Industrial Crops and Products 40 (2012) 1–12

Contents lists available at SciVerse ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Review

In vitro and *in vivo* studies of natural products: A challenge for their valuation. The case study of chamomile (*Matricaria recutita* L.)

Sílvia Petronilho[a], Marcelo Maraschin[b], Manuel A. Coimbra[a], Sílvia M. Rocha[a,*]

[a] *QOPNA, Universidade de Aveiro, Departamento de Química, 3810-193 Aveiro, Portugal*
[b] *Universidade Federal de Santa Catarina, Laboratório de Morfogênese e Bioquímica Vegetal, Santa Catarina, Brazil*

ARTICLE INFO

Article history:
Received 21 October 2011
Received in revised form 23 February 2012
Accepted 24 February 2012

Keywords:
Biological activities
Chamomile
In vivo studies
In vitro studies
Sesquiterpenic compounds

ABSTRACT

Medicinal plant research is universally on the rise. Researchers, as well as the general public, recognize that natural products, predominantly those derived from plants, may exhibit health benefits. The tendency is to consider natural products as non-toxic and presenting fewer side effects than those used by conventional medicine. However, information concerning the real human health benefits of natural products is yet seldom available, which is a drawback for their possible valuation. Chamomile is one of the most widely used medicinal plants and its sesquiterpenic-related products are an example of this informative weakness. Several health benefits have been claimed for chamomile extracts and for a large number of sesquiterpenic compounds known to occur in chamomile. However, a deep knowledge concerning the compounds responsible for each specific effect, as well as the mechanisms behind them has not been stated, or, if it exists, is dispersed in literature. Thus, this review comprises a deep survey on the reported potential health benefits of chamomile-related sesquiterpenic compounds, and takes into account the models used for their evaluation: *in vitro* or *in vivo*. In spite of the relevance of the *in vitro* and animal studies reported in literature, where the data obtained are very promising concerning the potential health benefits of chamomile-related sesquiterpenic compounds, their extension to human trials is essential. Several aspects related to this actual challenge are discussed.

Contents

* Corresponding author. Tel.: +351 234401508; fax: +351 234370084.
E-mail address: smrocha@ua.pt (S.M. Rocha).

0926-6690/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.indcrop.2012.02.041

1. Introduction

Focus on medicinal plant research has being increasing all over the world. People are turning to natural products; predominantly those derived from plants for their health care, due to the growing recognition that these natural products are mainly non-toxic, have lesser side effects than synthetic drugs, and are accessible at affordable prices (Milner, 1999; Chandrasekaran et al., 2010). In traditional medicine, plant formulations and combined extracts of plants are used for the treatment of a wide variety of diseases (Ansarullah et al., 2009). This therapeutic approach, although considered to be an alternative to conventional medicine, is still ignored by medical practice due to the lack of suitable scientific and clinical evidence (Fontanarosa and Lundberg, 1998; Yuan and Lin, 2000). In order to assess the efficacy and safety of products, numerous *in vitro* studies need to be carried out, which although very useful are not enough. The use of animal and human models is essential to scientifically prove the efficacy and safety of natural products on humans (Schneeman, 2007). The use of animals or humans to determine the toxicity of a pharmaceutical/biopharmaceutical or medical device is a required and determinant part for the validation and safe use of the natural products on humans (Alabaster, 2002).

The definition of *in vitro* and *in vivo* studies depends on the experimental model used. The *in vivo* studies refer to the characterization and analysis of molecules and biological systems in the context of intact organisms such as animals and humans. On the other hand, *in vitro* studies refer to the manipulation of organs, tissues, cells, and biomolecules under controlled artificial conditions (Lodish et al., 2004). *In vivo* studies are often employed for observing the overall effects of an experiment on a living organism (Novakofski, 2004). Unfortunately, *in vivo* studies are far more expensive, and often more difficult to control, than those performed *in vitro*. Despite the highlighted drawbacks of the *in vitro* model systems, they are more suitable than the *in vivo* models for the understanding of the mechanisms of drug-induced toxicity due to their lower structural and functional heterogeneity (Davila et al., 1998).

Plants have been used throughout history as the primary source of food, fuel, and medicine, among other purposes. Today, plants, plant extracts, and plant-derived components are being used in a multitude of herbal medicines and phytopharmaceuticals (Van der Kooy et al., 2009). Taking into consideration the Directive 2002/46/EC (Directive, 2002) on Food Supplements, and Directive 2004/24/EC (Directive, 2004) on herbal medicinal products, a medicinal product has to be identified by carefully interpreting the data on physiological *versus* pharmacological, and health *versus* disease conditions on dose/concentration bases.

For many of the medicinal plants of current interest, a primary focus of research to date has been in the areas of horticulture, phytochemistry, and pharmacognosy. Horticultural research on medicinal plants has focused on developing the capacity for optimal growth in cultivation. This has been especially pertinent as many medicinal plants are still harvested in the wild and, in several cases, conditions for growth in controlled cultivation have not been optimized with the purpose of increasing the yields of bioactive compounds. Wild harvesting of medicinal plants can be problematic in terms of loss of biodiversity, potential variation in medicinal plant quality and, occasionally, improper plant identification with potentially severe consequences. From the perspective of plant physiology, extensive opportunities exist for basic research on medicinal plants and for the study of their phytomedicinal chemical production (Briskin, 2000). In the area of phytochemistry, medicinal plants have been characterized for their possible bioactive compounds, which have been isolated and subjected to detailed structural characterization. Research on the pharmacognosy of medicinal plants has also involved assays of bio-activity, identification of potential modes of action, and target sites for active phytomedicinal compounds. *Matricaria recutita* L., known as chamomile, is one of the most widely used medicinal plants and is included in the pharmacopoeias of 26 countries all over the world (Shikov et al., 2008; Mohammad et al., 2010). This plant was used as a case study to discuss the importance that the development of *in vivo* studies has in the future valuation of natural products.

2. Biological activity of *M. recutita* L. and its sesquiterpenic compounds

M. recutita L. is an herbaceous plant that is indigenous to Europe and Western Asia (Mckay and Blumberg, 2006). Nowadays, it is mainly cultivated in Europe, South America and, to a lesser extent, in Africa (Povh et al., 2001). Several products obtained from chamomile are commercially available, such as soaps, detergents, fragrances, lotions, ointments, hair products, baked goods, confectionery, alcoholic beverages, and infusions (Mckay and Blumberg, 2006). The consumption of chamomile as infusion is rated as more than one million cups per day (Maschi et al., 2008).

A substantial part of the pharmacological effects is determined by the biologically active chemical constituents that have been identified, namely the sesquiterpenic and the phenolic compounds (Mann and Staba, 1986; Mckay and Blumberg, 2006). Sesquiterpenic compounds such as α-bisabolol, bisabolol oxides A and B and chamazulene and farnesene, and phenolic compounds namely the flavonoids apigenin, quercetin, patuletin, and luteolin, and their glucosides, and also coumarins (herniarin and umbelliferone), are considered to be the major bioactive compounds of chamomile (Habersang et al., 1979; Miller et al., 1996; Salamon, 2007; Lemberkovics et al., 1998; Baser et al., 2006). Among the flavonoids, apigenin is the most promising compound. It is present in very small quantities as free apigenin, but predominantly exists in the form of various glycosides (Avallone et al., 2000; Srivastava and Gupta, 2007; Srivastava et al., 2010).

The phenolic compounds of chamomile, namely the flavonoids, have been extensively studied and revised (Cushnie and Lamb, 2005; Patel et al., 2007). However, the information relating to the biological activities of sesquiterpenic compounds is still dispersed. Furthermore, chamomile inflorescences have been reported as a potential source of sesquiterpenic compounds (Orav et al., 2001; Szoke et al., 2004a,b; Rubiolo et al., 2006; Petronilho et al., 2011).

Sesquiterpenic compounds are extensively found in the essential oils of several plants and fruits, providing a wide spectrum of aromas, mostly perceived as very pleasant, and responsible for the aroma perception of several natural products. Sesquiterpenic compounds have also been related with medicinal plants and fruits with different health applications (Rocha et al., 2006).

Several biological effects have been attributed to *M. recutita* L., such as anti-microbial, antioxidant, anti-malarial, anti-mutagenic, anti-platelet, anti-chemotactic, anti-cancer, anti-inflammatory, anti-genotoxic, anti-spasmolytic, vulnerary, mildly sedative, hypocholesterolemic, beneficial for gastrointestinal, hepatic, central nervous system and autonomic nervous system, hemodynamic, and topical properties. It has been used for flatulent nervous dyspepsia, travel sickness, nasal catarrh, restlessness, and specifically for gastro-intestinal disorders associated with nervous irritability in children. It was also applied topically for haemorrhoids, mastitis, leg ulcer treatments, renal colic, nausea, skin eruption, constipation, as a sedative, for the expulsion of parasitic worms, stomach complaints, and skin diseases (Forster et al., 1980; Salamon and Honcariv, 1994; Kintzios and Michaelakis, 1999; Di Stasi et al., 2002; Franke and Schilcher, 2005; Crotteau et al., 2006; Kroll and Cordes, 2006; Gardiner, 2007; Srivastava et al., 2009; Bhaskaran

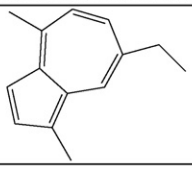
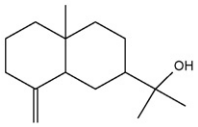
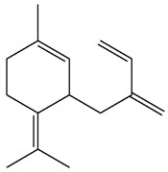
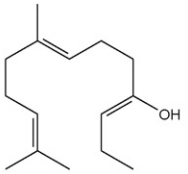
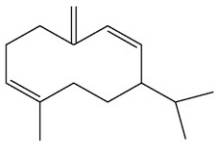
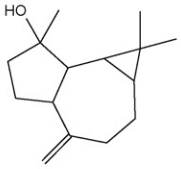
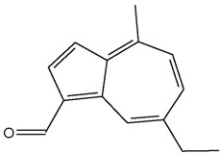
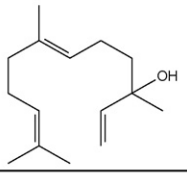
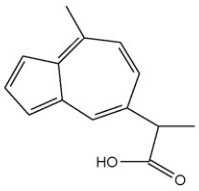
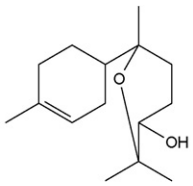
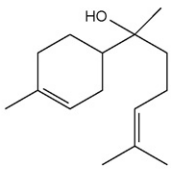
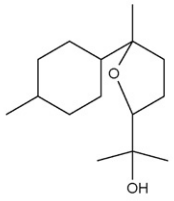
Compound	Chemical Structure	Compound	Chemical Structure
Chamazulene $C_{14}H_{16}$		β-Eudesmol $C_{15}H_{26}O$	
β-Farnesene $C_{15}H_{24}$		Farnesol $C_{15}H_{26}O$	
Germacrene D $C_{15}H_{24}$		Spathulenol $C_{15}H_{26}O$	
Chamaviolin $C_{14}H_{14}O$		Nerolidol $C_{15}H_{26}O$	
Chamazulene carboxylic acid $C_{14}H_{16}O_2$		Bisabolol oxide A $C_{15}H_{26}O_2$	
α-Bisabolol $C_{15}H_{26}O$		Bisabolol oxide B $C_{15}H_{26}O_2$	

Fig. 1. Sesquiterpenic compounds present in *Matricaria recutita* L.

et al., 2010; Hernández-Ceruelos et al., 2010; Rahimia et al., 2011). Chamomile extracts also revealed antioxidant activity (Hernández-Ceruelos et al., 2002; Koleckar et al., 2008). The essential oil of chamomile that contains several sesquiterpenic compounds such as α-bisabolol also revealed anti-inflammatory, anti-bacterial, anti-mycotic, and ulcer-protective properties (Isaac, 1979; Szelenyi et al., 1979; Yarosh et al., 2006).

These health benefits of *M. recutita* L. are associated with several groups of active components. In this review, although special attention is devoted to the chamomile-related sesquiterpenic compounds, it was considered useful to report the biological activity of *M. recutita* L. extracts, which comprise other components besides the sesquiterpenic compounds, as well as the effects reported in literature using chemical standards that correspond to those detected in chamomile. Studies that described biological effects of chemical standards like chamazulene, germacrene D, nerolidol, α-bisabolol, farnesol, bisabolol oxide A, chamaviolin and chamazulene carboxylic acid (Fig. 1) were considered (Rekka et al., 1996; Brehm-Stecher and Johnson, 2003; van Zyl et al., 2006; Simões et al., 2008; Ogata et al., 2010). For simplicity, the criterion used for systematizing bibliography was based on the experimental model used. Thus, biological activities of *M. recutita* L. are described in terms of *in vitro* and *in vivo* studies; the latter including animal models, and human trials. An overview of the existing biological activities of *M. recutita* L. is shown in Table 1.

2.1. In vitro studies

2.1.1. Anti-microbial activity

Several *in vitro* studies showed the anti-microbial activity of the essential oil, the aqueous and ethanolic extracts of chamomile, and also the chemical standards known to occur in chamomile, such as nerolidol, farnesol, α-bisabolol, germacrene D, and chamazulene. Some studies showed that the essential oil extracted from chamomile exhibits anti-microbial activity against some species of bacteria, fungi, and virus. The essential oil is effective against 25 different Gram-positive and Gram-negative bacteria, including 20 strains of *Listeria monocytogenes* (Lis-Balchin et al., 1998; Mckay and Blumberg, 2006). The anti-fungal activities of the essential oil (0.5–3 $g\,l^{-1}$) are also effective against *Aspergillus* and *Fusarium* species, where the highest concentration exhibited the highest inhibition against these microorganisms (91–95% inhibition) (Soliman and Badeaa, 2002; Mckay and Blumberg, 2006). It has also been reported that chamomile essential oil is active

Table 1
Potential biological activities of *Matricaria recutita* L. and its sesquiterpenic compounds.

Type of activity	Type of sample	Effects	References
1. *In vitro studies*			
Anti-microbial activity	Essential oil	Effective against 25 different Gram-positive and Gram-negative bacteria and 20 strains of *Listeria monocytogenes*	Lis-Balchin et al. (1998), Mckay and Blumberg (2006)
		Effective against *Aspergillus* and *Fusarium* species	Soliman and Badeaa (2002), Mckay and Blumberg (2006)
		Active against *Helicobacter pylori*	Stamatis et al. (2003)
		Influences the morphological and fermentative properties of *H. pylori* and also inhibits the its colony activity	Shikov et al. (2008)
	Alcoholic extracts	Effective against Gram-positive (*S. aureus* ATCC 12600, *S. mutans*, group B *Streptococcus*, and *S. salivarius*) and Gram-negative bacteria strains (*Kl. pneumonia*, *E. coli*, *B. megatherium* and *L. icterohaemorrhagia*)	Franke and Schilcher (2005)
		Inhibit the growth of both herpes and polio virus	Mckay and Blumberg (2006)
	Chemical standards	Nerolidol, farnesol, and α-bisabolol promote the disruption of the bacterial cell membrane – increase bacterial susceptibility	Brehm-Stecher and Johnson (2003), Kamatou and Viljoen (2010)
		α-Bisabolol and nerolidol are effective against *S. aureus*, *B. cereus*, *E. coli* and *C. albicans*	van Zyl et al. (2006), Kamatou and Viljoen (2010)
		Nerolidol and germacrene D sensitize pathogenic bacteria increasing their susceptibility	Simões et al. (2008)
		α-Bisabolol is active against *S. aureus*, *B. subtilis*, *E. coli*, *S. faecalis*, and *P. aeruginosa* and *B. phlei* and also fungistatic against *C. albicans*, *T. menthagrophytes*, and *T. rubrum* Chamazulene also had fungistatic activity	Franke and Schilcher (2005)
Antioxidant activity	Methanolic extracts	Moderate antioxidant activity in comparison with a selection of other medicinal plants	Dragland et al. (2003)
	Ethanolic extracts	Moderate antioxidant activity when compared with other medicinal plants	Koleckar et al. (2008)
	Aqueous extracts	Moderate antioxidant activity when compared with other medicinal plants	Lee and Shibamoto (2002)
	Chemical standards	Chamazulene inhibits Fe^{2+}/ascorbate-induced lipid peroxidation	Rekka et al. (1996)
		Nerolidol and α-bisabolol exhibit antioxidant activity	van Zyl et al. (2006)
		α-Bisabolol improves the antioxidant network and restore the redox balance by antagonising oxidative stress	Braga et al. (2009)
Anti-malarial activity	Chemical standards	Nerolidol and α-bisabolol interfer with malaria growth	van Zyl et al. (2006), Lopes et al. (1999)
Anti-mutagenic activity	Chemical standards	α-Bisabolol inhibits the effects of aflatoxin B1 and other indirect-acting mutagens	Gomes-Carneiro et al. (2005)
Interference on drug metabolism	Essential oil (chamazulene, α-bisabolol, *cis*-spiroether and *trans*-spiroether)	Inhibitory effect on cytochrome P450 enzymes. Modulate the metabolism of certain co-administered drugs	Ganzera et al. (2006), Rodriguez-Fragoso et al. (2008)
Anti-platelet activity	Aqueous extracts	Exhibit significant anti-platelet activity – inhibit ADP^{-} and collagen-induced platelet aggregation	Pierre et al. (2005)
Anti-chemotactic effect	Essential oil (β-farnesene, spathulenol, β-eudesmol, α-bisabolol, bisabolol oxides A and B, and chamazulene)	Inhibit casein-induced human leukocyte chemotaxis	Presibella et al. (2006)
	Aqueous-ethanolic extracts	Inhibit casein-induced human leukocyte chemotaxis	Presibella et al. (2007)
Osteoporosis prevention	Aqueous extracts	Induce osteoblastic cell differentiation	Kassi et al. (2004)
		Exhibit anti-estrogenic effect on breast cancer cells	
Anti-inflammatory activity	Chemical standard	Chamazulene presents anti-inflammatory activity	Safayhi et al. (1994)
Anti-cancer effect	Chemical standards	α-Bisabolol has efficient cytotoxic effect on human and rat malignant glioma cell lines	Cavalieri et al. (2004)
		Bisabolol oxide A present cytotoxic effect	Ogata et al. (2010)
2. *In vivo studies*			
2.1. *Animal model studies*			

Anti-inflammatory effect	Aqueous extracts	Effect on Wistar albino rats, similar to the effect of the anti-allergenic agent oxatomide	Mckay and Blumberg (2006)
	Ethanolic extracts (α-bisabolol, bisabolol oxides A and B, apigenin and its glucosides, dicycloethers, and azulenes)	Anti-inflammatory effect similar to benzydamine in Swiss rats	Tubaro et al. (1984)
	Extract of chamomile containing α-bisabolol, matricin and apigenin	Anti-inflammatory effect similar to of benzydamine (0.60 mg) in rats	Della Loggia et al. (1990)
	Isolated from *Matricaria recutita* L.	Chamazulene carboxylic acid presents anti-inflammatory activity	Ramadan et al. (2006)
	Chemical standard	Chamaviolin presents anti-inflammatory activity	Oehler et al. (2009)
		α-Bisabolol inhibits the dermatitis induced by the noxious agents	Leite et al. (2011)
Anti-genotoxic effect	Essential oil (α-bisabolol, bisabolol oxides A and B, chamazulene, α- and β-farnesene, germacrene D)	Attenuation of the induced genotoxic effects in rat bone marrow cells	Hernández-Ceruelos et al. (2002)
Hypo-cholesterolemic effect	Aqueous extracts	Reduction of the serum cholesterol level in hyperlipidemic Wistar rats	Mckay and Blumberg (2006)
Gastrointestinal effects	Aqueous extracts (α-bisabolol, bisabolol oxides A and B)	Effective anti-spasmodic effect in isolated guinea-pig ileum	Mckay and Blumberg (2006)
	Aqueous extracts	Reduction of the gastric damage	Bezerra et al. (2009)
	Essential oil (α-bisabolol, bisabolol oxides A and B)	Effective anti-spasmodic effect in isolated guinea-pig ileum	Mckay and Blumberg (2006)
	Chemical standard	α-Bisabolol shows a significant protective effect on gastric mucosa	Torrado et al. (1995), Franke and Schilcher (2005)
		α-Bisabolol protects the gastric mucosa from ethanol and indomethacin-induced ulcer	Rocha et al. (2010)
		α-Bisabolol attenuates the gastric lesions	Bezerra et al. (2009)
Drug metabolizing effects in liver	Infusions	Modulate the activity of hepatic cytochrome P450 in Wistar rats liver microsomes – modulate the activity of hepatic phases I and II metabolizing enzymes	Maliakal and Wanwimolruk (2001)
Central nervous system effects	Essential oil	Inhalation of chamomile essential oil reduced a stress-induced in ovarectomized rats by increasing in plasma ACTH levels Responsible for sedative effects	Mckay and Blumberg (2006)
2.2. *Human studies*			
Autonomic nervous system effects	Infusions	Relaxing effect in young Japanese males	Nakamura et al., 2002
Hemodynamic effects	Infusions	Increasing in brachial artery pressure in patients hospitalized for cardiac catheterization	Gould et al. (1973)
Topical effects	Chamomile cream extract	Anti-inflammatory effect on skin associated with atopic dermatitis or eczemas, radiation therapy and erythema	Maiche et al. (1991), Korting et al. (1993), Hempel et al. (1999), Patzelt-Wenczler and Ponce-Poschl (2000), Mckay and Blumberg (2006)
	Chamomile cream extract	Decreasing of wound area and increasing of drying wound in patients with weeping wounds following dermabrasion for tattoo removal	Mckay and Blumberg (2006)
Gastrointestinal effects	Chamomile aqueous oral rinse	Reduction of mucositis in patients submitted to cancer therapies	Mckay and Blumberg (2006), Rodriguez-Fragoso et al. (2008)

against *Helicobacter pylori* (Stamatis et al., 2003). Urease production by *H. pylori* is inhibited by *M. recutita* L. essential oil (Shikov et al., 2008). The minimal inhibitory concentration required to inhibit the growth of 90% of microorganisms (MIC_{90}) and the minimal inhibitory concentration required to inhibit the growth of 50% of microorganisms (MIC_{50}) were 125 g of essential oil l^{-1} and 62.5 g l^{-1}, respectively. *H. pylori* is able to resist the low pH of the stomach by producing urease which hydrolyses urea converting it into ammonia and carbon dioxide. Thus, an increase in the pH disturbs the mechanisms that lead to the survival of the bacteria within the stomach. The application of the oil extract influences the morphological and fermentative properties of *H. pylori* and also inhibits the colony activity of this microorganism (Shikov et al., 2008). Considering the high incidence of *H. pylori* infection around the world, mainly in less developed countries, the evaluation of the potential use of these compounds seems to be interesting, thus the extension of this test to *in vivo* studies is essential. This step should evaluate the concentrations of chamomile active components that guarantee the biological effect and prevent toxic effects. The data related to the anti-microbial activity of chamomile essential oil show that the necessary quantities of essential oil to inhibit the fungi *Aspergillus* and *Fusarium* activity (91% of inhibition – 3 g l^{-1}) is very different from that reported for the inhibition of the bacteria *H. pylori* activity (90% of inhibition – 125 g l^{-1}), suggesting that the essential oil is more effective against these fungi than against this bacterium.

An ethanol extract of chamomile is able to inhibit the growth of both herpes and polio virus. In general, aqueous extracts of chamomile are more effective against moulds and yeasts, while ethanolic extracts show higher activities against bacteria (Mckay and Blumberg, 2006). In a study reported by Franke and Schilcher (2005), a chamomile alcoholic extract (42 vol.%, v/v) also presents antibacterial activity against Gram-positive bacteria strains such as *Staphylococcus aureus* ATCC 12600, *Streptococcus mutans*, group B *Streptococcus*, and *Streptococcus salivarius*, and Gram-negative bacteria strains such as *Klebsiella pneumonia*, *Escherichia coli*, *Bacillus megaterium* and *Leptospira icterohaemorrhagiae*.

Several studies have been reported also using individual compounds detected in chamomile. Nerolidol, farnesol, α-bisabolol, germacrene D, and chamazulene have been investigated for their anti-microbial activity. Nerolidol, farnesol, and α-bisabolol promote the disruption of the bacterial cell membrane, allowing the permeation into the cell of exogenous solutes such as ethidium bromide (a membrane-impermeant model drug) and antibiotics (Brehm-Stecher and Johnson, 2003). The capability of these sesquiterpenoids to increase bacterial susceptibility to a number of clinically important antibiotics was also investigated. Treatment with concentrations of 0.11–0.44 g l^{-1} of these sesquiterpenoids enhances the susceptibility of *S. aureus* to ciprofloxacin, clindamycin, erythromycin, gentamicin, tetracycline, and vancomycin, suggesting a general role for these sesquiterpenoids as enhancers of nonspecific bacterial permeability to antibiotics and antimicrobials (Brehm-Stecher and Johnson, 2003; Kamatou and Viljoen, 2010). Furthermore, other studies carried out with α-bisabolol and nerolidol show that these compounds present by themselves anti-microbial activity against *S. aureus*, *Bacillus cereus*, *E. coli*, and *Candida albicans*. However, higher MIC_{50} values were obtained than those required to potentiate the antibiotic activity, namely, for α-bisabolol MIC_{50} ranged from 8 g l^{-1} for *C. albicans* to 32 g l^{-1} for all bacterial species under study, and the MIC_{50} value of nerolidol was *ca.* 32 g l^{-1} for all species under study (van Zyl et al., 2006; Kamatou and Viljoen, 2010). This result shows that α-bisabolol has higher anti-fungal activity than nerolidol and similar anti-bacterial activity. Nerolidol and germacrene D have been proposed as alternative or possible synergistic compounds for current antimicrobial chemotherapeutics, showing the practical utility of natural products, found in plants, to sensitize pathogenic bacteria by increasing their susceptibility (Simões et al., 2008). Other studies reported that α-bisabolol presented the strongest antibacterial activity when compared with its oxides. It is active against *S. aureus*, *Bacillus subtilis*, *E. coli*, *Streptococcus faecalis*, and *Pseudomonas aeruginosa* and inhibits the growth of *Bacillus phlei* that were resistant against standard anti-infectives. α-Bisabolol was also shown fungistatic activity against *C. albicans*, *Trichophyton mentagrophytes*, and *Trichophyton rubrum* at a concentration of 0.10 g l^{-1}. Chamazulene also has fungistatic activity, but at higher concentrations (Franke and Schilcher, 2005).

The determination of anti-microbial activity *in vitro* allows to predict the potential use of chamomile-related sesquiterpenic compounds in an actual and relevant issue that is the resistance to antibiotics of a continuously larger number of microorganisms, however it is imperative to evaluate the necessary quantities to make the desirable effect in humans.

2.1.2. Antioxidant activity

Several studies have been carried out in order to evaluate the antioxidant activity of *M. recutita* L. Essential oil, ethanolic extracts, and infusions of chamomile have been tested, and all of them exhibited antioxidant activity, although the aqueous extracts exhibited significantly higher activity than the ethanolic ones (Mckay and Blumberg, 2006). Aqueous and ethanolic extracts of chamomile have a moderate activity when compared with other medicinal plants (Lee and Shibamoto, 2002; Dragland et al., 2003; Koleckar et al., 2008). The antioxidant capacity of chamomile inflorescences methanolic extract was reported as 44 g trolox kg^{-1}. This value is higher than that exhibited by coriander (8.3 g kg^{-1}), but is lower than the values reported for peppermint (197 g kg^{-1}) and oregano (344 g $kg\,l^{-1}$) (Dragland et al., 2003). Lee and Shibamoto (2002) evaluated the antioxidant activity of an aqueous chamomile extract (20 g l^{-1} water) using two different assay systems. In the aldehyde/carboxylic acid assay, using butylated hydroxytoluene and α-tocopherol as standards, the highest dose (0.50 g l^{-1}) of the chamomile extract inhibited 50% of hexanal oxidation over a 40-day period. Compared with chamomile, the inhibition activities of thyme and basil were higher (100%), lavender and cinnamon were lower (5–6%), and that of rosemary was comparable (59%). In a conjugated diene assay measuring the inhibition of hydroperoxide formation from methyl linoleate (without initiators or metal catalysts), the trend of inhibition for the same extracts was similar to that observed for the hexanal oxidation. At the highest concentration (0.20 g l^{-1}), chamomile exhibited 31% inhibition of conjugated diene formation. Koleckar et al. (2008) carried out a study with 88 ethanolic extracts from various parts of plants from European Asteraceae, including *M. recutita* L. and Cichoriaceae. These plant extracts were analysed for radical scavenging activity by means of 1,1-diphenyl-2-picryl hydrazyl radical ($DPPH^{\bullet}$) test using the sequential injection analysis (SIA) methodology. The radical scavenging activity of all tested plant extracts was evaluated according to the 50% inhibitory concentration (IC_{50}). The IC_{50} values ranged from 0.046 g l^{-1} for the most active sample (leaves of *Leuzea carthamoides*) to 0.542 g l^{-1} for the least active sample (inflorescences of *Centaurea cyanus*). The IC_{50} value for *M. recutita* L. was 0.152 g l^{-1}, which suggests a potential antioxidant activity.

Data on the antioxidant activity of chamazulene, nerolidol and α-bisabolol are also available. Rekka et al. (1996) showed that chamazulene inhibits Fe^{2+}/ascorbate-induced lipid peroxidation, measured by the thiobarbituric acid reactive substances (TBARS) assay, in a concentration and time dependent mode (IC_{50} = 3.32 mg l^{-1} after 45 min incubation). In the same study, chamazulene was also shown to inhibit the autoxidation of dimethyl sulfoxide and to have a weak capacity to interact with the stable free radical diphenylpicrylhydrazyl ($DPPH^{\bullet}$). Similarly, nerolidol and α-bisabolol, both presenting IC_{50} >100 mg l^{-1},

have also exhibited antioxidant activity (van Zyl et al., 2006), which is, however, lower than that exhibited by chamazulene. Braga et al. (2009) investigated the antioxidant activity of α-bisabolol by studying the ability of this compound to interfere with the production of reactive oxygen species (luminol-amplified chemiluminescence, LACL) during human neutrophil bursts induced by both corpusculate (*C. albicans*) and soluble stimulants (*N*-formyl-methionyl-leucyl-phenylalanine, fMLP) and cell-free systems (SIN-1 and H_2O_2/$HOCl^-$). After *C. albicans* and fMLP stimulation, significant concentration-dependent LACL inhibition was observed with bisabolol concentrations ranging from 7.7 to 31 mg l^{-1} and 3.8 to 31 mg l^{-1}, respectively. A similar effect was observed in the SIN-1 and H_2O_2/$HOCl^-$ systems. These findings draw attention to the possible medical use of α-bisabolol as a means of improving the antioxidant network and restoring the redox balance by antagonising oxidative stress.

According to the referred data, the potential antioxidant activity of chamomile extracts and also of its individual constituents has been substantially investigated *in vitro*. The study of antioxidants is an actual issue as they play an important role in the prevention of cellular damage caused by free radicals that result in several human degenerative diseases such as cancer, heart diseases, and others. The study of this potential antioxidant activity *in vitro* is a first approach for its extension to *in vivo* studies in order to evaluate the real concentrations that guarantee this effect in humans.

2.1.3. Anti-malarial activity

Nerolidol was reported as interfering with malaria growth by inhibiting glycoprotein biosynthesis (Lopes et al., 1999). van Zyl et al. (2006) studied the biological activity of 20 chemical standards, including the sesquiterpenoids nerolidol and α-bisabolol. A chloroquine-resistant strain of *Plasmodium falciparum* (FCR-3) was continuously maintained *in vitro* and synchronized using 5% D-sorbitol. The anti-malarial activity was determined using the tritiated hypoxanthine incorporation assay. The concentration that inhibited 50% of the parasite growth was determined from the log sigmoid–dose response curve and quinine was used as the reference anti-malarial agent. The 20 substances tested were found to inhibit the growth of *P. falciparum* with IC_{50} values ranging from 0.20 to 334 mg l^{-1} and nerolidol was the compound that exhibited the highest activity (IC_{50} = 0.20 mg l^{-1}), even higher than quinine (IC_{50} = 23 mg l^{-1}). α-Bisabolol also exhibited anti-malarial activity, with an IC_{50} = 68 mg l^{-1}, although not as high as nerolidol. This result suggests that the observed effect is structure-dependent, and these two compounds are structurally different, as nerolidol presents an aliphatic structure, and α-bisabolol exhibits a cyclic structure combined with an aliphatic side chain (Fig. 1).

2.1.4. Anti-mutagenic activity

Gomes-Carneiro et al. (2005) investigated the mutagenicity and anti-mutagenicity of α-bisabolol in *Salmonella* microsome assay. Mutagenicity of α-bisabolol was evaluated with TA100, TA98, TA97a, and TA1535 *Salmonella typhimurium* strains, with and without the addition of a mixture of lyophilized rat liver (S9 mixture). Results obtained indicated that α-bisabolol do not present mutagenic activity. For the anti-mutagenicity assays, α-bisabolol was tested up to the highest nontoxic dose (*i.e.* 50 and 150 μg per plate, with and without S9 mixture, respectively) against direct-acting (sodium azide: SA; 4-nitroquinoline-N-oxide: 4-NQNO; 2-nitrofluorene: 2-NF; and nitro-o-phenylenediamine: NPD) as well as indirect-acting (cyclophosphamide: CP; benzo-α-pyrene: BαP; aflatoxin B1: AFB1; 2-aminoanthracene: 2-AA; and 2-aminofluorene: 2-AF) mutagens. α-Bisabolol did not alter the mutagenic activity of SA and NPD but, at the highest dose tested (50 μg per plate), showed an inhibitory effect lowering 40% on the mutagenicity induced by 4-NQNO (1.0 μg per plate) and 2-NF (2.5 and 1.0 μg per plate). The mutagenic effects of AFB1 (0.06 μg per plate), 2-AA (0.5 μg per plate), BαP (2.5 μg per plate), 2-AF (5.0 μg per plate), and CP (20 μg per plate) were attenuated and reduced by the highest dose of α-bisabolol (100 and 150 μg per plate) by 87, 84, 80, 57, and 52%, respectively. These results indicate that α-bisabolol inhibits the effects of aflatoxin B1 and of other indirect-acting mutagens. It was also found that in rat liver microsomes α-bisabolol inhibited pentoxyresorufino-dependentylase (PROD, IC_{50} = 0.61 mg l^{-1}) and ethoxyresorufin-o-deethylase (EROD, IC_{50} = 7.49 mg l^{-1}), which are markers for cytochrome P450 isoforms, *i.e.*, CYP2B and CYP1A subfamilies, a group of enzymes that use iron to oxidize potentially harmful substances by making them more water-soluble. Since CYP2B converts aflatoxin B1 and CP into mutagenic metabolites, and CYP1A activates BαP, 2-AA and 2-AF, results suggest that α-bisabolol-induced anti-mutagenicity could be mediated by an inhibitory effect on the metabolic activation of these promutagens. These results demonstrated that α-bisabolol has potential to inhibit the action of several mutagenic agents.

2.1.5. Interference on drug metabolism

Ganzera et al. (2006) investigated the inhibitory effect of chamomile essential oil on four selected human cytochrome P450 enzymes (CYP1A2, CYP2C9, CYP2D6, and CYP3A4). Essential oil (5 mg l^{-1}) demonstrated inhibition of each one of these enzymes, and CYP1A2 was more sensitive than the other isoforms. Three constituents of the essential oil, namely chamazulene (1.40 mg l^{-1}), *cis*-spiroether (1.39 mg l^{-1}), and *trans*-spiroether (1.61 mg l^{-1}) showed to be potent inhibitors of this enzyme, and also active towards CYP3A4, CYP2C9 and CYP2D6. Only chamazulene and α-bisabolol revealed a significant inhibition of the CYP2D6. This study demonstrated that the essential oil of chamomile may have the potential to modulate the metabolism of certain co-administered drugs, as it contains compounds that may inhibit the activities of major human drug metabolizing enzymes (Ganzera et al., 2006; Rodriguez-Fragoso et al., 2008). Further, α-bisabolol has already been reported in anti-mutagenic activity (Section 2.1.4) as a compound that also interferes with cytochrome P450, suggesting that this compound may underlie the metabolism of drugs and mutagenic agents.

2.1.6. Anti-platelet activity

The effect of aqueous extracts of several aromatic plants, including chamomile (100 g inflorescences per l of water), on human platelet aggregation has been evaluated. From among all the plants studied only chamomile, nettle, and alfalfa exhibit significant anti-platelet activity. Aqueous extracts of chamomile inhibit ADP^- and collagen-induced platelet aggregation. Chamomile inhibited platelet aggregation induced by ADP (60%) and collagen (84%) as well as the whole blood aggregation induced by collagen (30%), when compared to controls ($p < 0.05$). Chamomile strongly inhibits thromboxane B2 synthesis induced by either ADP or collagen (Pierre et al., 2005).

2.1.7. Anti-chemotactic effect

Recent studies investigated the ability of the essential oil (Presibella et al., 2006) and aqueous-ethanolic extract (Presibella et al., 2007) prepared from the inflorescences of *M. recutita* L. to interfere with human leukocyte chemotaxis (*e.g.*, the movement of leukocytes in reaction to a chemical stimulus) induced by casein, using the *in vitro* Boyden system. The analysis of the essential oil by GC–MS revealed the presence of β-farnesene, spathulenol, β-eudesmol, α-bisabolol, bisabolol oxides A and B, and chamazulene. The effects of exposing human leukocytes from peripheral blood to concentrations ranging from 0.1 to 1000 mg l^{-1} of chamomile essential oil, before inducing them to migrate towards a casein

gradient, resulted in an increased dose-related number of cells retained in the upper chamber compartment in comparison with the untreated control up to 10 mg l^{-1}, at which concentration it reached maximum significance, with only 66% ($n = 15$; $p < 0.005$) of the cells recovered from the lower chamber. The results showed a striking dose-related inhibition of the casein-induced human leukocyte migration after chamomile essential oil treatment in comparison with untreated controls (Presibella et al., 2006). The same results were observed for the chamomile aqueous-ethanolic extract, using the same range of concentrations (0.1–1000 mg l^{-1}) (Presibella et al., 2007). These results suggest that the exposure of human leukocytes to chamomile is associated with the destruction of leukocyte functions. These studies demonstrated that both essential oil and aqueous-ethanolic extract of chamomile may have the potential to interfere with human leukocyte chemotaxis, as they contain compounds that may inhibit the casein-induced human leukocyte migration.

2.1.8. Osteoporosis prevention

In order to research the chamomile potential as estrogen receptor modulators (SERMs – therapeutic agents), Kassi et al. (2004) studied the effect of aqueous extracts (10–100 mg l^{-1}) in a series of *in vitro* biological assays. Their ability to: (i) stimulate the differentiation and mineralization of osteoblastic cell culture; (ii) induce, like antiestrogens, the insulin growth; and (iii) inhibit cell proliferation of cervical adenocarcinoma cells was examined. The results demonstrated that the aqueous extracts of chamomile at the concentrations under study induced osteoblastic cell differentiation and exhibited anti-estrogenic effect on breast cancer cells without proliferative effects on cervical adenocarcinoma cells. These data opens the possibility to design functional foods based on the use of chamomile aqueous extracts in order to prevent osteoporosis.

2.1.9. Anti-inflammatory activity

Chamazulene showed anti-inflammatory activity, as according to Safayhi et al. (1994), chamazulene inhibits the formation of leukotriene B_4 (LTB_4) in intact cells and in the supernatant fraction in a concentration-dependent mode. Chamazulene ($IC_{50} = 0.37$ mg l^{-1}) blocks the chemical peroxidation of arachidonic acid. LTB_4 is a product of the 5-lipoxygenase pathway of arachidonic acid metabolism, and is a potent chemotactic factor for neutrophils. It has been postulated that chamazulene has an important role in a variety of pathological conditions including rheumatoid arthritis, psoriasis, and inflammatory bowel disease. These results are of interest as a focus on the effect of sesquiterpenic compounds on the immune system response associated with, for instance, the inflammatory process. Leukocyte activation, characterized by the respiratory burst, is an essential part of host defence against invading microorganisms. The respiratory burst leads to the formation of toxic oxidants, but since toxic oxidants cannot just be directed to kill microorganisms, the process of leukocyte activation must be strictly regulated. On the other hand, a systemic inflammation process and sepsis may lead to an exaggerated leukocyte response to stimuli and increased production of toxic oxidants, thus contributing to tissue damage and organ injury (Pascual et al., 1997). Thus, better understanding and control of leukocyte activation during inflammation and sepsis may help identify and select novel pro- or anti-inflammatory drugs, for example, sesquiterpenic compounds of *M. recutita* L. Moreover, as chamazulene is a compound present in the essential oil and also in chamomile aqueous-ethanolic extracts, all presenting effects on leukocyte chemotaxis, as reported in anti-chemotactic effect (Section 2.1.7), this compound might be related to the mechanism of leukocyte regulation during the inflammation process.

2.1.10. Anti-cancer effect

The anti-cancer effect of α-bisabolol and bisabolol oxide A was studied on human and rat glioma cell lines. α-Bisabolol was found to have a time- and dose-dependent cytotoxic effect on these cell lines (Cavalieri et al., 2004). After 24 h of treatment with 0.56–0.78 mg l^{-1} of α-bisabolol, the cells' viability was reduced by 50% compared to untreated cells. Glioma cells treated with higher concentration of α-bisabolol (2.22 mg l^{-1}) resulted in a 100% cell death. Furthermore, the viability of normal glial cells was not affected by treatment with α-bisabolol at the same concentrations as above. Since glioma is among the worst cancers against which no efficient and non-toxic treatments have so far been reported, the results obtained with α-bisabolol (no toxicity observed in cell lines and its fast accumulation in the brain), make the use of this substance very promising for the clinical treatment of this highly malignant cancer (Cavalieri et al., 2004). Ogata et al. (2010) study the cytotoxic effect of bisabolol oxide A. Bisabolol oxide A was cytometrically examined on rat thymocytes by using appropriate fluorescent dyes. When the cells were incubated with bisabolol oxide A for 24 h, at concentrations of 7.15 mg l^{-1} and 23.8 mg l^{-1}, significantly increased populations of dead cells (12.7% and 17.8% respectively), shrunken cells (39.4% and 68.4% respectively), and cells with phosphatidylserine exposed on membrane surface (33.1% and 59.3% respectively) were observed. Both cell shrinkage and externalization of membrane phosphatidylserine are general features at an early stage of apoptosis. In addition, bisabolol oxide A significantly increased the population of cells containing hypodiploid DNA (25.5% at 7.15 and 39.8% at 23.8 mg l^{-1} of bisabolol oxide A) (DNA with a chromosome number that is one or more lower than the normal haploid number of chromosomes characteristic for a species). This increase was completely attenuated by Z-VAD-FMK, a pan-inhibitor for caspases (cysteine proteases, which play an essential role in apoptosis, necrosis and inflammation), indicating an involvement of caspase activation. These studies demonstrated that both α-bisabolol and bisabolol oxide A may have cytotoxic effects, although the reported concentrations that may reduce the viability of malignant cells were significantly higher for bisabolol oxide A than for α-bisabolol.

All these studies reported *in vitro* are very hopeful in order to valorise and consequently enhance the use of *M. recutita* L. with medicinal proposes. However, their extension to *in vivo* studies to assess the authentic effects in humans is essential.

2.2. In vivo studies

2.2.1. Animal model studies

2.2.1.1. Anti-inflammatory effect. The anti-inflammatory effect of aqueous and ethanolic extracts of chamomile was evaluated. In Wistar albino rats, the aqueous extract of chamomile suppressed both the inflammatory effect and leukocyte infiltration induced by a simultaneous injection of carrageenan and prostaglandin E_1. The inhibitory effects of chamomile aqueous extracts were similar to those elicited by 10 mg kg^{-1} of the anti-allergenic agent oxatomide (Mckay and Blumberg, 2006). The similar results presented for *in vitro* studies that showed anti-chemotactic effects (Section 2.1.7) in chamomile essential oil and chamomile aqueous-ethanolic extracts, and also anti-inflammatory activity (Section 2.1.9) for chamazulene, support the possibility that this compound is linked to the mechanism of leukocyte activation during the inflammation process. In Swiss rats, the topical application of an ethanolic chamomile extract to the inner surface of the ear reduced edema induced by the application of a 2.5% emulsion of croton oil (oil prepared from the seeds of *Croton tiglium*). In this experiment, an ethanolic extract of chamomile containing 50 mg l^{-1} α-bisabolol, 450 mg l^{-1} of each, bisabolol oxide A and B, 400 mg l^{-1} apigenin and its glucosides, 800 mg l^{-1} dicycloethers, and 20 mg l^{-1} azulenes was

applied to test animals in doses equivalent to 0.75, 0.25, and 0.08 mg of dried flowers per animal. The 0.75 mg of chamomile extract induced a reduction similar to that obtained with 0.45 mg benzydamine (26.6%), a non-steroid anti-inflammatory agent (Tubaro et al., 1984). Another study found that topical treatment with an extract of chamomile containing α-bisabolol (518 mg kg^{-1}), matricin (296 mg kg^{-1}), and apigenin (53 mg kg^{-1}), at a dose equivalent to 0.75 mg of dry product, was as effective as the reference drug, 0.60 mg benzydamine ($n = 25$), in preventing inflammation in rats subjected to croton oil induced edema (Della Loggia et al., 1990). As both aqueous and ethanolic extracts of chamomile presented anti-inflammatory effects, which are in accordance with the evidence of chamazulene activity observed in *in vitro* studies previously discussed, they can be studied in-depth in order to design future anti-inflammatory agents.

α-Bisabolol also inhibits the mouse ear dermatitis induced by noxious agents (arachidonic acid, phenol and capsaicin), suggesting that α-bisabolol may be a topical anti-inflammatory agent (Leite et al., 2011). In studies carried out with chamaviolin and chamazulene carboxylic acid (Fig. 1), artefacts generated from matricin (dried inflorescences contain *ca.* 0.2% of matricin), demonstrated that these compounds present anti-inflammatory activity (Goeters et al., 2001; Franke and Schilcher, 2005; Ramadan et al., 2006; Oehler et al., 2009). Ramadan et al. (2006) evaluated the anti-inflammatory activity of chamazulene carboxylic acid in rat ear edema. Chamazulene carboxylic acid presents a chemical structure similar to profens (Goeters et al., 2001; Imming et al., 2001), known as anti-inflammatory compounds that inhibit the key enzymes of prostaglandin metabolism – cyclooxygenase-1 and 2. In this study chamazulene carboxylic acid was found to be an inhibitor of cyclooxygenase-2 (50% inhibition at 13 mg l^{-1}), but not of cyclooxygenase-1 (17.4% inhibition at 13 mg l^{-1}). In another study (Oehler et al., 2009), also carried out using rat ear edema, chamavioline acted as an inhibitor of cyclooxygenase-2 (57% at 0.23 mg l^{-1}), not inhibiting cyclooxygenase-1 up to a concentration of 2.3 mg l^{-1}. These results suggest that both compounds, chamazulene carboxylic acid and chamavioline, act as inhibitors of cyclooxygenase-2. However, chamavioline presents higher activity (57% at 0.23 mg l^{-1}) than chamazulene carboxylic acid (50% at 13 mg l^{-1}).

2.2.1.2. Anti-genotoxic effect. The inhibitory effect of chamomile essential oil on the sister chromatid exchanges produced by daunorubicin (antineoplastic, inductor of the free radicals, producing several types of genotoxic damages) and methyl methanesulfonate (alkylating agent that forms covalent bonds with DNA and modifies guanine residues causing genotoxic damage) in rat bone marrow cells was evaluated by Hernández-Ceruelos et al. (2002). Rats injected with 5, 50, and 500 mg kg^{-1} chamomile essential oil plus 10 mg kg^{-1} daunorubicin had 26%, 63%, and 76% fewer sister chromatid exchanges (SCE), respectively, than rats treated with daunorubicin alone. A similar effect was found in rats treated with 250, 500, and 1000 mg kg^{-1} chamomile essential oil plus 25 mg kg^{-1} methyl methanesulfonate had inhibition of SCE at 25%, 46%, and 63%, respectively, when compared with the control group treated with methyl methanesulfonate alone. The analysis of the essential oil revealed the presence of α-bisabolol and its oxides, chamazulene, α- and β-farnesene, germacrene D, among other sesquiterpenic compounds. These results are in accordance with those previously discussed with regard to the *in vitro* studies, showing that chamomile essential oil represents a source of potential anti-mutagenic agents.

2.2.1.3. Hypo-cholesterolemic effect. An aqueous extract of chamomile reduced the cholesterolemic level in hyperlipidemic Wistar rats. After 10 days, the hyperlipidemic rats treated orally with a 6% aqueous extract of chamomile (4 mg kg^{-1}) exhibited significant decrease of serum cholesterol when compared with the control rats and no changes have been observed in serum triglyceride levels. The aqueous extract of chamomile had no effect on the control rats with respect to either cholesterol or triacylglycerides levels (Mckay and Blumberg, 2006).

2.2.1.4. Gastrointestinal effects. Several studies analysed the gastrointestinal effects of aqueous extracts and essential oil of chamomile and α-bisabolol chemical standard. When compared to papaverine, a smooth muscle relaxing drug, α-bisabolol was 91% more effective on spasms induced with barium chloride, while bisabolol oxides A and B were 46–50% more effective (Mckay and Blumberg, 2006). These results demonstrated that the aqueous extracts and essential oil of chamomile, due to the presence of compounds such as α-bisabolol and its oxides, have the potential to be effective in relaxing spasms. Bezerra et al. (2009) also investigated the effects of chamomile aqueous extracts and α-bisabolol against gastric damage induced by ethanol (96%, 1 ml per animal) in rats. These effects were assessed by determining the changes of the gastric lesion area. Chamomile aqueous extract reduced gastric damage with all doses tested (100, 200, and 400 mg kg^{-1}) and α-bisabolol at oral doses of 50 and 100 mg kg^{-1} markedly attenuated the gastric lesions induced by ethanol to the extent of 87% and 96%, respectively. Another study carried out on rats demonstrated that α-bisabolol inhibits the development of gastric ulcers induced by indomethacin, stress, and alcohol (Mckay and Blumberg, 2006). α-Bisabolol also reduced healing times in ulcers induced by either chemical stress or heat coagulation (Mckay and Blumberg, 2006). Torrado et al. (1995) studied the gastrotoxic influence of acetylsalicylic acid mixed with α-bisabolol on rats. At a dose of 200 mg kg^{-1} acetylsalicylic acid, 0.8–80 mg kg^{-1} α-bisabolol showed a significant ($p < 0.05$) protective effect on gastric mucosa. Also, Rocha et al. (2010), evaluate the gastroprotective action of α-bisabolol on ethanol and indomethacin-induced ulcer models in rats, and further investigate the pharmacological mechanisms involved in this action. The oral administration of α-bisabolol at a concentration of 100 and 200 mg kg^{-1} was able to protect the gastric mucosa from ethanol (0.2 ml per animal) and indomethacin-induced ulcer (20 mg kg^{-1}). The dosage of gastric reduced glutathione levels showed that ethanol and indomethacin reduced the content of non-protein sulfhydryl (NP-SH) groups, while α-bisabolol significantly decreased the reduction of these levels on ulcer-induced mice, but not in ulcer-free mice. All the studies reported above demonstrate that α-bisabolol presents very interesting gastrointestinal protective effects but the real effect on humans should be further explored.

2.2.1.5. Drug metabolizing effects in liver. The effects of chamomile infusion on the activity of hepatic phases I and II metabolizing enzymes were studied using rat liver microsomes. In this experiment, a group of Wistar rats had free access to a tea solution (2%) for 4 weeks while the control group had water (Maliakal and Wanwimolruk, 2001). The activity of phase I CYP1A2 decreased significantly whereas the activity of phase II detoxifying enzyme UDP-glucuronosyl transferase increased more than twice in comparison to the control. This study indicates that chamomile infusions can modulate the activity of hepatic cytochrome P450, and suggests that they can cause modulation of phases I and II drug metabolizing enzymes, as reported by *in vitro* studies of anti-mutagenic activity (Section 2.1.4) and interference on drug metabolism (Section 2.1.5) when chamomile-related sesquiterpenic compounds were used.

2.2.1.6. Central nervous system effects. A study carried out on ovarectomized rats reported that the inhalation of chamomile

essential oil reduced induced stress by increasing adrenocorticotropic hormone (ACTH) levels in plasma. ACTH is a polypeptide tropic hormone that regulates the activity of various endocrine glands, produced and secreted by the anterior pituitary gland. It is an important component of the hypothalamic–pituitary–adrenal axis and is often produced in response to biological stress, along with corticotropin-releasing hormone from the hypothalamus. Diazepam, co-administered with the essential oil, reduces ACTH levels, while flumazenile (benzodiazepine antagonist) blocks the effect of chamomile oil on ACTH. The compounds present in extracts of chamomile can also bind benzodiazepines and γ-aminobutyric acid (GABA) receptors (receptors that respond to the neurotransmitter GABA, the chief inhibitory neurotransmitter in the vertebrate central nervous system) in the brain and are thought to be responsible for some sedative effects (Mckay and Blumberg, 2006).

2.2.2. Human studies

2.2.2.1. Autonomic nervous system effects. Effects of chamomile infusions on autonomic nervous system parameters including heart beat rate, peripheral skin temperature, and mood, were examined in young Japanese males. Hot water was used as control. After chamomile infusion consumption, a decrease in heart beating rate, ratings of depression, and "sadness and depression" (determined by electroencephalography by emotion spectral analysis system) was observed, as well as an increase in skin temperature. The results demonstrate that chamomile infusion has a relaxing effect (Nakamura et al., 2002), which are in accordance with the referred results discussed with regard to the animal models studies for chamomile essential oil. This work reports an interesting biological effect associated with the consumption of chamomile infusion but more experimental assays should be carried out in order to understand the observed effect.

2.2.2.2. Hemodynamic effects. Hemodynamics comprises all the mechanisms involved in blood circulation through the cardiovascular system. The hemodynamic effects of chamomile infusions were examined in an open study of 12 heart disease patients hospitalized for cardiac catheterization. Patients had a small but significant increase in mean brachial artery pressure (91–98 mm Hg) 30 min after drinking two cups of chamomile infusion. No other significant changes in cardiac function have been observed, although 10 of the 12 patients fell into a deep sleep within 10 min of consuming the infusion (Gould et al., 1973). No explanations have yet been given to support these effects.

2.2.2.3. Topical effects. Several studies reported the topical effects of chamomile cream on skin inflammation associated with atopic dermatitis or eczemas (Hempel et al., 1999; Patzelt-Wenczler and Ponce-Poschl, 2000), radiation therapy (Maiche et al., 1991) and erythema (Korting et al., 1993). Chamomile cream has been reported to be as effective as 0.25% hydrocorticoid and non-steroidal anti-inflammatory agents (Albring et al., 1983). Topical effects of chamomile cream have also been examined for its effectiveness on wound care (Mckay and Blumberg, 2006). In another study, 14 patients with weeping wounds following dermabrasion for tattoo removal were treated with a compress containing chamomile extract. A significant decrease of wound area and increase of drying wound have been observed (Mckay and Blumberg, 2006). The evaluation of the effects related to the topical application of chamomile cream and compresses containing chamomile extracts were carried out based on the observation of symptoms. However, there is no information available about the cream and extract composition, nor about the relation between compound *versus* observed effect.

2.2.2.4. Gastrointestinal effects. The effects of chamomile-containing oral rinse on oral mucositis or stomatitis induced by cancer therapies have been examined in a clinical trial. Results showed both prophylactic ($n = 66$ patients) and therapeutic ($n = 32$ patients) effects of chamomile mouthwash in a case series of 98 head and neck cancer patients treated with either radiation or systemic chemotherapy. After the treatment with chamomile oral rinse, in the prophylactic group, one of the 20 radiation therapy patients developed grade 3 mucositis in the final week of treatment, while 13 developed intermediate-grade and 6 developed low-grade mucositis. Of the 32 patients in the therapeutic treatment group (16 radiation, 16 chemotherapy), all experienced immediate relief from mouth discomfort and within one week nearly all patients had no clinical signs of mucositis. These results suggest that the resolution of mucositis was accelerated by chamomile oral rinse (Mckay and Blumberg, 2006; Rodriguez-Fragoso et al., 2008).

3. Potential health benefits of *M. recutita* L.: actual knowledge and future outlook

Several health benefits have been reported for *M. recutita* L. essential oil and its extracts; however, a deep knowledge concerning the chemical components of chamomile responsible for each specific effect, as well as the mechanisms behind each effect, have not yet been established. Besides, in most cases, a chamomile extract standardized as to its chemical constituents is not yet available, making it difficult to compare results and to draw more realistic conclusions.

Chamomile-related sesquiterpenic compounds seem to be involved in a network of biological effects, *i.e.* they may exhibit themselves specific health benefits, may have synergic effects with drugs such as antibiotics, and/or may underlie the metabolism of certain co-administered drugs and mutagenic agents. It is important to point out that the quantities necessary to have a biological effect, *i.e.*, the dose is effect-dependent, and for the studies reported above, the doses are in the order of mg l^{-1}. However, depending on the structure of the compound and on the biological effect, these can be as low as μg l^{-1}. When chamomile extracts, *i.e.*, a mixture of several chemical structures, were tested, the minimum concentration to have an effect can be higher.

The anti-microbial, antioxidant, anti-malarial, anti-mutagenic, anti-inflammatory, and anti-cancer activities are explained by the presence of sesquiterpenic compounds (Safayhi et al., 1994; Rekka et al., 1996; Lopes et al., 1999; Brehm-Stecher and Johnson, 2003; Cavalieri et al., 2004; Gomes-Carneiro et al., 2005; van Zyl et al., 2006; Simões et al., 2008). On the other hand, anti-chemotatic, anti-inflammatory, anti-genotoxic, and gastrointestinal activities were tested using aqueous and ethanolic extracts or essential oil of *M. recutita* L. (Hernández-Ceruelos et al., 2002; Ganzera et al., 2006; Mckay and Blumberg, 2006; Presibella et al., 2006; Rodriguez-Fragoso et al., 2008), which contain sesquiterpenic compounds among other components. Sesquiterpenic compounds present a low solubility in water but the amount that can be solubilised seems to be enough to promote their transference from the plant to the water. For example, the solubility in water for α-bisabolol, β-farnesene, farnesol, and nerolidol are, respectively, 0.0023, 0.0108, 1.7, and 2.07 mg l^{-1}, at 25 °C (Syracuse Research Corporation, 2010). Furthermore, the solubility increases with temperature, and usually the aqueous extraction occurs at a temperature higher than room temperature (*ca.* 70 °C). Nerolidol is usually reported as a constituent of chamomile aqueous extracts and exhibits anti-malarial activity at a concentration ten times lower (0.2 mg l^{-1}) than its solubility in water (van Zyl et al., 2006).

These results clearly suggest that in order to valorise chamomile, several approaches may be improved. One of them should include the development of efficient and solvent-free methodologies to obtain a sesquiterpenic-enriched extract like, for example, supercritical fluid extraction (Povh et al., 2001) for the specific evaluation of their biological effects. Another approach should be to plan *in vivo* studies using animal models, in order to in-depth study the mechanism behind each biological effect. After that, toxicological assays should be performed and the safe samples should be used in human trials carried out for the most promising effects. The evaluation of the effects associated with the use of chamomile products was usually based only on the observation of symptoms. Scarce information on real human health benefits is available, which is a drawback for the possible use of chamomile, namely the sesquiterpenic compounds. In spite of the relevance of all the studies reported, where the data obtained is very promising concerning the potential health benefits, their extension to human studies is essential.

4. *In vitro* and *in vivo* studies: drawbacks and future trends

This work gives data that open several opportunities for the valuation of chamomile raw material, by looking for the potential effects of its components, including the sesquiterpenic compounds. However, the lack of human trials to confirm the potential health benefits associated with chamomile components may bring some limitations to the valuation of this medicinal plant. Consequently, *in vivo* studies are essential to evaluate the safety of several natural products to which people may be exposed. Unfortunately, *in vivo* studies have a number of drawbacks: (1) ethical issues surrounding animal experimentation, namely, animal suffering and induction of diseases such as obesity, diabetes or cancer; (2) the need to extrapolate from animal to humans in terms of physiology, biochemistry, genetics and behaviour; (3) the economic costs of long-term studies; and (4) the possibility of missing idiosyncratic human reactions. To predict human *in vivo* responses based on *in vitro* studies, computational methods for prediction of hazards and determination of mechanistic information about the compounds under survey are required. A dynamic model of the human upper gastrointestinal tract has already been reported to simulate conditions of ingestion and digestion and to investigate interactions of probiotic bacteria with the human intestinal microflora and the effects of probiotic bacteria and symbiotic products on the human gastrointestinal microbiota (Mainville et al., 2005). This is an example of the computational models required to allow the analysis of several gastrointestinal diseases with the aim of appraising several natural products while minimizing the use of animals and human voluntaries. It is hoped that these practices become the operative form of preclinical safety assessment in the future.

References

Alabaster, V., 2002. *In Vivo* Pharmacology Training Group. The fall and rise of in vivo pharmacology. Trends Pharmacol. Sci. 23, 13–18.

Albring, M., Albrecht, H., Alcorn, G., Lüker, P.W., 1983. The measuring of the anti-inflammatory effect of a compound on the skin of volunteers. Meth. Find. Exp. Clin. Pharmacol. 5, 75–77.

Ansarullah, R.N.J., Thounaojam, M.C., Patel, V., Devkar, R.V., Ramachandran, A.V., 2009. Antihyperlipidemic potential of a polyherbal preparation on triton WR 1339 (Tyloxapol) induced hyperlipidemia: a comparison with lovastatin. Int. J. Green Pharm. 3, 119–124.

Avallone, R., Zanoli, P., Puia, G., Kleinschnitz, M., Schreier, P., Baraldi, M., 2000. Pharmacological profile of apigenin, a flavonoid isolated from *Matricaria chamomilla*. Biochem. Pharmacol. 59, 1387–1394.

Baser, K.H., Demirci, B., Iscan, G., Hashimoto, T., Demirci, F., Noma, Y., Asakawa, Y., 2006. The essential oil constituents and antimicrobial activity of *Anthemis aciphylla* BOISS. var. discoidea BOISS. Chem. Pharm. Bull. (Tokyo) 54, 222–225.

Bezerra, S.B., Leal, L.K.A.M., Nogueira, N.A.P., Campos, A.R., 2009. Bisabolol-induced gastroprotection against acute gastric lesions: role of prostaglandins, nitric oxide, and K^+ ATP channels. J. Med. Food 12, 1403–1406.

Bhaskaran, N., Shukla, S., Srivastava, J.K., Gupta, S., 2010. Chamomile: an anti-inflammatory agent inhibits inducible nitric oxide synthase expression by blocking RelA/p65 activity. Int. J. Mol. Med. 26, 935–940.

Braga, P.C., Dal Sasso, M., Fonti, E., Culici, M., 2009. Antioxidant activity of bisabolol: inhibitory effects on chemiluminescence of human neutrophil bursts and cell-free systems. Pharmacology 83, 110–115.

Brehm-Stecher, B.F., Johnson, E.A., 2003. Sensitization of *Staphylococcus aureus* and *Escherichia coli* to antibiotics by the sesquiterpenoids nerolidol, farnesol, bisabolol and apritone. Antimicrob. Agents Chemother. 47, 3357–3360.

Briskin, D.P., 2000. Medicinal plants and phytomedicines. Linking plant biochemistry and physiology to human health. Plant Physiol. 124, 507–514.

Cavalieri, E., Mariotto, S., Fabrizi, C., Carcereri de Prati, A., Gottardo, R., Leone, S., Berra, L.V., Lauro, G.M., Ciampa, A.R., Suzuki, H., 2004. α-Bisabolol, a nontoxic natural compound, strongly induces apoptosis in glioma cells. Biochem. Biophys. Res. Commun. 315, 589–594.

Chandrasekaran, C.V., Sundarajan, K., Kripalini, D., Agarwal, A., 2010. *In vitro* efficacy and safety of poly-herbal formulations. Toxicol. In Vitro 24, 885–897.

Crotteau, C.A., Wright, S.T., Eglash, A., 2006. Clinical inquiries: what is the best treatment for infants with colic? J. Fam. Pract. 55, 634–636.

Cushnie, T.P.T., Lamb, A.J., 2005. Review: antimicrobial activity of flavonoids. Int. J. Antimicrob. Agents 26, 343–356.

Davila, J.C., Rodriguez, R.J., Melchert, R.B., Acosta Jr., D., 1998. Predictive value of *in vitro* model systems in toxicology. Annu. Rev. Pharmacol. Toxicol 38, 63–96.

Della Loggia, R., Carle, R., Sosa, S., Tubaro, A., 1990. Evaluation of the anti-inflammatory activity of chamomile [*Chamomilla recutita*] preparations. Planta Med. 56, 657–658.

Di Stasi, L.C., Oliveira, G.P., Carvalhaes, M.A., Queiroz-Junior, M., Tiena, O.S., Kakinamia, S.H., Reis, M.S., 2002. Medicinal plants popularly used in the Brazilian Tropical Atlantic Forest. Fitoterapia 73, 69–91.

2002. Off. J. Eur. Union: L183 12 (July), 51–57.

2004. Off. J. Eur. Union: L183 51 (April), 85–90.

Dragland, S., Senoo, H., Wake, K., Holte, K., Blomhoff, R., 2003. Several culinary and medicinal herbs are important sources of dietary antioxidants. J. Nutr. 133, 1286–1290.

Fontanarosa, P.B., Lundberg, G.D., 1998. Alternative medicine meets science. J. Am. Med. Assoc. 280, 1618–1619.

Forster, H.B., Niklas, H., Lutz, S., 1980. Antispasmodic effects of some medicinal plants. Planta Med. 40, 309–319.

Franke, R., Schilcher, H., 2005. Chamomile: Industrial Profiles, II Series: Medicinal and Aromatic Plants, vol. 42. CRC Press/Taylor & Francis Group, Boca Raton/Broken Sound Parkway, NW.

Ganzera, M., Schneider, P., Stuppner, H., 2006. Inhibition effects of the essential oil of chamomile (*Matricaria recutita* L.) and its major constituents on human cytochrome P450 enzymes. Life Sci. 78, 856–861.

Gardiner, P., 2007. Complementary, holistic, and integrative medicine: chamomile. Pediatr. Rev. 28, 16–18.

Goeters, S., Imming, P., Pawlitzki, G., Hempel, B., 2001. On the absolute configuration of matricin. Planta Med. 67, 292–294.

Gomes-Carneiro, M.R., Dias, D.M.M., De-Oliveira, A.C.A.X., Paumgartten, F.J.R., 2005. Evaluation of mutagenic and antimutagenic activities of α-bisabolol in the *Salmonella*/microsome assay. Mutat. Res. 585, 105–112.

Gould, L., Reddy, C.V., Gomprecht, R.F., 1973. Cardiac effects of chamomile tea. J. Clin. Pharmacol. 11, 475–479.

Habersang, S., Leuschner, F., Isaac, O., Thiemer, K., 1979. Pharmacological studies with compounds of chamomile. IV. Studies on toxicity of (−)-alpha-bisabolol. Planta Med. 37, 115–123.

Hempel, B., Martino, V., Bandoni, A., Blaak, G., Capelle, N., 1999. CO_2-extract from German chamomile—proposed medicinal use. Acta Hortic. 503, 15–19.

Hernández-Ceruelos, A., Madrigal-Bujaidar, E., de la Cruz, C., 2002. Inhibitory effect of chamomile essential oil on the sister chromatid exchanges induced by daunorubicin and methyl methanesulfonate in mouse bone marrow. Toxicol. Lett. 135, 103–110.

Hernández-Ceruelos, A., Madrigal-Santillán, E., Morales-González, J.A., Chamorro-Cevallos, G., Cassani-Galindo, M., Madrigal-Bujaidar, E., 2010. Antigenotoxic effect of *Chamomilla recutita* (L.) Rauschert essential oil in mouse spermatogonial cells and determination of its antioxidant capacity *in vitro*. Int. J. Mol. Sci. 11, 3793–3802.

Imming, P., Goeters, S., Pawlitzki, G., Hempel, B., 2001. Absolute stereochemistry of guaianolides, of matricin and its epimers, of yarrow proazulenes, and of chamazulene carboxylic acid. Chirality 13, 337–341.

Isaac, O., 1979. Pharmacological investigation with compounds of chamomile on the pharmacology of (−)-alpha-bisabolol and bisabololoxides. Planta Med. 35, 118–124 (review).

Kamatou, G.P.P., Viljoen, A.M., 2010. A review of the application and pharmacological properties of α-bisabolol and α-bisabolol-rich oils. J. Am. Oil Chem. Soc. 87, 1–7.

Kassi, E., Papoutsi, Z., Fokialakis, N., Messari, I., Mitakou, S., Moutsatsou, P., 2004. Greek plant extracts exhibit selective estrogen receptor modulator (SERM)-like properties. J. Agric. Food Chem. 52, 6956–6961.

Kintzios, S., Michaelakis, A., 1999. Induction of somatic embryogenesis and *in vitro* flowering from inflorescences of chamomile (*Chamomilla recutita* L.). Plant Cell Rep. 18, 684–690.

Koleckar, V., Opletal, L., Brojerova, E., Rehakova, Z., Cervenka, F., Kubikova, K., Kuca, K., Jun, D., Polasek, M., Kunes, J., Jahodar, L., 2008. Evaluation of natural antioxidants of *Leuzea carthamoides* as a result of a screening study of 88 plant extracts from the European Asteraceae and Cichoriaceae. J. Enzyme Inhib. Med. Chem. 23, 218–224.

Korting, H.C., Schafer-Korting, M., Hart, H., Laux, P., Schmid, M., 1993. Anti-inflammatory activity of hamamelis distillate applied topically to the skin. Influence of vehicle and dose. Eur. J. Clin. Pharmacol. 44, 315–318.
Kroll, U., Cordes, C., 2006. Pharmaceutical prerequisites for a multi-target therapy. Phytomedicine 5, 12–19.
Lee, K.G., Shibamoto, T., 2002. Determination of antioxidant potential of volatile extracts isolated from various herbs and spices. J. Agric. Food. Chem. 50, 4947–4952.
Leite, G.O., Leite, L.H.I., Sampaio, R.S., Araruna, M.K.A., Menezes, I.R.A., Costa, J.G.M., Campos, A.R., 2011. (−)-α-Bisabolol attenuates visceral nociception and inflammation in mice. Fitoterapia 82, 208–211.
Lemberkovics, E., Kéry, A., Marczal, G., Simándi, B., Szöke, E., 1998. Phytochemical evaluation of essential oils, medicinal plants and their preparations. Acta Pharm. Hung. 68, 141–149.
Lis-Balchin, M., Deans, S.G., Eaglesham, E., 1998. Relationship between bioactivity and chemical composition of commercial essential oils. Flavour Frag. J. 13, 98–104.
Lodish, H., Berk, A., Matsudaira, P., Kaiser, C.K., Krieger, M., Scott, M.P., Zipursky, S.L., Darnell, J., 2004. Molecular Cell Biology, 5th ed. WH Freeman and Company, New York.
Lopes, N.P., Kato, M.J., Andrade, E.H., Maia, J.G., Yoshida, M., Planchart, A.R., Katzin, A.M., 1999. Antimalarial use of volatile oil from leaves of *Virola surinamensis* (Rol) Warb. by Waiapi Amazon Indians. J. Ethnopharmacol. 67, 313–319.
Maiche, A.G., Grohn, P., Maki-Hokkonen, H., 1991. Effect of chamomile cream and almond ointment on acute radiation skin reaction. Acta Oncol. 30, 395–396.
Mainville, I., Arcand, Y., Farnworth, E.R., 2005. A dynamic model that simulates the human upper gastrointestinal tract for the study of probiotics. Int. J. Food Microb. 99, 287–296.
Maliakal, P.P., Wanwimolruk, S., 2001. Effect of herbal teas on hepatic drug metabolizing enzymes in rats. J. Pharm. Pharmacol. 53, 1323–1329.
Mann, C., Staba, E.J., 1986. In herbs, spices and medicinal plants: recent advances in botany. In: Craker, L.E., Simon, J.E. (Eds.), Horticulture and Pharmacology. Oryx Press, Phoenix, Arizona, pp. 235–280.
Maschi, O., Dal Cero, E., Galli, G.V., Caruso, D., Bosisio, E., Dell'Agli, M., 2008. Inhibiton of human cAMP-phosphodiesterase as a mechanism of spasmolytic effect of *Matricaria recutita* L. J. Agric. Food Chem. 56, 5015–5020.
Mckay, D.L., Blumberg, J.B., 2006. A review of the bioactive and potential health benefits of chamomile tea (*Matricaria recutita* L.). Phytother. Res. 20, 519–530.
Miller, T., Wittstock, U., Lindequist, U., Teuscher, E., 1996. Effects of some components of the essential oil of chamomile, *Chamomilla recutita*, on histamine release from rat mast cells. Planta Med. 62, 60–61.
Milner, J.A., 1999. Functional foods and health promotion. J. Nutr. 129, 1395S–1397S.
Mohammad, R., Hamida, S., Adams An Norbert De, K., Patrick, V.D., 2010. Effects of planting date and seedling age on agro-morphological characteristics, essential oil content and composition of German chamomile (*Matricaria chamomilla* L.) grown in Belgium. Ind. Crops Prod. 31, 145–152.
Nakamura, H., Moriya, K., Oda, S., Yano, E., Kakuta, H., 2002. Changes in the parameters of autonomic nervous system and emotion spectrum calculated from encephalogram alter drinking chamomile tea. Aroma Res. 3, 251–255 (Japanese).
Novakofski, J., 2004. Adipogenesis: usefulness of *in vitro* and *in vivo* experimental models. J. Anim. Sci. 82, 905–915.
Oehler, Ch., Bensdorf, K., Gust, R., Imming, P., 2009. Chamavioline—antiedematous, but not a constituent of *Matricaria recutita*. Phytochem. Lett. 2, 171–175.
Ogata, I., Kawanai, T., Hashimoto, E., Nishimura, Y., Oyama, Y., Seo, H., 2010. Bisabololoxide A, one of the main constituents in *German chamomile* extract, induces apoptosis in rat thymocytes. Arch. Toxicol. 84, 45–52.
Orav, A., Kailas, T., Ivask, K., 2001. Volatile constituents of *Matricaria recutita* L. from Estonia. Proc. Estonian Acad. Sci. Chem. 50, 39–45.
Pascual, C., Karzai, W., Meier-Hellmann, A., Bredle, D.L., Reinhart, K., 1997. A controlled study of leukocyte activation in septic patients. Intensive Care Med. 23, 743–748.
Patel, D., Shukla, S., Gupta, S., 2007. Apigenin and cancer chemoprevention: progress, potential and promise. Int. J. Oncol. 30, 233–245 (review).
Patzelt-Wenczler, R., Ponce-Poschl, E., 2000. Proof of efficacy of Kamillosan® cream in atopic eczema. Eur. J. Med. Res. 5, 171–175.
Petronilho, S., Maraschin, M., Delgadillo, I., Coimbra, M.A., Rocha, S.M., 2011. Sesquiterpenic composition of the inflorescences of Brazilian chamomile (*Matricaria recutita* L.). Impact of the agricultural practices. Ind. Crops Prod. 34, 1482–1490.
Pierre, S., Crosbie, L., Duttaroy, A.K., 2005. Inhibitory effect of aqueous extracts of some herbs on human platelet aggregation *in vitro*. Platelets 16, 469–473.
Povh, N.P., Marques, M.O.M., Meireles, M.A.A., 2001. Supercritical CO_2 extraction of essential oil and oleoresin from chamomile (*Chamomilla recutita* [L.] Rauschert). J. Supercrit. Fluids 21, 245–256.
Presibella, M.M., Santos, C.A., Weffort-Santos, A.M., 2007. *In vitro* antichemotactic activity of *Chamomilla recutita* hydroethanol extract. Pharm. Biol. 45, 124–130.
Presibella, M.M., Villas-Bôas, L.B., Belletti, K.M.S., Santos, C.A.M., Weffort-Santos, A.M., 2006. Comparison of chemical constituents of *Chamomilla recutita* (L.) Rauschert essential oil and its anti-chemotactic activity. Braz. Arch. Biol. Technol. 49, 717–724.
Rahimia, E., Pradob, J.M., Zahedic, G., Meireles, M.A.A., 2011. Chamomile extraction with supercritical carbon dioxide: mathematical modeling and optimization. J. Supercrit. Fluids 56, 80–88.
Ramadan, M., Goeters, S., Watzer, B., Krause, E., Lohmann, K., Bauer, R., Hempelr, B., Imming, P., 2006. Chamazulene carboxylic acid and matricin: a natural profen and its natural prodrug, identified through similarity to synthetic drug substances. J. Nat. Prod. 69, 1041–1045.
Rekka, E.A., Kourounakis, A.P., Kourounakis, P.N., 1996. Investigation of the effect of chamazulene on lipid peroxidation and free radical process. Res. Commun. Mol. Pathol. Pharmacol. 92, 361–364.
Rocha, N.F.M., Venâncio, E.T., Moura, B.A., Silva, M.I.G., Neto, M.R.A., Riosa, E.R.V., Sousa, D.P., Vasconcelos, S.M.M., Fonteles, M.M.F., Sousa, F.C.F., 2010. Gastroprotection of (−)-α-bisabolol on acute gastric mucosal lesions in mice: the possible involved pharmacological mechanisms. Fundam. Clin. Pharmacol. 24, 63–71.
Rocha, S.M., Coelho, E., Vinholes, J., Coimbra, M.A., 2006. Grapes and wine from *Vitis vinifera* L. as a potential source of sesquiterpenoids. In: Singh, V.K., Govil, J.N., Ahmad, K., Sharma, R.Kr. (Eds.), Natural Products, Series Recent Progress in Medicinal Plants, vol. 15. Studium Press LLC, Huston, TX, USA, pp. 253–272 (Cap 12).
Rodriguez-Fragoso, L., Reyes-Esparza, J., Burchiel, S.W., Herrera-Ruiz, D., Torres, E., 2008. Risks and benefits of commonly used herbal medicines in Mexico. Toxicol. Appl. Pharmacol. 227, 125–135.
Rubiolo, P., Belliardo, F., Cordero, C., Liberto, E., Sgorbini, B., Bicchi, C., 2006. Headspace-solid-phase microextraction fast GC in combination with principal component analysis as a tool to classify different chemotypes of chamomile flower-heads (*Matricaria recutita* L.). Phytochem. Anal. 17, 217–225.
Safayhi, H., Sabieraj, J., Sailer, E.R., Ammon, H.P., 1994. Chamazulene: an antioxidant-type inhibitor of leukotriene B4 formation. Planta Med. 60, 410–413.
Salamon, I., Honcariv, R., 1994. Growing condition and breeding of chamomile (*Chamomilla recutita* L.) Rauschert regarding the essential oil qualitative–quantitative characterization in Slovakia. Herba Pol., 68–74.
Salamon, I., 2007. Effect of the internal and external factors on yield and qualitative–quantitative characteristics of chamomile essential oil. Acta Hortic. 749, 45–64.
Schneeman, B., 2007. FDA's review of scientific evidence for health claims. J. Nutr. 137, 493–494.
Shikov, A.N., Pozharitskaya, O.N., Makarov, V.G., Kvetnaya, A.S., 2008. Antibacterial activity of *Chamomilla recutita* oil extract against *Helicobacter pylori*. Phytother. Res. 22, 252–253.
Simões, M., Rocha, S., Coimbra, M.A., Vieira, M.J., 2008. Enhancement of *Escherichia coli* and *Staphylococcus aureus* antibiotic susceptibility using sesquiterpenoids. Med. Chem. 4, 616–623.
Soliman, K.M., Badeaa, R.I., 2002. Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. Food Chem. Toxicol. 40, 1669–1675.
Srivastava, J.K., Gupta, S., 2007. Antiproliferative and apoptotic effects of chamomile extract in various human cancer cells. J. Agric. Food Chem. 55, 9470–9478.
Srivastava, J.K., Pandey, M., Gupta, S., 2009. Chamomile, a novel and selective Cox-2 inhibitor with anti-inflammatory activity. Life Sci. 85, 663–669.
Srivastava, J.K., Shankar, E., Gupta, S., 2010. Chamomile: a herbal medicine of the past with bright future. Mol. Med. Rep. 3, 895–901.
Stamatis, G., Kyriazopoulos, P., Golegou, S., Basayiannis, A., Skaltsas, S., Skaltsa, H., 2003. *In vitro* anti-*Helicobacter pylori* activity of Greek herbal medicines. J. Ethnopharmacol. 88, 175–179.
Syracuse Research Corporation, 2010. The Physical Properties Database, Available at: http://www.syrres.com/esc/physdemo.htm (accessed 07.06.10).
Szelenyi, I., Isaac, O., Thiemer, K., 1979. Pharmacological experiments with compounds of chamomile. III. Experimental studies of the ulcerprotective effect of chamomile. Planta Med. 35, 218–227.
Szoke, É., Máday, E., Gershenzon, J., Lemberkovics, É., 2004a. Terpenoids in genetically transformed cultures of chamomile. Chromatographia 60, S269–S272.
Szoke, É., Máday, E., Tyihák, E., Kuzovkina, I.N., Lemberkovics, É., 2004b. New terpenoids in cultivated and wild chamomile (*in vivo* and *in vitro*). J. Chromatogr. B 800, 231–238.
Torrado, S., Torrado, S., Agis, A., Jiménez, M.E., Cadorniga, R., 1995. Effect of dissolution profile and (−)-alpha-bisabolol on the gastrotoxicity of acetylsalicylic acid. Pharmazie 50, 141–143.
Tubaro, A., Zilli, C., Redaelli, C., Della Loggia, R., 1984. Evaluation of antiinflammatory activity of a chamomile extract after topical application. Planta Med. 50, 359.
Van der Kooy, F., Maltese, F., Choi, Y.H., Kim, H.K., Verpoorte, H., 2009. Quality control of herbal material and phytopharmaceuticals with MS and NMR based metabolic fingerprinting. Planta Med. 75, 763–775.
van Zyl, R.L., Seatlholo, S.T., van Vuuren, S.F., Viljoen, A.M., 2006. The biological activities of 20 nature identical essential oil constituents. J. Essent. Oil Res. 18, 129–133.
Yarosh, D.B., Galvin, J.W., Nay, S.L., Pena, A.V., Canning, M.T., Brown, D.A., 2006. Anti-inflammatory activity in skin by biomimetic of *Evodia rutaecarpa* extract from traditional Chinese medicine. J. Dermatol. Sci. 42, 13–21.
Yuan, R., Lin, Y., 2000. Traditional Chinese medicine: an approach to scientific proof and clinical validation. Pharmacol. Ther. 86, 191–198.