho KH, Jung WS, Park SU, Han CH, Lee WC (2006). Clinical effects of Korean ginseng, Korean red ginseng, Chinese ginseng, and American ginseng on blood pressure in mild hypertensive subjects. Korean Journal of Oriental Medicine 27(4):198-208.

Choi YE, Kim YS, Yi MJ, Park WG, Yi JS, Chun SR, Han SS, Lee SJ (2007). Physiological and chemical characteristics of field and mountain cultivated ginseng roots. Journal of Plant Biology 50(2):198-205.

Chung IM, Kim JW, Seguin P, Jun YM, Kim SH (2012). Ginsenosides and phenolics in fresh and processed Korean ginseng (*Panax ginseng C.A. Meyer*): Effects of cultivation location, year and storage period. Food Chemistry 130(1):73-83.

Chung IM, Kim YO, Ali M, Kim SH, Park I, Kim EH, Yang YS, Park HR, Son ES, Ahmad A (2014). Triterpene glycosides from red ginseng marc and their anti-inflammatory activities. Bioorganic & Medicinal Chemistry Letters 24(17):4203-4208.

Drasar P, Moravcova J (2004). Recent advances in analysis of Chinese medical plants and traditional medicines. Journal of Chromatography B 812(1-2):3-21.

Fatmawati S, Ersam T, Yu H, Zhang C, Jin F, Shimizu K (2014). 20(S)-Ginsenoside Rh2 as aldose reductase inhibitor from *Panax ginseng*. Bioorganic & Medicinal Chemistry Letters 24(18):4407-4409.

Fishbein A, Wang CZ, Li XL, Mehendale S, Sun S, Aung H (2009). Asian ginseng enhances the anti-proliferative effect of 5-fluorouracil on human colorectal cancer: Comparison between white and red ginseng. Archives of Pharmacal Research 32(4):505-513.

Gillis CN (1997). *Panax ginseng* pharmacology: a nitric oxide link? Biochemical pharmacology 54(1):1-8.

Gray SL, Lackey BR, Boone WR (2016). Effects of *Panax ginseng*, zearalenol, and estradiol on sperm function. Journal of Ginseng Research 40(3):251-259.

Gui Y, Ryu GH (2014). Effects of extrusion cooking on physiochemical properties of white and red ginseng (powder). Journal of Ginseng Research 38(2):146-153.

Guo DJ, Cheng HL, Chan SW, Yu PH (2008). Antioxidant activities and the total phenolic contents of tonic Chinese medicinal herbs. Inflammopharmacology 16(5):201-207.

Guo Q, Cui SW, Kang J, Ding H, Wang Q, Wang C (2015). Non-starch polysaccharides from American ginseng: physicochemical investigation and structural characterization. Food Hydrocolloids 44:320-327.

Horacek M, Min JS, Heo SC, Soja G (2010). Discrimination between ginseng from Korea and China by light stable isotope analysis. Analytica Chimica Acta 682(1-2):77-81.

Hsu BY, Lu TJ, Chen CH, Wang SJ, Hwang LS (2013). Biotransformation of ginsenoside Rd in the ginseng extraction residue by fermentation with lingzhi (*Ganoderma lucidum*). Food Chemistry 141(4):4186-4193.

Hwang E, Park SY, Yin CS, Kim HT, Kim YM, Yi TH (2017). Antiaging effects of the mixture of *Panax ginseng* and *Crataeguspinnatifida* in human dermal fibroblasts and healthy human skin. Journal of Ginseng Research 41(1):69-77.

Jeong HC, Hong HD, Kim YC, RhoJ, Kim KT, Cho CW (2012). The research trend of ginseng processing technology and the status of ginseng industry. Food Science and Industry 45.

Jones BD, Runikis AM (1987). Interaction of ginseng with phenelizine. Journal of Clinical Psychopharmacology 7(3):201.

Keum YS, Park KK, Lee JM, Chun KS, Park JH, Lee SK, Kwon H, Surh YJ (2000). Antioxidant and anti-tumor promoting activities of the methanol extract of heat-processed ginseng. Cancer Letters 150(1):41-48.

Kiefer D, Pantuso T (2003). *Panax ginseng*. American Family Physician 68(8):1539- 1542.

Kim DH (2012). Chemical diversity of *Panax ginseng*, *Panax quinquifolium*, and *Panax notoginseng*. Journal of Ginseng Research 36(1):1.

Kim HG, Cho JH, Yoo SR, Lee JS, Han JM, Lee NH, Ahn YC, Son CG

(2013). Antifatigue effects of *Panax ginsengc.a. Meyer*. A Randomized, Double-Blind, Placebo-Controlled Trial. PloS One 8(4):e61271.

Kim HG, Yoo SR, Park HJ, Lee NH, Shin JW, Sathyanath R, Cho JH, Son CG (2011). Antioxidant effects of *Panax ginseng* c.a. Meyer in Healthy Subjects: A Randomized, Placebo-Controlled clinical trial. Food and Chemical Toxicology 49(9):2229-2235.

Kim MW, Ko SR, Choi KJ, Kim SC (1987). Distribution of saponin in various sections of *Panax ginseng* root and changes of its contents according to root age. Korean Journal of Ginseng Science 11:10-16.

Kim WY, Kim JM, Han SB, Lee SK, Kim ND, Park MK, Kim CK, Park JH (2000). Steaming of ginseng at high temperature enhances biological activity. Journal of Natural Products 63(12):1702-1704.

Kim YJ, Jeon JN, Jang MG, Oh JY, Kwon WS, Jung SK, Yang DC (2014). Ginsenoside profiles and related gene expression during foliation in *Panax ginseng Meyer*. Journal of Ginseng Research 38(1):66-72.

Kim YR, Yang CS (2018). Protective roles of ginseng against bacterial infection. Microbial Cell 5(11):472-481.

Kochan E, Kolodziej B, Gadomska G, Chmiel A (2008). Ginsenoside contents in *Panax quinquefolium* organs from field cultivation. Zeitschrift für Naturforschung C 63(1-2):91-95.

Komatsu K, Tohda C, Zhu S (2005). Ginseng drugs- molecular and chemical characteristics and possibility and antidementia drugs. Current Topics in Nutraceutical Research 3(1):47-64.

Kuo YH, Ikegami F, Lambein F (2003). Neuroactive and other free amino acids in seed and young plants of *Panax ginseng*. Phytochemistry 62:1087-1091.

Lakshmi T, Anitha R, Geetha RV (2011). *Panax Ginseng*- A universal Panacea in the herbal medicine with diverse pharmacological spectrum- A review. Asian Journal of Pharmaceutical and Clinical Research 4(1):14-18.

Lee CH, Kim JH (2014). A review on the medicinal potentials of ginseng and ginsenosides on cardiovascular diseases. Journal of Ginseng Research 38(3):161-166.

Lee FC, Ko JH, Park JK, Lee JS (1987). Effects of *Panax ginseng* on blood alcohol clearance in man. Clinical and Experimental Pharmacology & Physiology 14(6):543-546.

Lee H, Park D, Toon M (2013). Korean red ginseng (*Panax ginseng*) prevents obesity by inhibiting angiogenesis in high fat diet-induced obese C57BL/6J mice. Food and Chemical Toxicology 53:402-408.

Lee NH, Son CG (2011). Systematic review of randomized controlled trials evaluating the efficacy and safety of ginseng. Journal of Acupuncture and Meridian Studies 4(2):85-97.

Lee S, Rhee DK (2017). Effects of ginseng on stress-related depression, anxiety, and the hypothalamic-pituitary-adrenal axis. Journal of Ginseng Research 41(4):589-594.

Lee SH, Ahn YM, Ahn SY, Doo HK, Lee BC (2008). Interaction between warfain and *Panax ginseng* in ischemic stroke patients. The Journal of Alternative and Complementary Medicine 14(6):715-721.

Lee SM, Bae BS, Park HW, Ahn NG, Cho BG, Cho YL, Kwak YS (2015). Characterization of Korean Red Ginseng (*Panax ginseng Meyer*): history, preparation method, and chemical composition. Journal of Ginseng Research 39(4):384-391.

Lee Y, Oh S (2015). Administration of red ginseng ameliorates memory decline in aged mice. .Journal of Ginseng Research 39(3):250-256.

Leung K, Wong A (2010). Pharmacology of ginsenosides: A literature review. Chinese Medicine 5(1):20.

Li B, Zhao J, Wang CZ, Searle J, He TC, Yuan CS, Du W (2011). Ginsenoside Rh2 induces apoptosis and paraptosis-like cell death in colorectal cancer cells through activation of p53. Cancer Letters 301:185-192.

Li L, Luo GA, Liang QL, Hu P, Wang YM (2010). Rapid qualitative and quantitative analyses of Asian ginseng in adulterated American ginseng preparations by UPLC/Q-TOF-MS. Journal of Pharmaceutical and Biomedical Analysis 52(1):66-72

Li MR, Shi FX, Li YL, Jiang P, Jiao L, Liu B, Li LF (2017). Genome-wide variation patterns uncover the origin and selection in cultivated ginseng (*Panax ginseng Meyer*). Genome Biology and Evolution 9(9):2159-2169.

Liu D, Li YG, Xu H, Sun SQ, Wang ZT (2008). Differentiation of the root of cultivated ginseng, mountain cultivated ginseng and mountain wild

ginseng using FT-IR and two-dimensional correlation IR spectroscopy. Journal of Molecular Structure 883:228-235.

Lu C, Zhao S, Wei G, Zhao H, Qu Q (2017). Functional regulation of ginsenoside biosynthesis by RNA interference of a UDP-glycosyltransferase gene in *Panax ginseng* and *Panax quinquefolius*. Plant Physiology and Biochemistry 111:67-76.

Mahfuzur R, Zamir KP (2005). Biochemistry of ginseng root tissues affected by rusty root symptoms. Plant Physiology and Biochemistry 43(12):1103-1114.

Mitra SK, Chakraborti A, Bhattacharya SK (1996). Neuropharmacological studies on *Panax ginseng*. Indian Journal of Experimental Biology 34(1):41-47.

Mork SK, Son SY, Park H (1981). Root and top growth of *Panax ginseng* at various soil moisture regime. Han'guk Changmul Hakhoe chi.= Journal of the Korean Society of Crop Science.

Neto AG, Costa JMLC, Belati CC, Vinholis AHC, Possebom LS, Da Silva Filho A. AA, Cunha WR, Carvalho JCT, Bastos JK, e Silva MLA (2005). Analgesic and anti-inflammatory activity of a crude root extract of *Pfaffiaglomerata* (*Spreng*) *Pedersen*. Journal of Ethnopharmacology 96(1-2):87-91.

Neves CS, Gomes SSL, dos Santos TR, de Almeida MM, de Souza YO, Garcia RMG, Otoni WC, Chedier LM, Raposo NRB, Viccini LF, de Campos JMS (2016). Brazilian ginseng (*PfaffiaglomerataSpreng. Pedersen, Amaranthaceae*) methanolic extract: cytogenotoxicity in animal and plant assays. South African Journal of Botany 106:174-180.

Ogbaji PO, Li J, Xue X, Shahrajabian MH, Egrinya EA (2018). Impact of bio-fertilizer or nutrient solution on Spinach (*Spinacea Oleracea*) growth and yield in some province soils of P.R. China. Cercetari Agronomice in Moldova 51(2):43-52.

Oh MJ, Kim MW, Kim M (2015). Ginseng may modify the progression of degenerative cerebellar ataxia: A report of two case. Neurology Asia 20(3):313-318.

Ohh SH (1981). Diseases of Ginseng: Environmental and hose effect on disease outbreak and growth of pathogens. Korean Journal of Ginseng Science (Korea R.).

Ohh SH, Park CS (1980). Study on phytophthora disease of *Panax ginseng C.A. Meyer*; its causal agent and possible control measures. Korean Journal of Ginseng Science.

Olgun Y, Kirkim G, Altun Z, Aktas S, Kolatan E, Kiray M, Bagriyanik A, Olgun A, Kizmazoglu DC, Ozogul C, Ellidokuz H, Ercetin P, Serbetcioglu B, Yilmaz O, Guneri EA (2016). Protective effect of Korean red ginseng on cisplatin ototoxicity: Is it effective enough? Journal of International Advanced Otology 12(2).

Pace R, Martinelli EM, Sardone N, Combarieu ED (2015). Metabolomic evaluation of ginsenosides distribution in *Panax genus* (*Panax ginseng and Panax quinquefolius*) using multivariate statistical analysis. Fitoterapia 101:80-91.

Park H (1979). Physiological response of *Panax ginseng* to temperature. Korean Journal of Ginseng Science 3:156-157.

Park HJ, Kim DH, Park SJ, Kim JM, Ryu JH (2012). Ginseng in traditional herbal prescription. Journal of Ginseng Research 36(3):225.

Park JY, Choi P, Kim HK, Kang KS, Ham J (2016). Increase in apoptotic effect of *Panax ginseng* by microwave processing in human prostate cancer cells: *in vitro* and *in vivo* studies. Journal of Ginseng Research 40(1):62-67.

Park TY, Hong M, Sung H, Kim S, Suk KT (2017). Effect of Korean red ginseng in chronic liver disease. Journal of Ginseng Research 41(4):450-455.

Patel S, Rauf A (2017). Adaptogenic herb ginseng (Panax) as medical food: status quo and future prospects. Biomedicine & Pharmacotherapy 85:120-127.

Pengelly A, Bennett K (2011). Appalachian plant monographs: *Panax quinquefolius L.*, American ginseng.

Proctor JTA, Lee JC, Lee SS (1990). Ginseng Production in Korea. HortScience 25(7):746-750.

Proctor JTA, Palmer JW (2017). Optimal light for greenhouse culture of American ginseng seedlings. Journal of Ginseng Research 41(3):370-372.

Punja ZK (2011). American ginseng: research development, opportunities, and challenges. Journal of Ginseng Research

35(3):368.

Qi F, Zhao K, Zhou A, Zhang B, Li A, Wang Z, Han J (2015). The advantages of using traditional Chinese medicine as an adjunctive therapy in the whole course of cancer treatment instead of only terminal stage of cancer. Bioscience Trends 9(1):16-34.

Qi LW, Wang CZ, Yuan CS (2011). Ginsenosides from American ginseng: chemical and pharmacological diversity. Phytochemistry 72:689-699.

Radad K, Gille G, Rausch WD (2004). Use of ginseng in Medicine: Perspectives on CNS disorders. Iranian Journal of Pharmacology and Therapeutics 3(2):30-40.

Reay JL, Kennedy DO, Scholey AB (2005). Single doses of *Panax ginseng* (G115) reduce blood glucose levels and improve cognitive performance during sustained mental activity. Journal of Psychopharmacology 19(4):357-365.

Rocha HAC, Rocha TV, Nobrega FJF, Morais LCS, Diniz MFFM (2018). Randomized controlled trial of *Panax ginseng* in patients with irritable bowel syndrome. Revista Brasileira de Farmacognosia 28(2):218-222.

Ryu KR, Yeom MH, Kwon SS, Rho HS, Kim DH, Kim HK, Yun KW(2012). Influence of air temperature on the histological characteristics of ginseng (*Panax ginseng C. A. Meyer*) in six regions of Korea. Australian Journal of Crop Science 6(12):1637

Schlag EM, McIntosh MS (2006). Ginsenoside content and variation among and within American ginseng (*Panax quinquefolius L.*) populations. Phytochemistry 67(14):1510-1519.

Schlag EM, McIntosh MS (2013). The relationship between genetic and chemotypic diversity in American ginseng (*Panax quinquefolius L.*). Phytochemistry 93:96-104.

Seervi C, Kirtawade R, Dhabale P, Salve P (2010). Ginseng-Multipurpose Herb. Journal of Biomed Science and Research 2(1):6-17.

Sengupta S, Toh SA, Sellers LA, Skepper JN, Koolwijk P, Leung HW, Yeung HW, Wong RNS, Sasisekharan R, Fan TPD (2004). Modulating angiogenesis: the yin and the yang in ginseng. Circulation 110:1219-1225.

Shahrajabian MH, Wenli S, Qi C (2018). A review of goji berry (*Lyciumbarbarum*) in traditional Chinese medicine as a promising organic superfood and superfruit in modern industry. Academia Journal of Medicinal Plants 6(12):437-445.

Shahrajabian MH, Wenli S, Qi C (2019a). The power of natural Chinese medicine, ginger and ginseng root in an organic life. Middle-East Journal of Science 27(1):64-71.

Shahrajabian MH, Wenli, S., Qi C (2019b). Clinical aspects and health benefits of ginger (Zingiber officinale) in both traditional Chinese medicine and modern industry. Acta Agriculturae Scandinavica, Section B—Soil & Plant Science 1-11.

Sharma J, Goyral PK (2015). Chemoprevention of chemical-induced skin cancer by *Panax ginseng* root extract. Journal of Ginseng Research 39(3):265-273.

Shi W, Wang Y, Li J, Zhang H, Ding L (2007). Investigation of ginsenosides in different parts and ages of *Panax ginseng*. Food Chemistry 102(3):664-668

Shibata S (2001). Chemistry and cancer preventing activities of ginseng saponins and some related triterpenoid compounds. Journal of Korean Medical Science 16(Suppl):S28.

Shin BK, Kwon SW, Park JH (2015). Chemical diversity of ginseng saponins from *Panax ginseng*. Journal of Ginseng Research 39(4):287-298.

Shin SS, Yoon M (2018). Korean red ginseng (*Panaxginseng*) inhibits obesity and improved lipid metabolism in high fat diet-fed castrated mice. Journal of Ethnopharmacology 210:80-87.

Shiobara Y, Inoue SS, Kato K, Nishiguchi Y, Oishi Y, Nishimoto N, Oliveria F, Akisue G, Akisue MK, Hasaimoto G (1993). A nortriterpenoid, triterpenoids and ecdysteroids from *Pfaffiaglomerata*. Phytochemistry 32:1527-1530.

Sievenpiper JK, Arnason JT, Leiter LA, Vuksan V (2004). Asian and American ginsengs act differently on acute glycemia. Herbal Gram 64:24.

Silvestrini P, Beccaria C, Pereyra EAL, Renna MS, Ortega HH, Calvinho LF, Dallard BE, Baravalle C (2017). Intramammary inoculation of *Panax ginseng* plays an immunoprotective role in *Staphylococcus*

*aureus*infection in a murine model. Research in Veterinary Science 115:211-220.

Soleymani A, Shahrajabian MH (2012). Response of different cultivars of fennel (*Foeniculum vulgare*) to irrigation and planting dates in Isfahan, Iran. Research on Crops 13(2):656-660.

Soleymani A, Shahrajabian MH (2018). Changes in germination and seedling growth of different cultivars of cumin to drought stress. Cercetari Agronomice in Moldova 51(1):91-100.

Song HH, Moon JY, Ryu HW, Noh BS, Kim JH, Lee HK, Oh SR (2014). Discrimination of white ginseng origins using multivariate statistical analysis of data sets. Journal of Ginseng Research 38(3):187-193.

Sotaniemi EA, Haapakoski E, Rautio A (1995). Ginseng therapy in non-insulin-dependent diabetic patients. Diabetes Care 18(10):1373-1375.

Sun BS, Gu LJ, Fang ZM, Wang CY, Wang Z, Lee MR, Li Z, Li JJ, Sung CK (2009). Simultaneous quantification of 19 ginsenosides in black ginseng developed from *Panax ginseng* by HPLC-ELSD. Journal of Pharmaceutical and Biomedical Analysis 50(1):15-22.

Sun L, Ropartz D, Cui L, Shi H, Ralet MC, Zhou Y (2019). Structural characterization of rhamnogalacturonan domains from *Panax ginseng C. A. Meyer*. Carbohydrate Polymers 203:119-127.

Sun L, Wu D, Ning X, Yang G, Lin Z, Tian M, Zhou Y (2015). α-Amylase-assisted extraction of polysaccharides from *Panax ginseng*. International Journal of Biological Macromolecules 75:152-157.

Sun S, Qi LW, Du GJ, Mehendale SR, Wang CZ, Yuan CS (2011). Red not ginseng: higher ginsenoside content and stronger anticancer potential than Asian and American ginseng. Food Chemistry 125(4):1299-1305.

Szymanska G, Kochan E, Szymczyk P (2013). Field cultivation and in vitro cultures, root-forming callus cultures and adventitious root cultures, of *Panax quinquefolium* as a source of ginsenosides. Zeitschrift für Naturforschung C 68(11-12):482-488.

Uluisik D, Keskin E (2016). Hepatoprotective effects of ginseng in rats fed cholesterol rich diet. Acta Scientiae Veterinariae 44:1-5.

Van Kampen JM, Baranowski DB, Shaw CA, Kay DG (2014). *Panax ginseng* is neuroprotective in a novel progressive model of Parkinson's disease. Experimental Gerontology 50:95-105.

Vardanega R, Carvalho PIN, AlbarelliJQq, Santos DT, Meireles MAA (2017). Techno-economic evaluation of obtaining Brazilian ginseng extracts in potential production scenarios. Food and Bioproducts Processing 101:45-55.

Vinh LB, Lee Y, Han YK, Kang JS, Park JU, Kim YR, Yang SY, Kim YH (2017). Two new dammarane-type triterpene saponins from Korean red ginseng and their anti-inflammatory effects. Bioorganic & Medicinal Chemistry Letters 27(23):5149-5153.

Wan JY, Fan Y, Yu QT, Ge YZ, Yan CP, Alolga RN, Li P, Ma ZH, Qi LW (2015). Integrated evaluation of malonyl ginsenosides, amino acids and polysaccharides in fresh and processed ginseng. Journal of Pharmaceutical and Biomedical Analysis 107:89-97.

Wan JY, Yao H, Zhang CF, Huang WH, Zhang Q, Liu Z, Bi Y, Williams S, Wang CZ, Yuan CS (2018). Red American ginseng enhances the effect of fluorouracil on human color cancer cells via both paraptosis and apoptosis pathways. Journal of Applied Biomedicine 16(4):311-319.

Wang CZ, Zhang B, Song WX, Wang A, Ni M, Luo X, Aung HH, Xie JT, Tong R, He TC (2006). Steamed American ginseng berry: ginsenoside analyses and anticancer acitivites. Journal of Agricultural and Food Chemistry 54(26):9936-9942.

Wang HX, Ng TB (2004). A ribonuclease from Chinese ginseng (*Panax ginseng*) flowers. Protein Expression and Purification 33(2):195-199.

Wei Y, Zhao W, Zhang Q, Zhao Y, Zhang Y (2011). Purification and characterization of a novel and unique ginsenoside Rg1-hydrolyzing b-D-glucosidase from Penicillium sclerotiorum. Acta Biochim Biophys Sin 43(3):226-231.

Wen J, Zimmer EA (1996). Phylogeny and biogeography of *Panax L.* (the ginseng genus, araliaceae): inferences from ITS sequences of nuclear ribosomal DNA. Molecular Phylogenetics and Evolution 6(2):167-177.

Wu YY, Cui YN, Zhang TY, Li W, Zhang MY, Cheng J, Wang Y, Wang J, Zhao YQ, Zhang YX (2018). Transformation of ginsenoside Rh4 and its aglycone from the total saponins of stems and leaves of *Panax ginseng* by *Aspergillus tubingensis*. Phytochemistry Letters 27:123-128.

Xie JT, McHendale S, Yuan CS (2005). Ginseng and diabetes. The American Journal of Chinese Medicine 33(03):397-404.

Xie YY, Luo D, Cheng YJ, Ma JF, Wang YM, Liang QL, Luo GA (2012). Steaming-induced chemical transformations and holistic quality assessment of red ginseng derived from *Panax ginseng* by means of HPLC-ESI-MS/MS n-based multicomponent quantification fingerprint. Journal of Agricultural and Food Chemistry 60(33):8213-8224.

Xu XF, Gao Y, Xu SY, Liu H, Xue X, Zhang Y, Zhang H, Liu MN, Xiong H, Lin RC, Li XR (2018). Remarkable impact of steam temperature on ginsenosides transformation from fresh ginseng to red ginseng. Journal of Ginseng Research 42(3):277-287.

Youssef GA (2016). Role of Ginseng as Hepatoprotective, Antioxidant and Anti-Inflammatory against Methotrexate Induced Liver Injury in Rats. The Egyptian Journal of Hospital Medicine 62:105-108.

Yun TK (2001). Brief introduction of *Panax ginseng C.A. Meyer*. Journal of Korean Medical Science 16(Suppl):S3.

Zhang H, Jiang JM, Zheng D, Yuan M, Wang ZY, Zhang HM, Zheng CW, Xiao LB, Xu HX (2019). A multidimensional analytical approach based on time-decoupled online comprehensive two-dimensional liquid chromatography couples with ion mobility quadrupole time-of-flight mass spectrometry for the analysis of ginsenosides from white and red ginsengs. Journal of Pharmaceutical and Biomedical Analysis 163:24-33

Zhang HM, Li SL, Zhang H, Wang Y, Zhao ZL, Chen SL, Xu HX (2012). Holistic quality evaluation of commercial white and red ginseng using a UPLC-QTOF-MS/MS-based metabolomics approach. Journal of Pharmaceutical and Biomedical Analysis 62:258-273.

Zhang X, Yu L, Bi H, Li X, Ni W, Han H (2009). Total fractionation and characterization of the water-soluble polysaccharides isolated from *Panax ginseng C. A. meyer*. Carbohydrate Polymers 77(3):544-552.

Zhang Y, Zhang J, Liu C, Yu M, Li S (2017). Extraction, isolation, and aromatase inhibitory evaluation of low-polar ginsenosides from *Panax ginseng* leaves. Journal of Chromatography A 1483:20-29.

Zhang Y, Zhang Y, Aboueloyoun Taha A, Ying Y, Li X, Chen X, Ma C (2018). Subcritical water extraction of bioactive components from ginseng roots (*Panax ginseng C.A. Mey*). Industrial Crops and Products 117:118-127.

Zhao H, Xu J, Ghebrezadik H, Hylands PJ(2015). Metabolomic quality control of commercial Asian ginseng, and cultivated and wild American ginseng using ^{1}H NMR and multi-step PCA. Journal of Pharmaceutical and Biomedical Analysis 114:113-120.

Zhao Q, Zhao M, Ye X, He M, Yang Y, Gao H, Zhang X (2019). Rapid discrimination between red and white ginseng based on unique mass-spectrometric features. Journal of Pharmaceutical and Biomedical Analysis 164:202-210.

Zheng BC (1985). Shennong's herbal--one of the world's earliest pharmacopoeia. Journal of traditional Chinese medicine= Chung i tsa chih ying wen pan 5(3):236-236.

Zhou QL, Yang XW (2015). Four new ginsenosdies from red ginseng with inhibitory activity on melanogenesis in melanoma cells. Bioorganic & Medicinal Chemistry Letters 25(16):3112-3116.

Zhou QL, Zhu DN, Yang XW, Xu W, Wang YP (2018). Development and validation of a UFLC-MS/MS method for simultaneous quantification of sixty-six saponins and their six aglycones: Application to comparative analysis of red ginseng and white ginseng. Journal of Pharmaceutical and Biomedical Analysis 159:153-165.

AJ ACADEMIC JOURNALS
expand your knowledge

Journal of Medicinal Plants Research

Full Length Research Paper

In vitro anti-inflammatory activity of ginger (*Zingiber officinale* Rosc.) rhizome, callus and callus treated with some elicitors

Ammar Mohammed Ahmed Ali[1,2]*, Mawahib ElAmin Mohamed El-Nour[2], Owais Mohammad[3] and Sakina Mohamed Yagi[4]

[1]Department of Biology, Faculty of Education, Hajjah University, Yemen.
[2]Department of Biology and Biotechnology, Faculty of Science and Technology, AL Neelain University, Khartoum, Sudan.
[3]Molecular Immunology Lab1, Biotechnology Unit, Aligarh Muslim University, Aligarh, India.
[4]Department of Botany, Faculty of Science, University of Khartoum, Sudan.

This study evaluated the effect of ginger rhizome and its callus as well as callus elicited by yeast extract, glycine and salicylic acid on the production of pro-inflammatory (TNF-α, IL-1 and IL-6) and anti-inflammatory (IL-10 and TGF-β) cytokines *in vitro*. Petroleum ether (PE) and chloroform; methanol (1:1) (CM) extracts of rhizome and callus were shown to significantly ($P < 0.05$) suppress in a dose-dependent manner the LPS-induced production of TNF-α, IL-1 and IL-6. Both callus extracts showed significantly ($P < 0.05$) higher ability than the rhizome extracts. CM extract of ginger callus treated with elicitors showed significant ($p < 0.05$) capacity to inhibit IL-1, IL-6 and TNF-α secretion at highest concentration used (100 µg/mL) when compared to control (untreated callus). Elicitors improved significantly ($P < 0.05$) the callus capacity to produce the IL-10 and TGF-β anti-inflammatory cytokines. HPLC analysis showed that 6-gingerol and 6-shogaol were found in both extracts of rhizome, but were not detected in the callus extracts. Furthermore, gallic acid was found only in CM extracts of rhizome (34.05 ± 0.39 µg/mg) and callus (17.88 ± 0.01 µg/mg). Yeast extract, salicylic acid and glycine elicitors enhanced significantly ($p < 0.05$) the production of gallic acid in callus CM extract where the highest content was obtained from callus elicited with 100 mg/L of yeast extract followed by callus elicited with 50 mg/L of salicylic acid and 200 mg/L of glycine, respectively. Therefore, ginger callus could be included in nutraceutical formulations where it could provide valuable protection against inflammatory diseases.

Key words: Anti-inflammatory, ginger, callus, elicitors.

INTRODUCTION

Zingiber officinale or ginger (Zingiberaceae), is indigenous to tropical Asia, and occur particularly in southern China and India. It was introduced by the Arab traders to Africa continent (Semwal et al. 2015). In Yemen it is used as an aphrodisiac and an aromatic stimulant (Fleurentin and Pelt, 1982). A number of

scientific studies have proven the biological properties of ginger such as antioxidant (Stoilova et al., 2007; Mošovskáa et al., 2015), anti-inflammatory (Minghetti et al., 2007; Barari, 2016), anticancer (Cheng et al., 2011) and anti-diabetic (Afshari et al., 2007) activities. The safety of *Z. officinale* has been investigated. Acute and chronic toxicity studies have demonstrated a broad safety of *Z. officinale* (Daily et al*.,* 2015).The nutraceutical value of ginger is mainly attributed to gingerols, shogaols and zingiberene (Butt and Sultan, 2011).

Plant tissue and cell suspension cultures have been used to produce interesting bioactive secondary metabolites (Sák et al., 2014). Furthermore, it is reported that application of elicitors like salicylic acid, yeast extract and glycine induces the production of bioactive molecules from *in vitro* cultures (Namdeo, 2007). Elicitors including yeast extract, glycine and salicylic acid augmented significantly the total phenolic content of ginger callus, and consequently its antioxidant capacity (Ali et al., 2018).

The use of ginger infusions for relieving the pain of rheumatism and arthritis have prompted researchers to investigate the anti-inflammatory activity of ginger and the chemical compounds responsible for this activity (Tripathi et al., 2008; Ahn et al*.,* 2009; Semwal et al., 2015; Srinivasan, 2017). However, there is no report on the effect of ginger callus or its callus treated with specific elicitors on the production of cytokines which play the major role in the inflammatory process. The present study was performed to investigate the effect of ginger rhizome and its callus/treated callus with different concentrations of yeast extract, glycine and salicylic acid on the *in vitro* production of pro-inflammatory and anti-inflammatory cytokines.

MATERIALS AND METHODS

Plant materials

Fully matured rhizomes of ginger were collected from the botanical garden at the Biology and Biotechnology Department, Faculty of Science and Technology, Al-Neelain University, Khartoum, Sudan. Rhizomes were well cleaned, cut into thin slices, and dried at room temperature.

Callus induction and proliferation

Ginger callus was initiated and proliferated as described by Ali et al. (2016). Highest fresh weight of callus was induced from shoot tip explants on MS medium fortified with 0.5 mg/L 2,4-dichloro-phenoxyacetic acid (2,4-D) and proliferated on the same medium and concentration of 2,4-D.

Elicitor's treatments

Callus elicited by yeast extract (100, 300 and 500 mg/L), glycine (100, 200 and 300 mg/L) and salicylic acid (100 and 200 mg/L) was prepared using the protocol published by Ali et al. (2018).

Preparation of extracts

Two types of extracts were prepared by sequential maceration of each sample (rhizome, callus and treated callus) in petroleum ether (PE) and chloroform: methanol (1:1, v/v) (CM) (Ali et al., 2018).

Cell culture

THP cells (human acute monocytic leukemia cell line) were acquired from American type culture collection. Culture medium was Dulbecco's modified eagle's medium (DMEM) containing glucose (4.5 g/L), fetal bovine serum (10% v/v), nonessential amino acids (1%), glutamine (1%), streptomycin (10 µg/mL) and penicillin (100 U/mL). Cell line was cultured at 37°C with 5% humidified CO_2. Cells were activated with Vitamin D3 (0.1 µM) and PMA (100 ng/mL). After that, the cells were exposed to different concentrations (25, 50 and100 µg /mL) of ginger rhizome, callus, treated callus extracts and 50 µg/mL of 6-gingerol and 6-shogaol in a fresh serum-free medium in the presence of LPS (1 µg/mL).

Cytokine enzyme linked immunosorbent assay (ELISA)

Sterile solutions of samples (DMSO dissolved) were incubated with THP cells for 72 h. Afterward, the levels of various cytokines in culture supernatants were measured using an ELISA via cytokine ELISA set (R and D Systems, USA). ELISA was performed according to the manufacturer's protocol. The absorbances were read at 450 nm with a microtiter plate reader (Genetix GMB-580). Cytokine levels, expressed as pg/mL, were calculated from the standard curve (Ahmad et al., 2017).

HPLC analysis of ginger rhizome and callus extracts

Preparation of sample solutions

Accurate weight (20 mg) of ginger rhizome and callus extracts was dissolved separately in HPLC grade chloroform: Methanol 1:1 (10 mL). Volume of extracts was prepared with methanol up to 20 mL to give stock solutions (1 mg/mL), and filtered through 0.45 µ filter. Further, all solutions were subjected to HPLC analysis for simultaneous estimation of 6-Gingerol, 6-Shogaol and gallic acid. To prepare the standards solution, 6-gingerol, 6-shogaol and gallic acid (0.0625, 0.125, 0.250, 0.500 and 1 mg/mL) were dissolved in HPLC grade methanol. All standards solutions were stored at 4°C until used.

HPLC conditions

Analysis of ginger rhizome and callus extracts, along with reference compounds was performed using a gradient HPLC system (Waters, USA) as described by Mahadik et al. (2013). Liquid chromatographic separations were performed on an Inertsil ODS-3 column with a dimension of 250 x 4.6 mm, 5 µm particles size (Thermo Scientific, Waltham, USA). The mobile phase was consisting of acetonitrile and (0.05%) ortho-phosphoric acid (85:15, v/v). Flow rate was 1.0 mL/min, column temperature was 30°C and detection wavelength was set at 227 nm. All operations and data analysis were controlled using the Chemstation software (Empower). Identification of the three compounds in the extracts was achieved by comparison of their retention times with standards, UV spectra and UV absorbance ratios after co-injection of samples and standards. The content of 6-gingerol, 6-shogaol and gallic acid were calculated by using the standard calibration curves. The calibration curves of 6-gingerol, 6-shogaol and gallic acid were

constructed with the correlation coefficients (R^2) 0.9998, 0.9983 and 0.9994 respectively and regression equations; y=24356x + 296180, y=46021x - 108135 and y= 41274x + 155145 respectively.

Statistical analysis

All data were analyzed using SPSS version 19. The values were expressed as mean ± standard deviation (SD). Significant differences between samples were detected by analysis of variance (ANOVA) followed by Duncan's multiple-range test ($p < 0.05$).

RESULTS AND DISCUSSION

Effect of ginger rhizome and callus extracts on pro-inflammatory and anti-inflammatory cytokines

Biological therapies for inflammatory diseases involve generally the suppression of members of the inflammatory cascade, like cytokines (Kim et al., 2013). In this study, PE and CM extracts of ginger rhizome and callus induced a dose-dependent inhibition of the production of pro-inflammatory cytokines (TNF-α, IL-1 and IL-6) by LPS activated macrophages.

Rhizome PE and CM extracts displayed more or less the same capacity to inhibit TNF-α. However, both callus extracts showed significantly ($p < 0.05$) higher activity than the rhizome extracts. At highest concentration (100 µg/mL) the activity of the PE and CM callus extracts to inhibit TNF-α production increased significantly ($P < 0.05$) by 19 and 58% respectively than their corresponding rhizome extracts as depicted in Figure 1A. Moreover, both rhizome extracts displayed significant ($P < 0.05$) capacity to inhibit IL-1 production with higher activity observed for PE. The ability of callus PE and CM extracts to inhibit IL-1 at concentration 100 µg/mL, increased significantly ($P < 0.05$) by 30.4 and 61.2% respectively compared to their corresponding rhizome extracts as shown in Figure 1B.

PE extracts of ginger rhizome and callus displayed more or less the same capacity to inhibit the IL-6 production; however, the inhibition capacity for IL-6 production of the CM callus extract at concentrations 50 and 100 µg/mL, increased significantly ($P < 0.05$) by 23.8 and 75.9% respectively than those of the rhizome as presented in Figure 1C. TGF-β involved in cell growth and differentiation where it is known to act as a multifunctional cytokine (Derynck et al., 2001). In addition, IL-10 and TGF-β are known to play an important role in tolerance induction and immune regulation (Van Kooyk et al., 2004). In this study, extracts of ginger rhizome and callus did not induce the production of IL-10 while CM extracts of rhizome and callus slightly, but not significantly, induced the production of TGF-β anti-inflammatory cytokine. These results supported earlier reports on the ginger anti-inflammatory effect. However, many previous studies showed that gingerols were the most typical active components contributing to the anti-

inflammatory effect of ginger (Tripathi et al., 2008; Ahn et al., 2009; Semwal et al., 2015).

Some other studies demonstrated that shogaols were more potent than gingerols (Dugasani et al., 2010). Ha et al. (2012) reported that 6-shogaol displayed higher anti-inflammatory activity than 6-gingerol by inhibiting prostaglandin E2 and pro-inflammatory cytokines production. Other researchers like Ho et al. (2013) showed that 10-Gingerol as well as shogaols inhibited neuro-inflammation in a LPS-activated BV2 microglia. In our study, 6-gingerol and 6-shogaol showed strong significant ($P < 0.05$) activity to inhibit TNF-α, IL-1 and IL-6 production with higher capacity for 6-gingerol towards IL-1 inhibition.

Moreover, 6-gingerol promoted significantly ($P < 0.05$) the production of IL-10 and TGF-β, while 6-shogaol induced only TGF-β production and with lower effect than 6-gingerol as shown in Figure 1 D and F. It was also observed that the rhizome extracts possessed lower activity than the pure 6-gingerol and 6-shogaol suggesting the presence of molecules in rhizome that may interfere with activation of these compounds and their ability to suppress the production of pro-inflammatory cytokines; IL-1, IL-6 and TNF-α. Although 6-gingerol was not detected in both callus extracts, the callus showed significant ($P < 0.05$) higher capacity to inhibit the production of pro-inflammatory cytokines than that exhibited by the rhizome extracts suggesting the presence of other anti-inflammatory agents. The effect of callus tissues treated with different levels of yeast extract, glycine and salicylic acid elicitors on anti-inflammatory activity was also carried out and results are presented in Figure 2

In general, CM extract of ginger callus treated with elicitors showed significant ($P < 0.05$) capacity to inhibit the production of pro-inflammatory cytokines (IL-1, IL-6 and TNF-α) at highest concentration used (100 µg/mL) compared to untreated callus. Callus elicited with 100, 300, 500 mg/L yeast extracts improved significantly ($P < 0.05$) its capacity, at 100 µg/mL to inhibit TNF-α production by 18, 39 and 75% respectively, while callus elicited with 50, 100 mg/L salicylic acid enhanced significantly ($P < 0.05$) its capacity, at 100 µg/mL by 19 and 41% respectively. However, the glycine only showed significant ($P < 0.05$) effect at concentration 300 mg/L where it improved the inhibition capacity of callus to produce TNF-α cytokine by 51% as presented in Figure 2A. On the other hand, glycine at 300 mg/L and salicylic acid (50, 100 mg/L) elicitors improved significantly ($P < 0.05$) the callus capacity, at 100 µg/mL to inhibit IL-1 production by 66, 58 and 52% respectively, as shown in Figure 2B.

Inhibition of IL-6 production was significantly ($P < 0.05$) obtained from callus elicited with 300 and 500 mg/L yeast and 100 mg/($P < 0.05$) salicylic acid where it increased the callus capacity by 74, 59 and 14% respectively, as presented in Figure 2C. Interestingly, elicitors improved
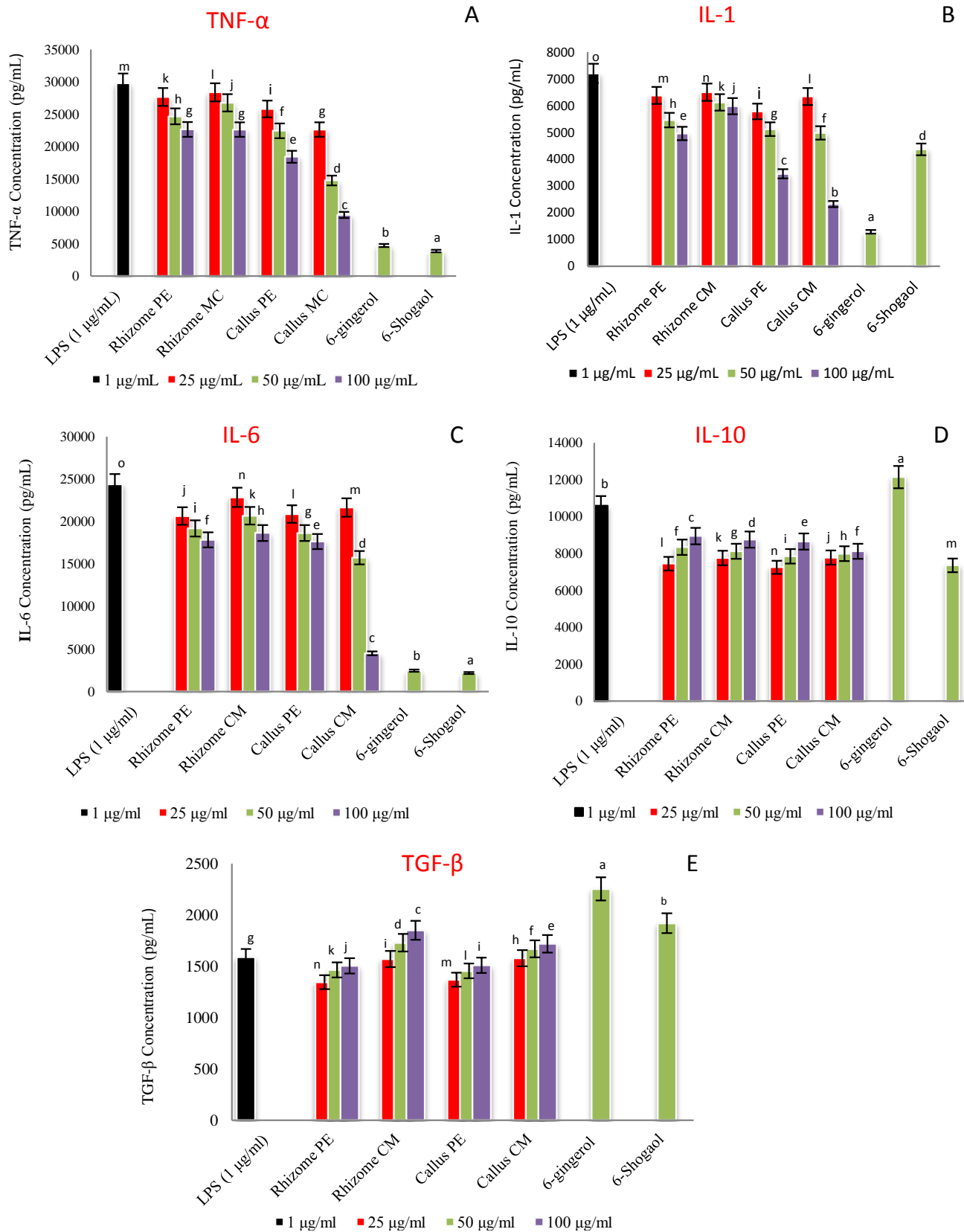
Figure 1. Effect of ginger rhizome and untreated callus petroleum ether (PE) and chloroform: methanol (1:1, v/v) (CM) extracts on pro-inflammatory and anti-inflammatory cytokines in lipopolysaccharide activated macrophages. Different letters indicate groups that differ statistically p<0.05.
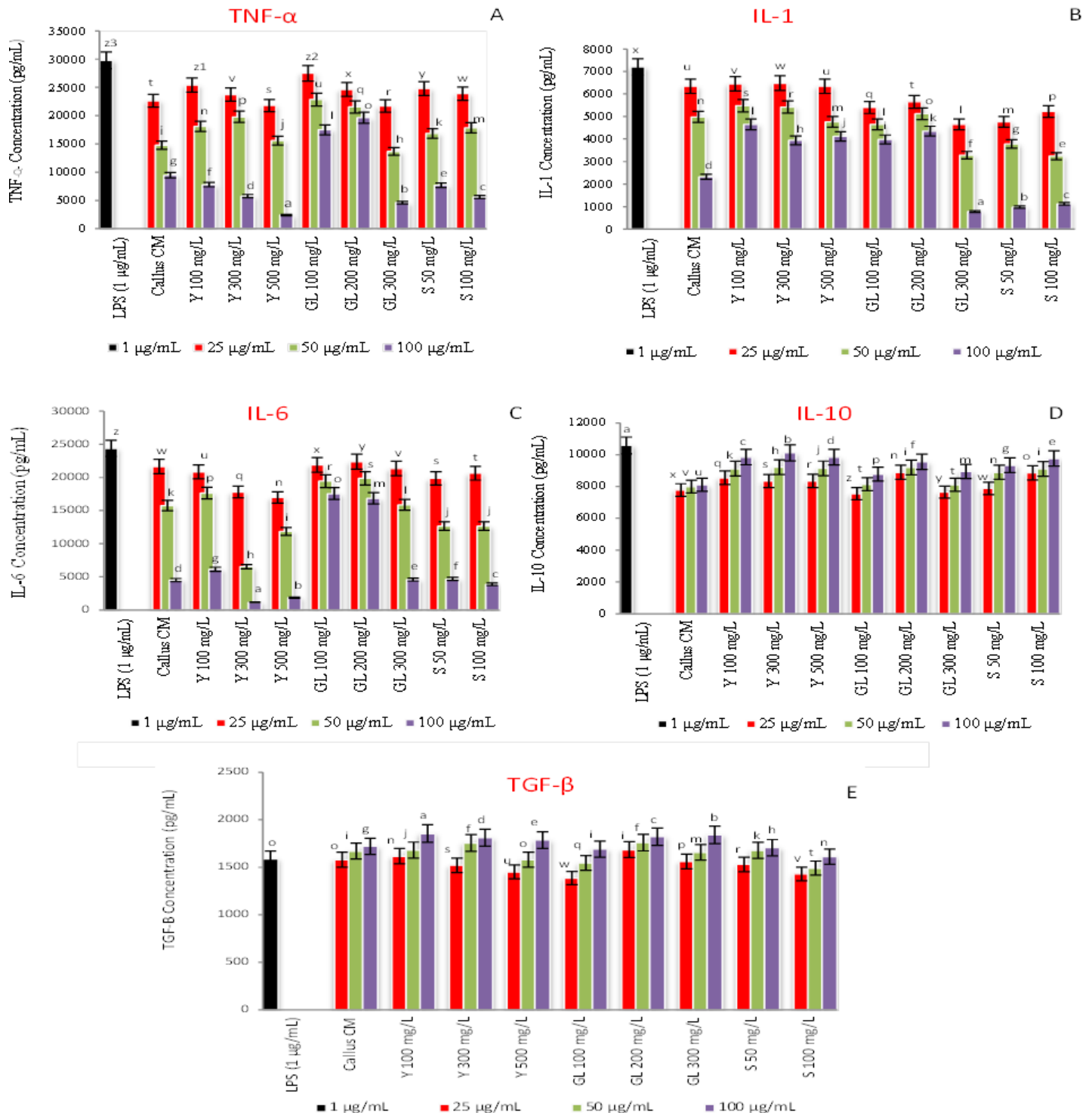
Figure 2. Effect of ginger callus CM extracts treated with elicitors on pro-inflammatory and anti-inflammatory cytokines in lipopolysaccharide activated macrophages. Y, Yeast extract; GL, glycine; S, salicylic acid. Different letters indicate groups that differ statistically p<0.05.

the callus capacity to produce the IL-10 and TGF-β anti-inflammatory cytokines. Callus treated with yeast extract (100, 300, 500 mg/L) increased significantly (*P* < 0.05) the production of IL-10 in a concentration dependent manner where highest increase (21, 25 and 21%

respectively) was obtained at 100 mg/L of CM extract. Glycine (100, 200, 300 mg/L) improved significantly (*P* < 0.05) the IL-10 production of callus by 8, 18 and 10% respectively, while salicylic acid (50, 100 mg/L) by 15 and 20% respectively as shown in Figure 2D.
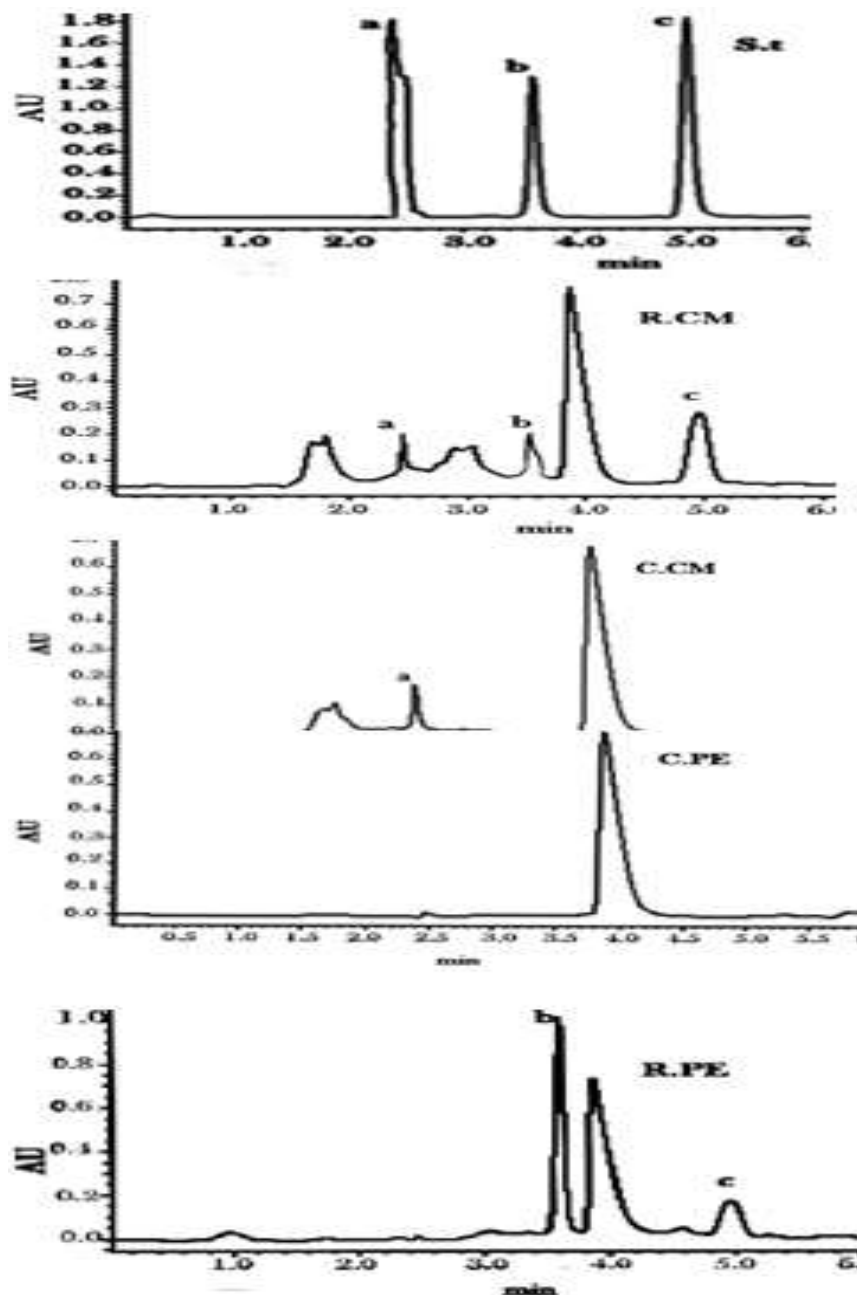
Figure 3. HPLC analysis of ginger rhizome and untreated callus extracts. Standards (St), gallic acid (a), 6-gingerol(b), 6-shogaol (c); rhizome chloroform: methanol (R.CM), callus chloroform: methanol (C.CM), callus petroleum ether (C. PE), rhizome petroleum ether (R. PE) extract.

However, yeast (100, 300, 500 mg/L) and glycine (200 and 300 mg/L) elicitors slightly enhanced the capacity of callus at concentration 100 mg/L of CM extract to produce TGF-β anti-inflammatory cytokine by 8, 5 and 4% respectively for yeast elicitor and by 6 and 7% respectively for glycine elicitor as depicted in Figure 2E. These results suggested that the type and concentration of elicitor may have influenced the secondary metabolite synthesis and thus influence their anti-inflammatory

potentiality (Karwasara et al., 2011).

HPLC profile of ginger rhizome and callus extracts

Qualitative and quantitative analysis for presence of 6-gingerol, 6-shogaol and gallic acid in ginger rhizome and callus extracts was also carried out using HPLC, and results are illustrated in Figures 3 and 4. Comparing the

Table 1. Concentration of gallic acid, 6-gingerol and 6-shogaol in rhizome and callus extracts of *Zingiber officinale.*

Extract of *Zingiber officinale*		Extract	Gallic acid (µg/mg)	6-Gingerol (µg/mg)	6-Shogaol (µg/mg)
Rhizome		PE	-	191.21 ± 0.00[b]	48.34 ± 0.00 [a]
Rhizome		CM	34.05 ± 0.39[i]	61.67 ± 0.00[a]	80.46 ± 0.02[b]
Callus		PE	-	-	-
Callus		CM	17.88 ± 0.01[d]	-	-
Callus treated with yeast extract	100 mg	CM	24.97 ± 0.41[h]	-	-
	300 mg	CM	18.01 ± 0.01[d]	-	-
	500 mg	CM	16.34 ± 0.52[c]	-	-
Callus treated with Glycine	100 mg	CM	8.77 ± 0.00[a]	-	-
	200 mg	CM	21.3 ± 0.66[f]	-	-
	300 mg	CM	12.22 ± 0.10[b]	-	-
Callus treated with salicylic acid	50 mg	CM	23.04 ± 0.09[g]	-	-
	100 mg	CM	20.43 ± 0.01[e]	-	-

PE, Petroleum ether; CM, chloroform: methanol (1:1, v/v). Different letters indicate groups that differ statistically (p<0.05).

chromatograms of rhizome extracts with that of the standards, it was observed that 6-gingerol and 6-shogaol were only detected in both extracts (PE and CM) of ginger rhizome, while callus extracts were devoid of these two standards as illustrated in Figure 3. 6-gingerol was higher than that of 6-shogaol where the former had the highest concentration (191.21 ± 0.00 µg/mg) in the PE extract of the rhizome and the latter displayed highest content (80.46 ± 0.02 µg/mg) in the CM extract as illustrated in Table 1.

These results were consistent with those reported by Kizhakkayil and Sasikumar (2012) who determined the levels of gingerols and shogaols in the oils of 46 ginger accessions using HPLC. They found that 6- gingerol was the predominant one among the identified gingerols and 6-shogaol was also present in all the samples but with relatively low concentration when compared with 6-gingerol. The absence of 6-gingerol and 6-shogaol incallus extracts might be attributed to the dedifferentiation of callus culture. Pawar et al. (2015) investigated the content of 6-gingerol in ginger callus, micro-propagated rhizome and rhizomes growing conventionally using HPLC analysis. They found that, callus culture and micro-propagated plants contained lower amounts of 6-gingerol than that amounted in conventionally grown plants. These results suggested that, the dedifferentiation of callus and cells cultured *in vitro* is often associated with reduction of secondary metabolites content.

Furthermore, HPLC analysis of ginger rhizome and callus extracts showed that gallic acid was found in CM extracts of rhizome (34.05 ± 0.39 µg/mg) and callus (17.88 ± 0.01 µg/mg) and was not detected in their PE extracts as illustrated in Table 1. Gallic acid was reported as one of the most abundant phenolic acid in ginger (Ghasemzadeh and Ghasemzadeh, 2011). Treatment of callus with different concentrations of elicitors (yeast extract, glycine and salicylic acid) showed variable effect on the production of gallic acid in callus CM extracts as illustrated in Table 1 and Figure 4.

Highest content in gallic acid was obtained in callus elicited with yeast at concentration 100 mg/L where gallic acid content production increased significantly ($P < 0.05$) by 40% when compared with untreated callus. Elicitation of callus with yeast at concentration 300 mg/L did not affect the production of gallic acid while at higher concentration (500 mg/L), the production of gallic acid was reduced slightly. The effect of yeast extract, which is known as biotic elicitors mainly composed of amino acids, minerals and vitamins is attributed to its role in increasing the activity of phenylalanine ammonia lyase, a key enzyme of phenylpropanoid pathway that catalyses' deamination of L-phenylalanine and trans-cinnamic acid production that links primary metabolism to the secondary one, and consequently the formation of vast secondary metabolites with phenylpropanoid skeleton (Seidel et al., 2002). Furthermore, the elicitation effects of yeast extract might be due to the presence of cations such as Ca, Zn and Co (El-Nabarawy et al., 2015).

Elicitation of callus with glycine showed that only at concentration 200 mg/L the production of gallic acid was increased significantly ($P < 0.05$) by 19%, while it reduced significantly ($P < 0.05$) at concentrations 100 and 300 mg/L. Molnár et al. (2011) reported that some amino acids like glycine, arginine and lysine, and vitamins such as nicotinic acid and thiamine, could serve as replacements for yeast extract, where it enhances growth in media containing relatively low concentrations of
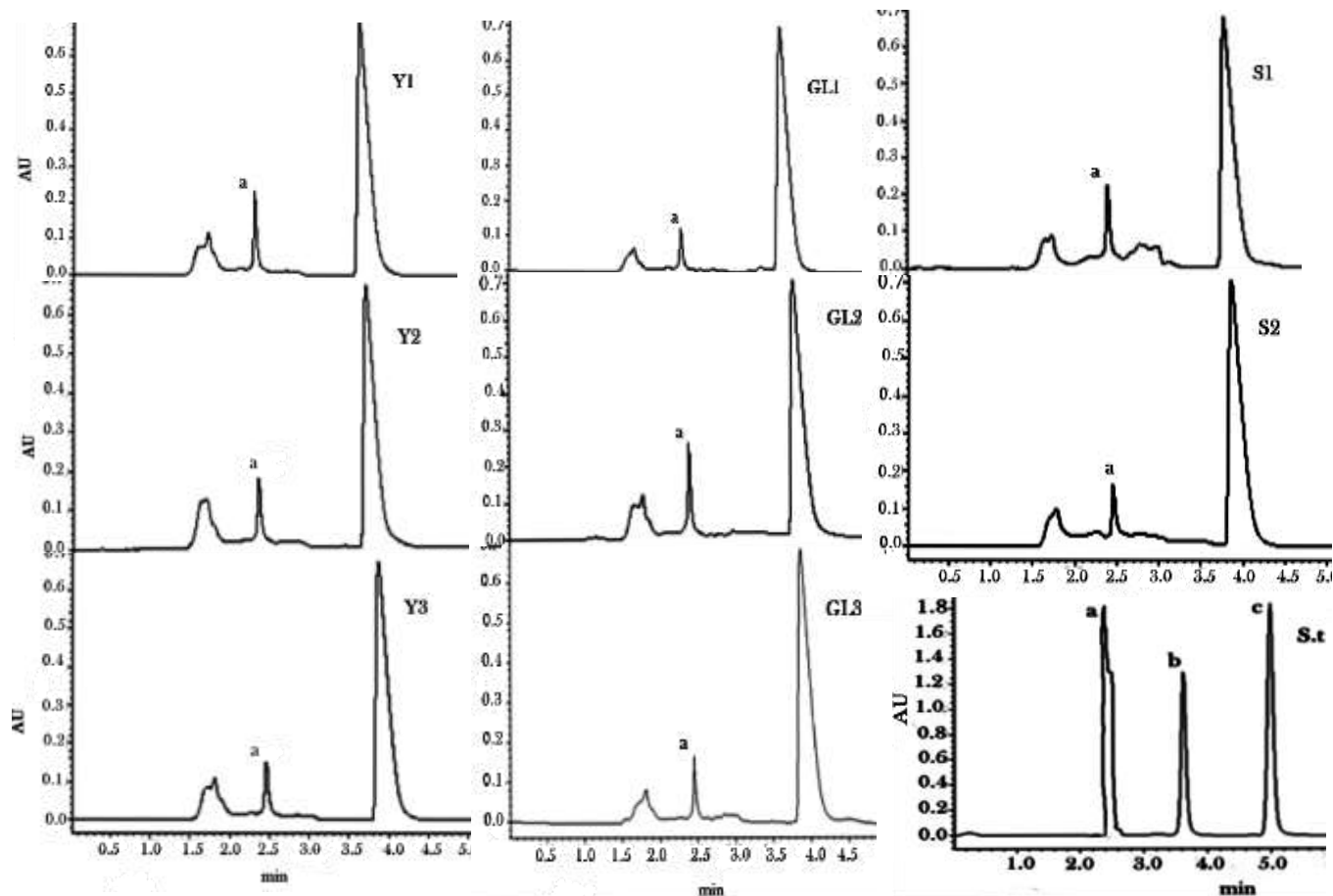
Figure 4. HPLC analysis of ginger callus treated with elicitors. Standards (St), gallic acid (a), 6-gingerol(b), 6-shogaol (c); yeast extract 100 mg (Y1), yeast extract 300 mg (Y2), yeast extract 500 mg (Y3), glycine 100 mg (GL1), glycine 200 mg(GL2), glycine 300 mg(GL3), salicylic acid 50 mg(S1), salicylic acid 100 mg(S2).

nitrogen or where vitamins are lacking.

The production of gallic acid was increased significantly ($P < 0.05$) by 29 and 14% in callus elicited with salicylic acid at concentrations 50 and 100 mg/L respectively. This result was in agreement with that of Ghasemzadeh and Jaafar (2012) who found that the content of phenolic acids including gallic acid increased significantly in two ginger varieties treated with different concentrations of salicylic acid. Moreover, they observed that the increase in phenolic compounds was associated with an increase in total soluble carbohydrate and a decrease in total flavonoids.

Thus, it was clear that yeast, salicylic acid and glycine elicitors enhanced the production of gallic acid where the highest content was obtained from callus elicited with 100 mg/L of yeast followed by callus elicited with 50 mg/L of salicylic acid and 200 mg/L of glycine respectively. Thus, it could be suggested that gallic acid participated in the anti-inflammatory activity of ginger callus.

Conclusion

In summary, the data of the present study demonstrated

that, callus CM extract showed significant ($P < 0.05$) higher ability than the rhizome extracts to inhibit TNF-α, IL-1 and IL-6 production. Gallic acid was found in rhizome and callus while 6-ginger and 6-shogaol were only detected in the rhizome. Yeast, salicylic acid and glycine elicitors enhanced the production of gallic acid in callus and consequently its ability to modulate pro-inflammatory and anti-inflammatory cytokines release.

Furthermore, the results suggested that in addition to gingerols and shogaols, gallic acid might also contribute in the immunomodulatory effect of ginger rhizome, while in callus; gallic acid might play a major role in their anti-inflammatory activity. We speculate that, ginger callus could be included in nutraceutical formulations where it could provide valuable protection against inflammatory diseases.

Higher amounts of biomass accumulation could be obtained in cell suspension culture of ginger on a commercial level. Other biotic elicitors like glycoproteins and abiotic elicitors like ultraviolet radiation and heavy metals could be tested in order to generate more secondary metabolism from ginger culture for pharmaceutical and food industries.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

REFERENCES

Afshari AT, Shirpoor A, Farshid A, Saadatian R, Rasmi Y, Saboory E (2007). The effect of ginger on diabetic nephropathy, plasma antioxidant capacity and lipid peroxidation in rats. Food Chemistry 101:148-153.

Ahmad F, Zubair S, Gupta P, Gupta UD, Patel R, Owais M (2017). Evaluation of aggregated Ag85B antigen for its biophysical properties, immunogenicity, and vaccination potential in a murine model of tuberculosis infection. Frontiers in Immunology 8:1-19.

Ahn SI, Lee JK, Youn HS (2009). Inhibition of homodimerization of toll-like receptor 4 by 6-shogaol. Molecules and Cells 27:211-215.

Ali AMA, El-Nour M EM, Yagi SM (2018). Total phenolic and flavonoid contents and antioxidant activity of ginger (*Zingiber officinale* Rosc.) rhizome, callus and callus treated with some elicitors. Journal of Genetic Engineering and Biotechnology 16:67-682.

Ali AMA, El-Nour MEM, Yagi SM (2016). Callus induction, direct and indirect organogenesis of ginger (*Zingiber officinale* Rosc). African Journal of Biotechnology 38:2106-2114.

Barari AR (2016). Effect of ginger supplementation and endurance training on serum levels of IL-1α and TNF-α. Medical Laboratory Journal 10:58-63. https://doi.org/ 10.18869/acadpub.mlj.10.4.63.

Butt MS, Sultan MT (2011). Ginger and its health claims: molecular aspects. Critical reviews in food Science and Nutrition 51(5):383-393.

Cheng X, Liu Q, Peng Y, Qi L, Li P (2011). Steamed ginger (*Zingiber officinale*): Changed chemical profile and increased anticancer potential. Food Chemistry 129(4):1785-1792.

Daily JW, Yang M, Kim DS, Park S (2015). Efficacy of ginger for treating Type 2 diabetes: A systematic review and meta-analysis of randomized clinical trials. Journal of Ethnic Foods 2:36-43.

Derynck R, Akhurst RJ, Balmain A (2001). TGF-beta signaling in tumor suppression and cancer progression. Nature Genetics 29:117-129.

Dugasani S, Pichika MR, Nadarajah VD, Balijepalli MK, Tandra S, Korlakunta JN (2010). Comparative antioxidant and anti-inflammatory effects of [6]-gingerol, [8]-gingerol, [10]-gingerol and [6]-shogaol. Journal of Ethnopharmacology 127:515-520.

El-Nabarawy MA, El-Kafafi SH, Hamza MA, Omar MA (2015). The effect of some factors on stimulating the growth and production of active substances in *Zingiber officinale* callus cultures. Annals of Agricultural Sciences 1:1-9.

Fleurentin J, Pelt JM (1982). Repertory of drugs and medicinal plants of Yemen. Journal of Ethnopharmacology 6:85-108.

Ghasemzadeh A, Ghasemzadeh N (2011). Effects of shading on synthesis and accumulation of polyphenolic compounds in ginger (*Zingiber officinale* Roscoe) varieties. Journal of Medicinal Plants Research 5(11):2435-2441.

Ghasemzadeh A, Jaafar HZE (2012). Effect of salicylic acid application on biochemical changes in ginger (*Zingiber officinale* Roscoe). Journal of Medicinal Plants Research 6(5):790-795.

Ha SK, Moon E, Ju MS, Kim DH, Ryu JH, Oh MS, Kim SY (2012). 6-Shogaol, a ginger product, modulates neuroinflammation: a new approach to neuroprotection. Neuropharmacology 63(2):211-223.

Ho SC, Chang KS, Lin CC (2013). Anti-neuroinflammatory capacity of fresh ginger is attributed mainly to 10-gingerol. Food Chemistry 141:3183-3191.

Karwasara VS, Tomar P, Dixit VK (2011). Influence of fungal elicitation on glycyrrhizin production in transformed cell cultures of *Abrus precatorius* L. Pharmacognosy Magazine 7:307-313.

Kim Y, Lee SK, Bae S, Kim H, Park Y, Chu NK (2013). The anti-inflammatory effect of alloferon on UVB-induced skin inflammation through the down-regulation of pro-inflammatory cytokines. Immunology Letters 149:110-118.

Kizhakkayil J, Sasikumar B(2012). Characterization of ginger (*Zingiber officinale* Rosc.) germplasm based on volatile and non-volatile components. African Journal of Biotechnology 11(4):777-786.

Mahadik KR, Nikam AR Sathiyanarayanan L (2013). Validation of reversed-phase high-performance liquid chromatography method for simultaneous determination of 6-,8-, and 10-shogaol from ginger preparations. International Journal of Pharmacy and Pharmceuetical Science 5:432-437.

Minghetti P, Sosaz S, Cilurzo F, Casiraghi A, Alberti E, Tubaro A (2007). Evaluation of the topical anti-inflammatory activity of ginger dry extracts from solutions and plasters. Planta Medica 73:1525-1530.

Molnár Z, Virág E, Ördög V (2011). Natural substances in tissue culture media of higher plants. Acta Biologica Szegediensis 1:123-127.

Mošovskáa S, Novákováa D, Kaliňákb M (2015). Antioxidant activity of ginger extract and identification of its active components. Acta Chimica Slovaca 8:115-119.

Namdeo AG (2007). Plant cell elicitation for production of secondary metabolites: A review. Pharmacognosy Reviews 1:69-79.

Pawar P, Pai S, Nimbalkar M, Dixit G (2015). RP-HPLC analysis of phenolic antioxidant compound 6-gingerol from *in vitro* cultures of *Zingiber officinale* Roscoe. Plant Science Today 1:24-28.

Sák M, Dokupilova` I, Mihalik D, Lakatosova J, Gubisova M, Kraic J (2014). Elicitation of phenolic compounds in cell culture of *Vitis vinifera* L. by *phaeomoniella chlamydospora.* Nova Biotechnologica et Chimica 2:162-171.

Seidel V, Windho¨ vel J, Eaton G, Alfermann AW, Arroo RRJ, Medarde M, Petersen M, Wolley JG (2002). Biosynthesis of podophyllotoxin in Linum album cell cultures. Planta 215:1013-1039.

Semwal R B, Semwal, D K, Combrinck, S, Viljoen, AM (2015). Gingerols and shogaols:Important nutraceutical principles from ginger. Phytochemistry 117:554-568.

Srinivasan K (2017). Ginger rhizomes (*Zingiber officinale*): A spice with multiple health beneficial potentials. Pharma Nutrition 5:18-28.

Stoilova I, Krastanov A, Stoyanova A, Denev P, Gargova S (2007). Antioxidant activity of a ginger extract (*Zingiber officinale*). Food Chemistry 102:764-770.

Tripathi S, Bruch D, Kittu DS (2008). Ginger extract inhibits LPS induced macrophage activation and function. BMC Complementary and Alternative Medicine 8:1-7.

Van Kooyk Y, Engering A, Lekkerkerker AN, Ludwig IS, Geijtenbeek TB (2004). Pathogens use carbohydrates to escape immunity induced by dendritic cells. Current Opinion in Immunology 16:488-493.

Journal of Medicinal Plants Research

Full Length Research Paper

Antihistaminic action of *Melaleuca armillaris* ointment

Kassandra Elfrida Pauliello, Diba Maria Sebba Tosta Souza, Marcos Mesquita Filho,
Manoel Araújo Teixeira and Adriana Rodrigues dos Anjos Mendonça*

Universidade do Vale do Sapucaí, Brazil.

Melaleuca oil has great medicinal properties. The objective is to develop a product based on *Melaleuca armillaris* to evaluate its antihistaminic action and to compare the antihistaminic effect and protective effect of *M. armillaris* oil and ointment. Pruritus intensity was assessed at sensitization points. Eighty volunteers, aged between 21 and 60 years (male and female) participated in the study, without contraindications to the *Prick* test. The left forearm was sensitized at four points: A: control (+) histamine, B: control (-) *M. armillaris* oil, C: histamine and after 2 min, drying and application of *M. armillaris*. D: *M. armillaris* and after 2 min the application of histamine. The right forearm was sensitized at four points. A: control (+) histamine; B: control (-) *M. armillaris* ointment. C: histamine and after 2 min, drying and application of *M. armillaris*. D: *M. armillaris* ointment and after 2 min, drying and application of histamine. Reading was taken after 15 min. Areas obtained were scanned and analyzed by Image J. The pruritus was evaluated through the Visual Analogue Scale. There were differences in the left and right forearm between the points A: control (+) and C: effect of post-chopped oil and post-chopped effect of ointment rearmillarisectively. The oil of *M. armillaris*, 100% and the ointment of *M. armillaris,* 50% presented antihistaminic action and did not influence the intensity of pruritus. There was no protective effect of the oil and ointment of *M. armillaris*.

Key words: Histamine antagonists, phytotherapy, *Melaleuca*, bites, stings.

INTRODUCTION

Reactions due to insects of the order Diptera are due to allergens in insect saliva and not due to toxin. Saliva contains active compounds that inhibit the body's immune responses causing coagulation, platelet formation, vasodilation and anti-inflammatory activities to be impaired (Singh and Mann, 2013).

Generally occurring in children 2 to 10 years old, papular urticaria occasionally affects adolescents and adults. Sensitization takes time, so it is not seen in newborns. The higher prevalence in children may result from immune mechanisms and / or behaviors that predispose them to contact with insects. Most children overcome the disease probably due to desensitization by repeated exposures. There are no racial or gender predilections (Singh and Mann, 2013). Insect bite reactions are common, but information on their prevalence is limited. Children under 14 at the Dermatology Outpatient Clinic in Pondicherry had a

*Corresponding author. E-mail: drijar@hotmail.com.

prevalence of papular urticaria of 5.3%. Children under 5 years old attending clinic in Calcutta had a prevalence of 10.6% papular urticaria, with seasonal variation (rainy season 16.7%, summer 6.7%, winter 5.8%) (Singh and Mann, 2013). Phytotherapy is the area of study that evaluates the drug action of active ingredients from plants used in the treatment and prevention of diseases. *Phyton*, in Greek, means plant and *therapeia* comes from the verb therapeuo, which means to treat, to take care of. According to Portaria 971, dated 05/05/2006, of the Ministry of Health, Phytotherapy is a therapy characterized by the use of medicinal plants in their different pharmaceutical forms, without the use of isolated active substances, although of vegetal origin (Panizza, 2016).

The genus *Melaleuca* belongs to the family *Myrtaceae* (Oliveira et al., 2011). It usually presents simple, coriaceous, acute-lanceolate leaves and, sometimes, in scythe format, 1 - 2.5 cm length, with oil glands (Monteiro et al., 2013). This genus is formed by several species standing out the *Melaleuca alternifolia* (Vieira et al., 2004). It is known as tea tree (Oliveira et al., 2011). The trees can reach seven meters in height, have thin bark and long pointed leaves that, when broken, emit a strong aroma. They can be cut after 15 months of cultivation and cropped each year (Simões et al., 2002).

Native to Australia and Indian Ocean Islands. Flowering mainly in marsh areas, near rivers (Oliveira et al., 2011). *M. alternifolia* belongs to the group of species of *Melaleuca armillaris* Sm. (Which also includes *Melaleuca dissitiflora* F. Muell, *Melaleuca linophylla* F. Muell., *Melaleuca trichostachya* Lindl), of wide distribution and occurrence in several climates. Terpinenol-4 chemotypes occur in species of the group. Species grown in Brazil (Monteiro et al., 2013). The oil of the tree is colorless or pale yellow in color and has a spicy earthy odor (Fahlbusch et al., 2003). Terpinenol-4 is mainly responsible for its medicinal properties, mainly antifungal and antibacterial (Garcia et al., 2009).

Chemically terpenes can be divided into two major groups: terpene derivatives (menthol and citronellol) and phenylpropane derivatives (anethole and eugenol). The main pharmacological characteristics of terpenes are related to the use as antiseptic, anti-inflammatory and antipyretic agents (Lima et al., 2005). The main product of the *Melaleuca* plant is TTO tea tree oil, of great medicinal importance because it has a proven bactericidal and antifungal action against several human pathogens, being used in topical formulations (Oliveira et al., 2011).

Among their great properties, they emphasize their bactericidal, healing, expectorant, fungicidal, anti-infective, balsamic, anti-inflammatory, antiseptic, antiviral, febrifuge, insecticide, immunostimulant, diaphoretic and parasiticide actions (Maluf, 2009). However, this oil has interesting therapeutic characteristics against certain pathologies, such as antiacne action, onychomycosis,

dermatitis, eczema, toothache, bad breath, among others (Garcia et al., 2009). It can also be incorporated into formulations such as the intima liquid soaps, where it seeks the prevention of diseases of the female genito-urinary tract, as against candidiasis (Garcia et al., 2009). *Melaleuca* oil has wide applicability in products such as disinfectants, capillary products, oral and personal hygiene, deodorants, burns, insect bites, post-sun, veterinary products, aromatherapy and flavoring (Souza, 2009).

Topical use of *Melaleuca* oil is considered safe for most adults. It is commonly indicated for skin infections, cuts and abrasions, boils, recurrent cold sores, infections of the mouth and nose, sore throat and ear infections such as otitis media and otitis externa, cough additive, bronchial congestion and the pulmonary inflammation (Oliveira et al., 2011). There are no reports of health hazards if given in an appropriate therapeutic manner. However, contact dermatitis may occur. In pediatric use, the oil can not be applied around the nose of infants and children, because of the risk of glottal edema (Monteiro et al., 2013). In view of the need for further studies and research on the medicinal importance and applicability of *M. armillaris*. The objective of this study was to evaluate the antihistaminic effectiveness of this oil in order to provide an adequate treatment for allergic reactions and subsequent development of a new pharmaceutical formulation.

MATERIALS AND METHODS

Clinical, analytical, observational, transversal and controlled study. The research was carried out in the city of Pouso Alegre, MG, at the Universidade do Vale do Sapucaí, Fátima Unit, from June to September 2016. The study population consisted of eighty volunteers of both sexes who agreed to participate in the study and signed the Free and Informed Consent Term (FICT). Inclusion criteria: individuals who signed the FICT, aged between 18 and 60 years old and who did not present contraindications to the Prick Test (history of previous anaphylaxis, extensive dermatitis using antihistamine) (Mendonça et al., 2014).

Exclusion criteria: Individuals who have had a negative reaction to histamine or who withdraw their consent at any stage of the study.

Oil of *M. armillaris*

Melaleuca oil was obtained from leaves taken from *M. armillaris*, using a hydrodistillation extracted methods. The leaves were dried at room temperature in a rustic solar dryer with clear plastic cover for six days. 150 g of dried leaves was transferred to a volumetric flask with 400 ml of distilled water. The flask was then fitted to the condenser and connected to the refrigeration system. The heating mantle was switched on and set at the boiling temperature of the water at 100°C until it reached boiling and then reduced to 75°C in admixture with the biomass, initiating the extraction process for a period of 50 min.

When the mixture of water and leaves (biomass) was boiled, the water vapors and volatiles were conducted towards the condenser, where the heat exchange was carried out, condensing the vapors with the cooling water. At this stage, this mixture of oil and

Table 1. Description of the ointment components of *M. armillaris*.

Component	Concentration
Oil of *Melaleuca armillaris*	50%
Petroleum jelly	q.s.p. 100 g

hydrolyzate (by-product) was cooled and returned to the liquid phase. The mixture reached the last stage of the process, separating the oil from the hydrolate by means of the polarity and density differences of these substances through the separating funnel. To determine the yield of the essential oil, the empty 1000 ml flask (320 g) was weighed in analytical balance and the flask was then charged with the sheets (470 g). At each extraction of 150 g of *M.armillaris*, 6.5 ml of oil was extracted. The oil was then transferred to an amber glass vial and stored in a cool place without the direct incidence of light.

Preparation of *M. armillaris* ointment.

The semi-solid pharmaceutical form was made according to Table 1.

Procedures for data collection

The *Prick* Test is the safest and easiest to perform, has good reproducibility and is considered the best for use in clinical allergy practice (Motta et al., 2005). The skin test is very important for the verification of IgE-mediated hypersensitivity reactions, which are attributed to mediators, including histamine. For this reason, the method chosen, based on this test, was used in humans to compare the antihistaminic effect of *M. armillaris* and the oil of *M. armillaris* incorporated in ointment. Each individual was their own control (paired sample). And the place for the test used the ventral midface (volar) of the forearms of the participants. Antisepsis of the skin was carried out with cotton soaked in 70% alcohol. The region to be applied the test could not present any type of tissue injury. After cleaning, the points (A, B, C and D) were marked with a watermark on the left and right forearms. In the left forearm the antihistaminic action of the oil was evaluated and in the right forearm the antihistaminic action of the *Melaleuca* ointment. Each point had a distance of approximately 2 cm in a predetermined sequence:

i) Point A (positive control) application of histamine at the concentration of 10 mg / ml acquired from the Laboratory Alergolatina. In order to simulate an allergic reaction to the insect bite.
ii) Point B (negative control) had the objective of verifying that the oil of *M. armillaris*. (left forearm) and ointment (right forearm) would have an irritating effect on the skin.
iii) Point C simulated the antihistaminic effect of *M. armillaris* (left forearm) and the ointment (right forearm) after the insect sting. The oil of *M. armillaris*. and ointment were applied 2 min after histamine application.
iv) Point D simulated the protective antihistaminic effect of *M. armillaris* (left forearm) and ointment (right forearm) for the insect bite. The oil of *M. armillaris* and the ointment were applied 2 min before histamine application.

The left forearm was sensitized at four points (A, B, C and D). Point A: Positive control (+) sensitized with a drop of histamine, causing redness, papule (swelling) and pruritus, point B: negative control

(-) sensitized with only one drop of *M. armillaris*. Point C was sensitized with one drop of histamine and after 2 minutes, drying of the histamine with paper napkin and application of one drop of the oil of *M. armillaris*. Point D was sensitized with one drop of the *M. armillaris* oil and after 2 minutes the application of one drop of histamine.

The right forearm was sensitized at four points (A, B, C and D). Point A: Positive (+) control sensitized with a drop of histamine, which may cause redness, papules (swelling) and pruritus; point B: negative control (-) sensitized only with the ointment of *M. armillaris*. Point C was sensitized with a drop of histamine and after 2 minutes, drying with paper napkin and application of the ointment of *M. armillaris*. The D spot was sensitized with *M. armillaris* ointment and after 2 min, paper napkin drying and application of a drop of histamine. The application of *M. armillaris* and histamine were performed with the bead dropper from each vial. And the ointment of *M. armillaris* was applied with wooden spatula.

A puncture (plastic device that limited the degree of skin penetration) was used and allowed the penetration of histamine through the skin of the participant for each point. The reading was performed after 15 min after puncture. The papules obtained were delimited with a fine-tipped dermographic pen and covered by transparent adhesive tape, forming a mold of the areas. These areas were scanned and analyzed by the Image J program which is a scientific image processor available in a virtual environment. The pruritus was evaluated through the Visual Analogue Scale to measure the degree of intensity at each point on the forearms of the participants. The Visual Analogue Scale consisted of a 10 cm line, marked the numbers from 0 to 10. And the participants reported their note for pruritus. From 0 to 3 it was considered mild pruritus, between 4 and 8 moderate pruritus and between 9 and 10 severe pruritus (Welter et al., 2008).

Ethical aspects

Before starting any procedure, the present study was submitted to the Research Ethics Committee of the Universidade do Vale do Sapucaí and approved on 12/16/15. Under the Supported Opinion: 1,372,740, CAAE: 50703515.1.0000.5102. Performed in accordance with the precepts established by Resolution 466/12 of 12/12/12 of the Ministry of Health in Brazil.

Data analysis

The obtained data were arranged in tables of Microsoft Office Excel 2007 and analyzed quantitatively. The program used for Statistical Analysis was PASW Statistics 18. Descriptive procedures for quantitative variables through measures of central tendency (mean and median) and by measures of dispersion (standard deviation). Qualitative variables were described by proportions. Inferential analysis was conducted for parametric continuous variables by paired T tests (for two samples). For the non-parametric variables Wilcoxon and Friedman tests were used. The level of significance was set at 5% ($p < 0.05$).

Table 2. Age range of the study population.

Age range (years old)	Nº	%
21-29	24	30.0
30-39	20	25.0
40-49	19	23.8
50-60	17	21.3
Total	80	100.0

Table 3. Areas of the papules in cm² formed at the application sites - left and right forearms.

Point	Average	SD
Point A: Histamine (left arm)	0.521	0.242
Point B: *Melaleuca armillaris*	0.014	0.050
Point C: Histamine + *Melaleuca armillaris*	0.389	0.213
Point D: *Melaleuca armillaris*. + Histamine Ponto	0.465	0.208
Point A: Histamine (right arm)	0.489	0.218
Point B: *Melaleuca armillaris* ointment	0.006	0.317
Point C: Histamine + *Melaleuca armillaris* ointment	0.415	0.200
Point D: *Melaleuca armillaris* ointment + Histamine	0.484	0.245

SD: Standard deviation.

RESULTS

Characterization of the sample

Of the eighty participants, six had a negative reaction to histamine (positive control) at point A of the right forearm and were excluded from the sample. Thus the sample for the left forearm was n: 80 and for the right forearm n: 74. Regarding gender, 49 were females and 31 were males. Age was divided into age groups in four subgroups: 21-29 years, 30-39 years, 40-49 years and 50-60 years, with a predominance of young adults (Table 2).

Comparison of application and effect of oil and ointment of *M. armillaris*

In Table 3 it is possible verify the averages of the areas of the papules in cm² formed at the points of application on the left and right forearms.

Standard deviation

It was observed in points B: negative controls that the oil of *M. armillaris* and the ointment of *M. armillaris* did not cause any irritating effect on the skin. At points A and C (left forearm) there was a difference between the positive control: histamine and the effect of post-chopped oil (p: 0.000), demonstrating the antihistaminic effect of *M. armillaris* and suggesting its post-pricking insect use. At

points A and D (left forearm) there was no difference between the positive control: histamine and the protective antihistaminic effect of oil (p: 0.057). At points A and C (right forearm) there was a difference between the positive control: histamine and the post-pricked effect of the ointment (p: 0.003), proving the antihistaminic effect of *M. armillaris* and suggesting its post-pricking insect use. At points A and D (right arm) there was no significant difference between the positive control: histamine and the protective antihistamine effect of the ointment (p: 0.887). At points C and D (right arm), the post-pricked and protective effect of the ointment was compared (p: 0.017) and there was a significant difference evidencing the antihistaminic effect of *M. armillaris* post-chopped. Comparing point C (left arm) and point C (right arm), it was verified that there was no difference in the post-chopped effect between oil and ointment (p: 0.496). Comparing D (left arm) and D (right arm), there was no difference in the protective effect between oil and ointment (p: 0.497).

Measurement of pruritus degree

The results in relation to the intensity of pruritus (Table 4) showed that the majority of participants reported moderate itching in the application of oil and ointment of *M. armillaris*. There were no differences between the points of the left and right forearms. It was also verified in points B: negative controls that the oil of *M. armillaris* and the ointment of *M. armillaris* did not cause itching on the

Table 4. Average, standard deviation and p value of the application points in relation to the intensity of pruritus.

Point	Average	SD	p value
Point A: Histamine (left arm)	5.03	3.036	0.296
Point B: *Melaleuca armillaris*	0.28	0.900	0.000
Point C: Histamine + *Melaleuca armillaris*	4.72	3.069	0.281
Point D: *Melaleuca armillaris* + Histamine	4.50	3.174	0.149
Point A: Histamine (right arm)	5.11	3.234	0.119
Point B: *Melaleuca armillaris* ointment	0.34	1.262	0.000
Point C: Histamine + *Melaleuca armillaris* ointment	4.61	3.184	0.252
Point D: *Melaleuca armillaris* ointment + Histamine	4.41	3.329	0.289

SD: Standard deviation.

skin.

DISCUSSION

In the present study there were differences between the A points (positive control: histamine) and the C points that simulated the post-prickly antihistamine effect of the insect of *M.armillaris* to 100% (left forearm) and *M. armillaris* to 50% (right forearm). The C points showed a decrease in the size of the papule area formed in relation to point A. The antihistaminic effect of the oil and ointment of *M. armillaris* and their post-pricking insect use was suggested.

Comparisons were also made between the C-points that simulated the post-chopped antihistamine effect and the D-points that simulated the protective effect of *M. armillaris* to 100% (left forearm) and *M. armillaris* to 50% (right forearm) and there were differences. The antihistaminic effect of the oil and ointment of *M. armillaris* post-chopped. The effect of topically applied tea tree oil (TTO) reduced histamine-induced edema in the ears of mice. Topical application of TTO, and in particular terpinenol-4, may be effective in controlling the histamine-induced edema frequently associated with Type I immediate hypersensitivity allergic reactions (Brand et al., 2002).

Topical application of 100% TTO may have therapeutic benefit in nickel-induced contact hypersensitivity on human skin. The mode of action of TTO requires additional investigation (Pearce et al., 2005). When applied 20 min after histamine injection into the skin of the human forearm, tea tree oil (TTO) reduces the developing cutaneous vascular response (Khalil et al., 2004). It was verified in points B: negative controls that the oil of *M. armillaris* to 100% and *M. armillaris* to 50% did not cause any irritation after topical use. The topical use of *Melaleuca* oil is relatively safe and adverse effects are minor and occasional (Hammer et al., 2006). Both 100% *M. armillaris* oil and 50% *M. armillaris* ointment cause any pruritus on the skin through evaluation. It is fundamental to attenuate or eliminate pruritus, since it is the act of scratching one of the factors, not only of

increasing the duration of the lesions but also the one responsible for possible secondary infections and residual hyperpigmentation. Anti-histamines (cetirizine, ebastine) have been shown to be associated with a 50% reduction in the maculae caused by the immediate reaction after mosquito bites and a 70% decrease in pruritus accompanying the bites. The use of emollients / moisturizers with additional pruritic and healing action may also be useful in some patients (Moreira et al., 2014).

In an experimental study on contact dermatitis and urticaria, the efficacy of traditional topical therapeutic agents and tea tree oil was compared. The effects of 10% ictamol, 20% zinc oxide, 20% camphor, 10% levomenthol, 20 or 50% tea tree oil and 0.05% clobetasone were studied in the following experimental models: nickel allergic contact dermatitis, irritative contact dermatitis of benzalkonium chloride, and in immediate reactions to histamine and benzoic acid), respectively. Tea tree oil reduced allergic contact dermatitis by 40.5%, zinc oxide by 17.4% and clobetasone by 23.5%. Zinc oxide reduced histamine-induced enlargement by 18.5%, ictamol by 19.2% and clobetasone by 44.1%. The histamine-induced pruritus was assessed using Visual Analog Scale and also remained unchanged. In this study, the author states that tea tree oil appears to be a more effective anti-eczematous agent than zinc oxide and clobetasone in the topical treatment of urticaria reactions (Wallengren, 2011).

Popular knowledge has brought great contribution to the dissemination of the use of plants in the treatment of diseases. In recent decades, there has been an increase in interest in alternative medicines, mostly from natural extracts, triggering the search for validation of the use of these drugs, given the favorable therapeutic effects in vitro and in vivo (Oliveira et al., 2011). Preparations based on *Melaleuca* essential oil obtained by steam distillation of foliage are indicated for the treatment of small surface wounds and insect bites. Semi-solid dosage forms are suitable for cutaneous use (European Medicines Agency, 2013).

In addition to being vectors of infectious diseases, insects can be a cause of discomfort for their bites. Some

species of mosquitoes may cause local irritation, pruritus, secondary infection, cellulitis, pain and sleep disturbances (Stefani et al., 2009). One of the reactions to insect bites is the pruritus that occurs in the region of the papules. When immunological sensitization to these bites occurs a more extensive picture called estropole (papular urticaria) (Rodrigues et al., 2010).

Although the exact prevalence of papular urticaria is unknown, it is a frequent dermatosis (estimated if it occurs in about 10% of the population) and is characteristic of children, appearing mainly from 2 years of age, with spontaneous disappearance, in most cases, up to 7 10 years. However, it can also affect adolescents and adults and can cause considerable impact on the quality of life of affected patients. Its diagnosis implies a high clinical suspicion and it is carried out based on the clinical history and identification of the characteristic cutaneous lesions. Regarding its treatment, this is mainly based on symptomatic relief, control of hypersensitivity reaction and measures of eradication and prophylaxis of bites by causative agents. In this context, antihistamines play a determining role, as the main drugs to be used (Moreira et al., 2014). The aim of this study is to study the medicinal plant *M. armillaris* and evaluating the antihistaminic effect of *M. armillaris* oil and compare it with *M. armillaris* ointment and thus to develop a herbal medicine for the treatment of patients suffering from allergies to insect bites.

In view of the limitations encountered during the manipulation in the incorporation of *M. armillaris* and obtaining the stability of the pharmaceutical form. Taking into account children with hypersensitivity to insect bites and information from the European Medicines Agency (EMA), 2013 that the use of oil in children under 12 years of age has not been established due to lack of adequate data. The future prospects of in-depth studies on the absorption, toxicity of this active principle and pharmaceutical technology aimed at obtaining a stable and appropriate formulation for pediatric topical use are highlighted.

Conclusion

The oil of *M. armillaris,* 100% and *M. armillaris* ointment, 50% presented antihistaminic action and did not influence the severity of pruritus. There was no protective effect of the oil and ointment of *M. armillaris.*

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Brand C, Townley SL, Finlay-Jones JJ, Hart HP (2002). Tea tree oil reduces histamine-induced o edema in mirine ears. Inflammation Research 51(6):283-289.

European Medicines Agency (2013). Committee on herbal medicinal products. Community herbal monograph on Melaleuca alternifolia (Maiden and Betch). Cheel, M. *armillaris* Smith, *M. dissitiflora F. Mueller* and/or other species of *Melaleuca, aetheroleum*.

Fahlbusch KG, Hammerschmidt FJ, Panten J, Picknhagen W, Schatkowski D, Bauer K, Garbe D, Surburg H (2003). Flabors and fragrances. Ullmann's Encyclopedia of Industrial Chemistry.

Garcia CC, Germano C, Osti NM, Chorilli M (2009). Desenvolvimento e avaliação da estabilidade físico-química de formulações de sabonete líquido íntimo acrescidas de óleo de Melaleuca. Revista Brasileira de Farmácia 90(3):236-240.

Hammer KA, Carson CF, Riley TV, Nielsen JB (2006). A review of the toxicity of Melaleuca alternifolia (tea tree) oil. Food and Chemical Toxicology 44(5):616-625.

Khalil Z, Pearce AL, Satkunanathan N, Storer E, Finlay-Jones JJ, Hart PH (2004). Regulation of wheal and flare by tea tree oil: complementary human and rodent studies. Journal of Investigative Dermatology 123(4):683-690.

Lima JF Jr, Vieira LB, Leite MJVF, Lima KC (2005). O uso de fitoterápicos e a saúde bucal. Rev Saúde em Revista 16:11-17.

Maluf S (2009). Aromaterapia uma abordagem sistêmica. São Paulo: do autor.

Mendonça ARA, Paschoal GRM, Barbosa S, Santos WJ (2014). Efeito anti-histamínico do óleo de Melaleuca (Melaleuca alternifolia) em testes imunológicos em humanos. Trabalho de conclusão de curso. Pouso Alegre: Universidade do Vale do Sapucaí, Curso de Ciências Biológicas.

Monteiro MHDA, Macedo HW, Silva AJr, Paumgartten FJR (2013). Óleos essenciais terapêuticos obtidos de espécies de Melaleuca L.(Myrtaceae Juss.). Revista Fitos 8(1).

Moreira A, Rosmaninho I, Silva JPM (2014). Urticária papular: Revisão da literatura. Revista Portuguesa de Imunoalergologia 22(4):279-287.

Motta AA, Kalil J, Barros MT (2005). Testes cutâneos. Brazilian Journal of Allergy and Immunology 28(2):73-83.

Oliveira ACM, Fontana A, Negrini TC, Nogueira, MNM, Bedran TBL, Andrade CR, Spolidorio LC, Spolidorio DMP (2011). Emprego do óleo de Melaleuca alternifolia Cheel (Myrtaceae) na odontologia: perspectivas quanto à utilização como antimicrobiano alternativo às doenças infecciosas de origem bucal. Revista Brasileira de Plantas Medicinais,pp. 492-499.

Panizza ST (2016). Fitoterapia e terapias complementares: plantas medicinais e fitoterápicos. Available at: http://www.fitoterapia.com.br

Pearce G, Finlay-Jones JJ, Hart HP (2005). Reduction of nickel-induced contact hypersensitivity reactions by topical tea tree oil in humans. Inflammation Research 54(1):22-30.

Rodrigues DA, Tomimori J, Floriano MC, Mendonça S (2010). Atlas de dermatologia em povos indígenas. SciELO-Editora Fap-Unifesp.

Simões RP, Groppo FC, Sartorato A, Del Fiol FS, Mattos Filho TR, Ramacciato JC, Rodrigues MVN (2002). Efeito do óleo de Melaleuca alternifolia sobre a infecção estafilocócica. Lecta-USF 20(2):143-152.

Singh S, Mann BK (2013). Insect bite reactions. Indian Journal of Dermatology, Venereology and Leprology 79(2):151.

Souza VM (2009). Ativos dermatológicos, volumes 1 a 4: guia de ativos dermatológicos utilizados na farmácia de manipulação para médicos e farmacêuticos. São Paulo: Pharmabooks Editora, pp. 402-403.

Stefani GP, Pastorino AC, Castro APBM, Fomin ABF, Jacob CMA (2009). Repelentes de insetos: recomendações para uso em crianças. Revista Paulista de Pediatria 27(1):81-89.

Vieira TR, Barbosa LCA, Maltha CRA, Paula VF, Nascimento EA (2004). Constituintes químicos de Melaleuca alternifolia (Myrtaceae). Química Nova 27(4):536-539.

Wallengren J (2011). Tea tree oil attenuates experimental contact dermatitis. Archives of Dermatological Research 303(5):333-338.

Welter EQ, Bonfá R, Petry W, Moreira LL, Weber MB (2008). Relação entre grau de prurido e qualidade de vida de pacientes em hemodiálise Relationship between pruritus and quality of life in patients on hemodialysis. Brazilian Annals of Dermatology 83(2):137-40

Related Journals:

Clinical Reviews and Opinions

Journal of
Medicinal Plant Research

African Journal of
Pharmacy and Pharmacology

Journal of
Dentistry and Oral Hygiene

Journal of
Parasitology and Vector Biology

Journal of
Pharmacognosy and Phytotherapy

Journal of
Medical Laboratory and Diagnosis

Journal of
Diabetes and Endocrinology

Medical Practice and Reviews

www.academicjournals.org