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Review Article

A review on pharmacological potentials of phenolic diterpenes carnosic acid and carnosol obtained from *Rosmarinus officinalis* L. and modern extraction methods implicated in their recovery

Dhananjay Singh[1], Nishu Mittal[2], And Mohammed Haris Siddiqui[3,*]

[1]Department of Biosciences, Integral University, Kursi Road, Lucknow, Uttar Pradesh-226026, India
[2]Faculty of Biosciences, Institute of Biosciences and Technology, Shri Ramswaroop Memorial University, Barabanki, Uttar Pradesh-225003, India
[3]Department of Bioengineering, Integral University, Kursi Road, Lucknow, Uttar Pradesh-226026, India

ABSTRACT

Rosmarinus officinalis L. is a perennial herb, known for culinary as well as medicinal properties. It has been shown that bioactive compounds like rosmarinic acid, carnosic acid, and carnosol are responsible for the medicinal properties of this plant species. Carnosic acid is a phenolic diterpene synthesized in young leaves of rosemary, whereas carnosol is produced after the oxidation of carnosic acid. Several studies have confirmed their antiangiogenic, anti-inflammatory, antimicrobial, antidiabetic, antioxidant, antitumor, neuroprotective, and gastroprotective properties. Ethanol, a mixture of ethanol-acetone, and hexane have been recommended as the best solvents for the extraction of carnosic acid, but advanced extraction techniques such as microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), pressurized liquid extraction (PLE), supercritical fluid extraction (SFE), etc. have been used to extract these phenolic antioxidant compounds in higher yield. This report is a kind of first study that emphasizes the recent research on the pharmacological potentials of carnosic acid and carnosol and summarizes the studies on modern extraction procedures.

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1. Introduction

Rosmarinus officinalis L., commonly called rosemary and belonging to the Lamiaceae family, is a plant used worldwide as a culinary herb. The Lamiaceae family is the sixth largest among angiosperms with a reported 236 genera having over 7,000 species (Halmschlag et al., 2022) with a diverse morphology among species which may be herbs, shrubs, and trees (Harley, 2012). These species are bisexual, have flowers with apparent sepals and petals, inflorescence, show bilateral symmetry, their corolla tube has a characteristic "lip shape" where it is divided into two distinct parts, and their fruits are of schizocarp type (Ramos da Silva et al., 2021). The species which show aromatic properties are basil, thyme, oregano, sage, lemon balm, and rosemary (Bekut et al., 2018). A total of 4 species of the genus *Rosmarinus* have been identified as *R. lavandulaceus* Noë, *R. eryocalix* Jord. & Fourr., *R. tomentosus* Hub.-Mor. & Maire, and *R. officinalis* L., but only *R. officinalis* L. and *R. eryocalix* Jord. & Fourr. are widely known medicinal and culinary plants in the Mediterranean, Asia, and Latin America regions (Hernández et al., 2016; Pieracci et al., 2021; WFO-2023 http://www.worldfloraonline.org/). *R. eriocalyx* Jord. & Fourr. is a well-known medicinal plant in Algeria and Morocco and is found as a potential source of antioxidant compounds (Boudiar et al., 2019). *R. officinalis* L. is native to the Mediterranean region, also grown in Europe, USA, Algeria, China, Romania, the Middle East, Morocco, Russia, Serbia, Tunisia, Turkey, and some regions of India. Rosemary is a perennial shrub that grows about 1-2 m in height. Their leaves are dark green in color, shiny, linear, like curved needles, and about 1 cm long. Rosemary plant has also been used for several medicinal purposes (Aziz et al., 2022). Like other reported medicinal plants *i.e.*, *Urtica dioica* L., *Bellis perennis* L., *Allium sativum* L., *Morus nigra* L., *Juniperus oxycedrus* L., *Allium cepa* L., *Vitis vinifera*

Corresponding author: Mohammed Haris Siddiqui
Tel: 0522-2890730; Fax: 0522-2890812
E-mail address: mohdharis.siddiqui22@gmail.com, **doi: 10.30495/tpr.2023.1990761.1365**

L., and *Ajuga chamaepitys* (L.) Schreb., *R. officinalis* L. is also used to treat hemorrhoids and other diseases (Çakılcıoğlu and Türkoğlu, 2007; Medini et al., 2013; Tudu et al., 2022; Tarasevičienė et al., 2023).

Rosemary contains a broad spectrum of essential oil constituent components, *e.g.*, 1,8-cineole, α-pinene, and camphor, phenolic diterpenes, *e.g.*, carnosic acid, carnosol, and rosmanols as well as phenolic esters, *e.g.*, rosmarinic acid (Choi et al., 2019; Oualdi et al., 2023). Seasonal variations in phenolic, flavonoid, and antioxidant compounds of rosemary have also been reported (Afshar et al., 2022a; Afshar et al., 2022b). Carnosic acid ($C_{20}H_{28}O_4$) is a benzenediol abietane diterpenoid that is abieta-8,11,13-triene substituted by hydroxy groups at positions 11 and 12 and a carboxy group at position 20 (Fig. 1-a), obtained from rosemary (*R. officinalis* L.), common sage (*Salvia officinalis* L.) and other species of the Lamiaceae plant family (Birtić et al., 2015; Loussouarn et al., 2017). Carnosic acid biosynthesis occurs in young leaves at the branch apices and the consumption of diterpene molecule partially takes place during leaf development and aging (Brückner et al., 2014; Božić et al., 2015). Besides carnosic acid, another phenolic diterpene measured in rosemary leaves is carnosol ($C_{20}H_{26}O_4$) (Fig. 1-b) which is a major product after the oxidation of carnosic acid (Loussouarn et al., 2017). Carnosic acid and carnosol are terpenoids (isoprenoids or terpenes), the largest class of plant secondary metabolites (Hill and Connolly, 2013). Carnosic acid is often classified among polyphenols because it contains a phenolic group but its cellular distribution, biosynthetic pathway, solubility properties, and other properties differ from the majority of polyphenolic compounds. Therefore, it resembles terpenoids such as tocopherols and carotenoids (Birtić et al., 2015).

Carnosic acid is a lipid-soluble compound known for its high antioxidative capacities having an IC_{50} over the range of 24-96 µM and possessing several industrial applications in food and beverage disciplines, personal care, nutrition, and for health (Barni et al., 2012; Birtić et al., 2015). Carnosic acid shows several pharmacological properties such as antiadipogenic, antiatherosclerosis, antiangiogenic, anti-inflammatory, antimicrobial, antidiabetic, antioxidant, antiplatelet, hypolipidemic, antitumor, and neuroprotective characteristics, while carnosol has antiangiogenic, anti-inflammatory, antimicrobial, antioxidant, antitumor, and gastroprotective properties (González-Vallinas et al., 2015; Chan et al., 2022). The presence of catechol moiety is presumably responsible for the remarkable antioxidant effect of carnosic acid and carnosol (Richheimer et al., 1996). Carnosol has also been reported to display promising anti-inflammatory properties (Poeckel et al., 2008). The antibacterial activity of carnosic acid and carnosol was also reported in several studies (Del Campo et al., 2000; Cushnie and Lamb, 2005; Moreno et al., 2006).

In the conventional extraction procedures, several parameters such as extraction time, liquid-to-solid ratio, and the kind of extracting solvent play an important role. The conventional solid-liquid extraction procedure is popular for antioxidant extraction because it is safe, cheap, and easy to scale up (Oliveira Gde et al., 2016). The correct operational conditions are much needed for higher recovery of compounds, which additionally give the concentrated extract using minimum energy, time, raw material, and solvent. The antioxidant extraction procedure requires optimization of all such parameters, even for different plants or the same plant if it has undergone different pretreatments (Dorta et al., 2013; Oliveira Gde et al., 2016). In comparison to conventional Soxhlet extraction (CSE), the eco-footprint of modern extraction procedures, like pressurized liquid extraction (PLE) was detected by considering the consumption of raw material, solvent, energy, and time where PLE was found as a rapid, and environmentally friendly technique for the detection of active contents in the plant (Hirondart et al., 2020). The other modern methods optimized for more robust and environmentally friendly antioxidant extraction from *R. officinalis* L. are ultrasound-assisted extraction (Paniwnyk et al., 2009), pressurized green solvent extraction (Herrero et al., 2010), accelerated solvent extraction (Hossain et al., 2011), and CO_2 supercritical fluid extraction (Visentín et al., 2011). Dielectric heating has also been proposed to dry rosemary leaves to minimize the reduction in quality by effective heat distribution throughout the plant material (Sui et al., 2012).

The main objective of this review is to describe an overview of the therapeutic effects of primary medicinal constituents obtained from *R. officinalis* L. herb, carnosic acid and carnosol, considering the previously published reports in the literature. In this connection, our focus is on the recent studies representing the pharmacological properties of carnosic acid, and modern extraction procedures to maximize the yield of these phenolic diterpenes. Furthermore, the modern biotechnological applications have also been discussed to increase the yield of carnosic acid.

2. Results and Discussion

2.1. Biologically active compounds obtained from *R. officinalis* L.

R. officinalis L. has been widely used as a medicinal plant to prevent and cure colds, rheumatoid arthritis, along with muscle and joint pain (Calvo et al., 2011; Zhang et al., 2014). It is one of the popularly known sources of bioactive natural compounds representing antibacterial, antidiabetic, anti-inflammatory, antitumor, antioxidant, effectiveness against multidrug-resistant bacteria, amoebicidal, acaricidal, and other activities (Bozin et al., 2007; Cheung and Tai, 2007; Bakirel et al., 2008; Takaki et al., 2008; Pérez-Fons et al., 2010; Yesil-Celiktas et al., 2010; Tai et al., 2012; Yu et al., 2013; Zhang et al., 2014; Santomauro et al., 2018; Anacarso et al., 2019; Iseppi et al., 2019; Guellouma et al., 2023; Lopes et al., 2023). Recently, *R. officinalis* L. extract was found to be the protective agent against the risk factors caused by some neurodegenerative disorders (Zappalà et al., 2021). It has been well documented that *R. officinalis* L. contains 2 types of potentially active compounds: *i*) one involves small molecular weight aromatic compounds, which evaporate rapidly and produce the characteristic

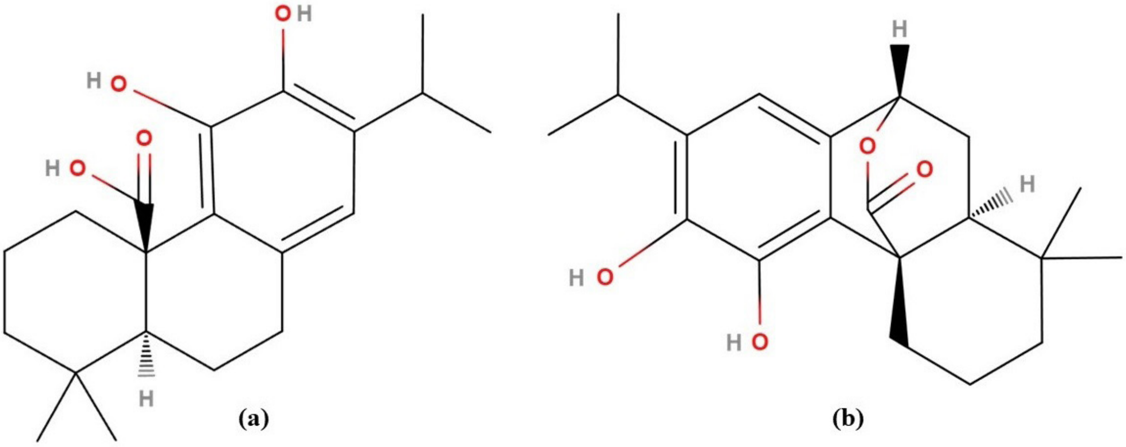

Fig. 1. Structures of (**a**) Carnosic acid and, (**b**) Carnosol.

smell and taste of rosemary, commonly known as essential oils and *ii*) the other type includes polyphenolic compounds such as rosmarinic acid, carnosic acid and carnosol, which have been known to exert indirect antioxidant actions, anticancer and other activities (de Oliveira, 2016; Loussouarn et al., 2017; Andrade et al., 2018; Jesus et al., 2023). Carnosic acid is the most investigated bioactive compound in *R. officinalis* L. followed by carnosol, rosmarinic acid, and ursolic acid (Andrade et al., 2018). Other reported compounds are betulinic acid, oleanolic and micromeric acid, rosmanol, epirosmanol, luteolin, etc.

2.2. Pharmacological activities of carnosic acid

Traditionally, rosemary oil has been known to cure several diseases such as inflammatory diseases and diabetes mellitus (Bakirel et al., 2008; Arranz et al., 2015). The bioactivities of rosemary extracts include anti-inflammatory, anti-diabetic, hepatoprotective, and antimicrobial activity (Fig. 2) and these bioactivities are most probably due to the presence of phenolic compounds such as carnosic acid (Andrade et al., 2018).

2.2.1. Antitumor activity

R. officinalis L. polyphenols can modulate cell growth and differentiation; thus interfering with cancer progression and development (Kar et al., 2012). Several studies have reported the antitumor activity of *R. officinalis* L. which is rich in phenolic compounds (Huang et al., 1994; Dörrie et al., 2001; Tsai et al., 2011; Barni et al., 2012). Carnosic acid and carnosol represent ~5% of *R. officinalis* L. dried leaves' weight, and these compounds have greater antitumor potential (Andrade et al., 2018). Several studies confirm the cytotoxicity of carnosol and carnosic acid on human, breast, and colon cancer cells (Dörrie et al., 2001; Bai et al., 2010; Barni et al., 2012). A study also reported a decrease in cell viability in resistant tumor cells using carnosic acid in a dose-dependent manner (González-Vallinas et al., 2013). The use of carnosic acid and its ester derivatives in an *in vivo* experiment was reported as a compound to prevent gastric lesions in HCl/EtOH-induced gastric lesions model in mice serving as a chemopreventative in 7,12-dimethylbenz anthracene (DMBA)-induced mammary tumorigenesis in rats (Singletary et al., 1996; Theoduloz et al., 2011; Birtić et al., 2015).

2.2.2. Antioxidant activity

The antioxidant activity of several compounds from rosemary such as carnosol, carnosic acid, rosmanol, rosmarinic acid, oleanolic acid, and ursolic acid were reported in previous studies. Carnosol was found as a suppressor of inducible nitric oxide synthase by down-regulating nuclear factor-kappaB (NFkB) in mouse macrophages (Lo et al., 2002). In an *in vitro* study, carnosic acid reduced acrylamide-induced neurotoxicity in rat and PC12 cells through inhibition of oxidative stress and apoptosis (Ghasemzadeh Rahbardar et al., 2022) and was also found as a protective agent against acrylamide-induced liver damage (Donmez et al., 2020). Carnosic acid offers a neuroprotective effect against organophosphate pesticide chlorpyrifos-induced oxidative stress as well as inflammation of mice brain and eye tissues (AlKahtane et al., 2020). A study on the protective mechanism of carnosic acid revealed that it protected neuronal cells under ischemia/hypoxia by reducing reactive oxygen species (ROS) and nitric oxide (NO), inhibiting COX-2 and MAPK pathways confirming its anti-inflammatory and antioxidative properties (Hou et al., 2012). Carnosic acid has also been found as a protective agent against 6-hydroxydopamine (6-OHDA)-induced neurotoxicity through increasing the antioxidant enzymes expression such as c-glutamate-cysteine ligase catalytic (GCLC) subunit, c-glutamate-cysteine ligase modifier (GCLM) subunit, superoxide dismutase (SOD), and glutathione reductase (Wu et al., 2015). It is also noteworthy that carnosic acid protects the mitochondria of human neuroblastoma SH-SY5Y cells from the pro-oxidant effects of chlorpyrifos through the activation of the PI3K/Akt/Nrf2 axis, which is responsible for the upregulation of the mitochondrial glutathione content and antioxidant effects (de Oliveira et al., 2016). In a recent study, carnosic acid was

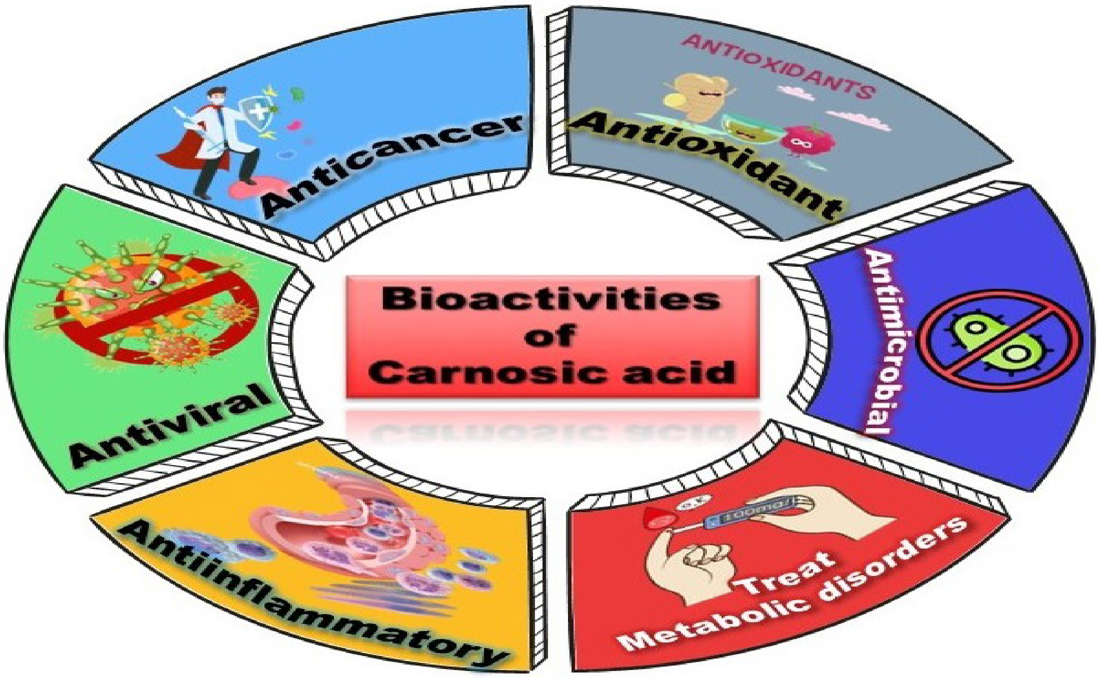

Fig. 2. Some of the most important known bioactivities of carnosic acid.

found effective against ROS-dependent neutrophil extracellular trap formation which is a hallmark of acute respiratory distress syndrome (ARDS), and significantly improved pulmonary neutrophil infiltration, oxidative damage, and alveolar damage (Tsai et al., 2023). The activation of Nrf2-ARE (Fig. 3) and PI3K/Akt signaling pathways are the most significant and widely studied mechanisms of carnosic acid antioxidant activity (Mirza et al., 2023).

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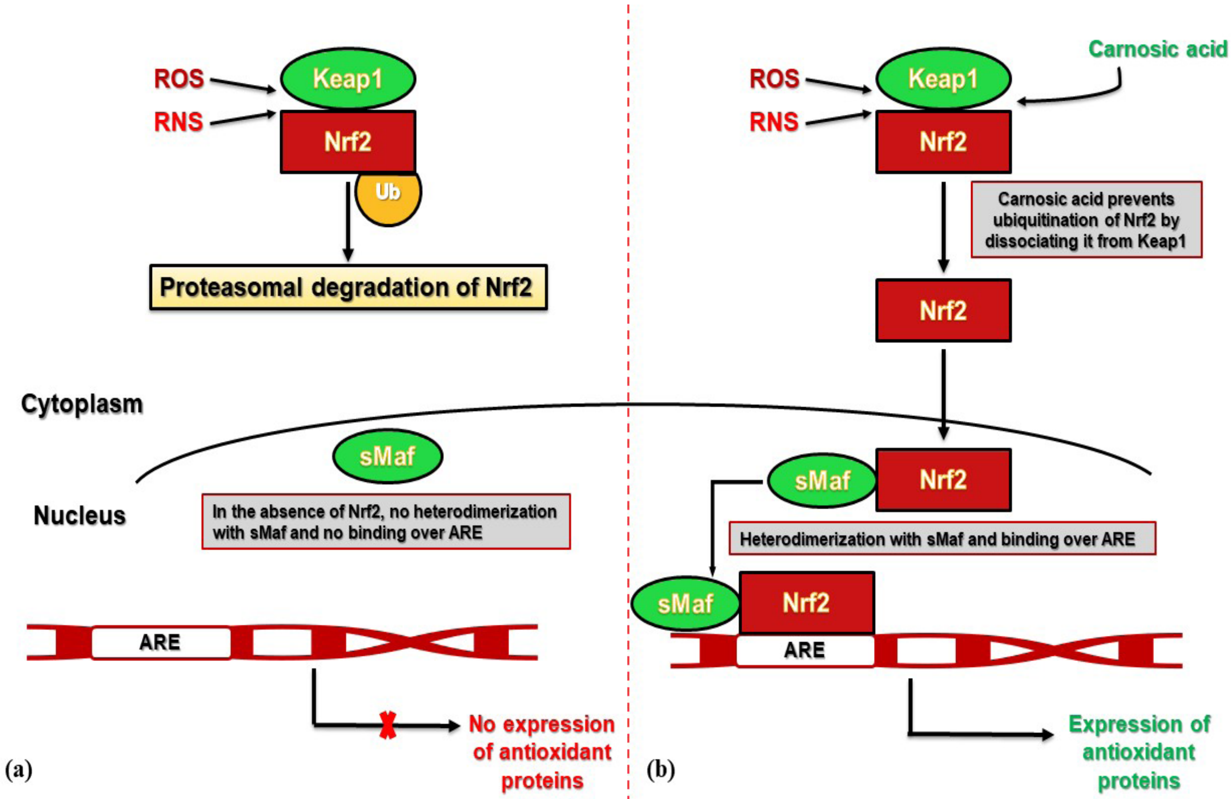

Fig. 3. Carnosic acid-based regulation of Nrf2-ARE pathway: (**a**) Under normal conditions, Nrf2 remains sequestered in the cytoplasm by a regulatory protein Keap1 which targets Nrf2 for ubiquitination but during cellular stress (Injury, toxicity, or cellular stress) Keap1 ends the inhibition of Nrf2 and translocate it into the nucleus, where it binds over the AREs and induces the transcription of antioxidant genes. In the case of neurodegenerative disorders/injury-induced changes in ROS/RNS, nuclear translocation of Nrf2 is impaired and it causes ubiquitination of Nrf2, same as normal conditions, even during oxidative stress and (**b**) Carnosic acid dissociates the Nrf2 from Keap1 and blocks the ubiquitination of Nrf2, followed by their translocation into the nucleus and binding over the AREs to facilitate the transcription of antioxidant genes (ROS: Reactive oxygen species; RNS: Reactive nitrogen species; Keap1: Kelch-like ECH-associating protein 1; Nrf2: Nuclear factor erythroid 2-related factor 2; Ub: Ubiquitination; sMafs: Small musculoaponeurotic fibrosarcoma oncogene family and ARE: Antioxidant response element).

2.2.3. Anti-infectious activity

Carnosic acid, carnosol and rosmarinic acid compounds obtained from *R. officinalis* L. have been reported as an inhibiter to the *agr* quorum sensing pathway of *Staphylococcus aureus* involved in atopic dermatitis (Nakagawa et al., 2020). Carnosic acid was found as a potential prebiotic to alleviate inflammation in human inflammatory bowel disease (IBD) by modulating the composition and metabolic function of the gut microbiota (Du et al., 2023). More recently, it has been shown that carnosic acid and carnosol have the potential to be used against prion diseases and could be developed as therapeutic agents against prion and other neurodegenerative diseases (Karagianni et al., 2022). Argüelles et al. (2021) reported that the combination of propolis with carnosic acid induced a stronger fungicidal and cytotoxic impact against *Cryptococcus neoformans*. In another study dealing with the combination of carnosic acid with gentamicin antibiotic, the final product was found as a potential alternative against the infections caused by methicillin-resistant *S. aureus* (MRSA) (Vázquez et al., 2016).

2.2.4. Anti-inflammatory activity

Several studies have reported the anti-inflammatory and analgesic activities of carnosic acid (Fig. 4), carnosol, ursolic acid and betulinic acid, rosmarinic acid, rosmanol and oleanolic acid (Benincá et al., 2011).

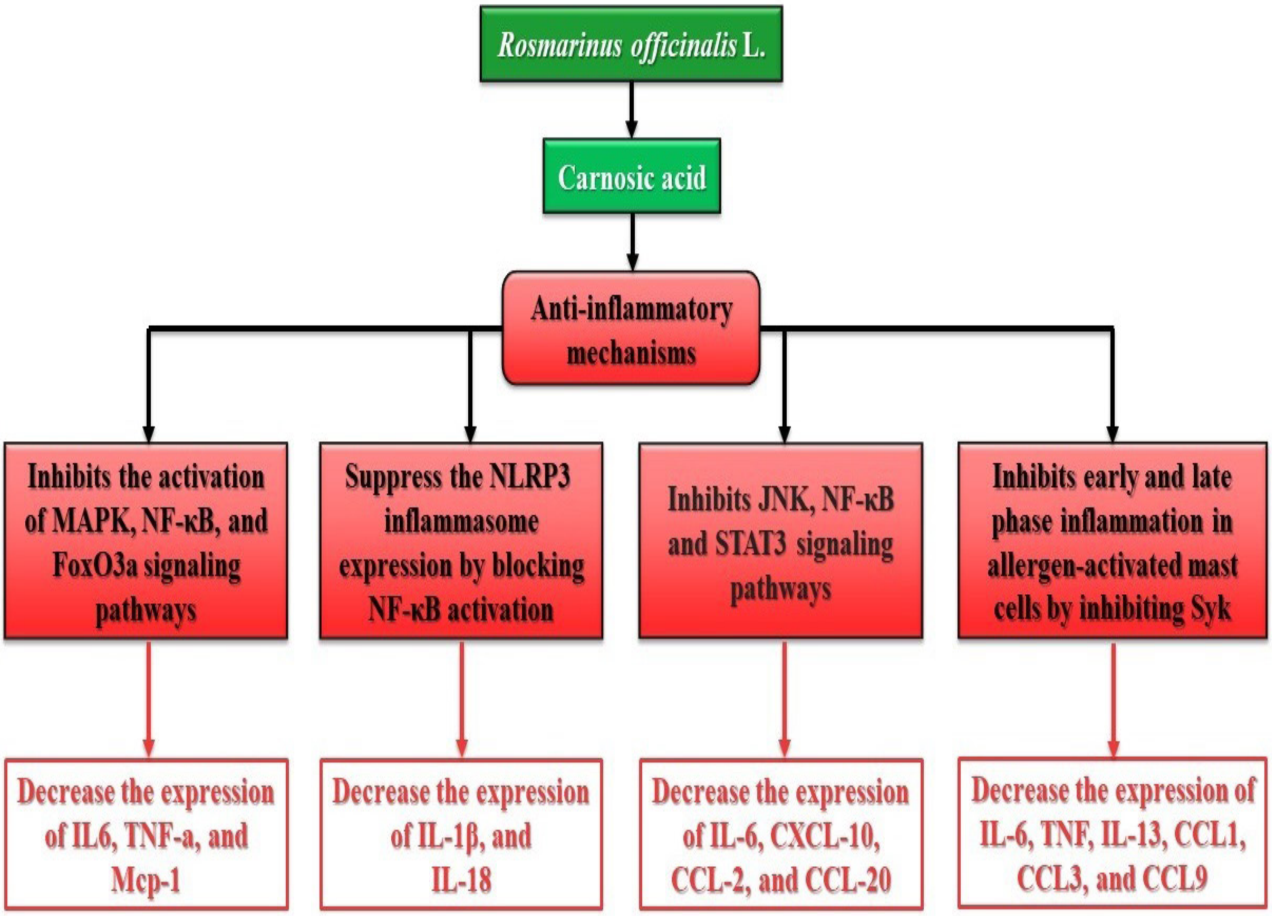

Fig. 4. Anti-inflammatory mechanisms shown by carnosic acid (MAPK: Mitogen-activated protein kinase; NF-κB: Nuclear factor kappa B; FoxO3a: Forkhead box O3a; IL-6: Interleukin-6; TNF-a: Tumour Necrosis factor-alpha; Mcp-1: Monocyte chemoattractant protein-1; NLRP3: Nucleotide-binding domain, leucine-rich containing family, pyrin domain containing-3; IL-1β: Interleukin-1β; IL-18: Interleukin-18; JNK: c-Jun N-terminal kinases; STAT3: Signal transducer and activator of transcription 3; CXCL-10: C-X-C motif chemokine ligand 10; CCL-2: Chemokine ligand 2; CCL-20: Chemokine ligand 20; syk: spleen tyrosine kinase; TNF: Tumour Necrosis Factor; IL-13: Interleukin-13; CCL-1: Chemokine ligand 1; CCL-3: Chemokine ligand 3 and CCL-9: Chemokine ligand 9).

Wang et al. (2018) have shown that carnosic acid inhibits the NO and TNFα level, downregulated COX2 protein expression, and the expression level of inflammatory genes like Nos2, Tnfα, Cox2, and Mcp1 in LPS-stimulated RAW264.7 cell lines and a murine model. In a related study, carnosic acid reduced NLRP3 expression by blocking NF-κB activation, and also inhibited the activation of NLRP3 inflammasome assembly by inhibiting mitochondrial ROS production and interrupting NLRP3-NEK7 interaction (Lin et al., 2023). de Oliveira et al. (2018) studied the effects of carnosic acid in paraquat (PQ)-induced inflammation-related alterations in human neuroblastoma SH-SY5Y cells and found that it exerted an anti-inflammatory action through an Nrf2/HO-1 axis-dependent manner associated with downregulation of NF-κB. The outputs of another report suggested that carnosic acid has anti-inflammatory effects in human periodontal ligament cells by inhibiting the Jun-N-terminal kinase (JNK) pathway, nuclear factor (NF)-κB pathway, signal transducer and also serving as the activator of transcription (STAT)3 pathway activation in IL-1β or TNF-α-stimulated human periodontal ligament cells (Hosokawa et al., 2020). An interesting study on bone marrow-derived mast cells sensitization with anti-tri nitrophenyl IgE, followed by activation with TNP-BSA allergen under stem cell factor potentiation revealed that carnosic acid treatment reduced the release of all cytokines, modulated the mast cell activation, and subsequently inhibited the secretion of allergic inflammatory mediators (Crozier et al., 2023).

2.2.5. Metabolic disorders-related activities

Olanzapine is an atypical antipsychotic medication, that has shown side effects like weight gain and metabolic toxicity in long-term usage. Carnosic acid is reported against olanzapine-induced metabolic toxicity by activating the AMPK, which increases fat consumption and regulates glucose hemostasis in the liver (Razavi et al., 2020). Recently, it has been implied that carnosic acid and carnosol attenuate both cAMP-induced gluconeogenic and lipogenic gene expressions. In addition, carnosic acid and carnosol induced the expressions of PGC1α and CPT1a genes via AMPK activation to promote fatty acid oxidation in HepG2 cells and showed protective effects against diabetes and fatty liver disease (Hasei et al., 2021). A recent finding suggested that carnosic acid exerts protective effects in diabetic nephropathy (DN) conditions, which is one of the most serious complications in diabetes patients causing diabetes-induced kidney complications (Xie et al., 2018). Wang et al. (2019) found that inhibiting the activity of glycosidase from carnosic acid is an effective method for the prevention of diabetes. The binding of carnosic acid to the amino acid residues of glycosidase leads to the change in the molecular conformation of glycosidase and reduces the activity of glycosidase which could reduce postprandial blood glucose in mice. A parallel study indicated that carnosic acid can be used as a potential therapeutic agent in case of high-fat diet-induced non-alcoholic fatty liver disease (NAFLD)-related metabolic diseases, which comprises liver damage, abnormal hepatic fat accumulation, and inflammatory response (Song et al., 2018).

2.3. Methods implicated in the extraction of carnosic acid and carnosol

Various extraction techniques have been implicated to obtain the antioxidant extracts from *R. officinalis* L. but, sometimes the extraction process can be costly. The main objectives of the separation technique are to give high yield, purity, and reproducibility (Azmir et al., 2013; Pizani et al., 2022). Modern features like less environmental impact using green solvents or reducing energy consumption are needed to develop extraction procedures (Chemat et al., 2019). The extraction parameters like temperature, solvent type, and pressure influence the recovery of compounds with antioxidative properties such as carnosic acid, carnosol, and rosmarinic acid (Ali et al., 2019). Since the carnosic acid and carnosol content determine the quality of *R. officinalis* L. extract (Ali et al., 2019), the selection of appropriate solvent with appropriate polarity is crucial regardless of the applied technique. The European Union (EU) recommended either ethanol or the mixture of ethanol and acetone as acceptable solvents for the extraction of carnosic acid and carnosol from *R. officinalis* L. because these compounds are of medium polarity (Younes et al., 2018; Oreopoulou et al., 2021). Solid-liquid extraction, also called maceration, is a conventional method of extraction. The major disadvantages of conventional extraction methods are the decomposition of natural compounds, and long processing time (Rasul, 2018). So, several promising non-conventional and technically advanced green extraction techniques such as microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), pressurized liquid extraction (PLE) /accelerated solvent extraction (ASE), and supercritical fluid extraction (SFE), pulse electric field (PEF) extraction, enzyme-assisted extraction and molecular distillation (MD) have been used to extract phenolic extracts (Selvamuthukumaran and Shi, 2017; Lefebvre et al., 2021).

The most common conventional processes include steam distillation (SD) and hydrodistillation (HD) (Azwanida, 2015). SD was proven to be superior over HD for the isolation of essential oils from *R. officinalis* L. as hydrolysis reactions at high temperatures resulted in reduced volatile fraction and degraded bioactive compounds (Boutekedjiret et al., 2003; Conde-Hernández et al., 2017). Microwave technology integrated with distillation methods gives rise to a method termed microwave-assisted hydrodistillation (MAHD) (Fig. 5). MAHD combines the mechanism of HD and MAE for extracting essential oils from aromatic plants (Hashemi-Moghaddam et al., 2014; Hashemi-Moghaddam et al., 2015; Mohammadhosseini, M., 2017; Hashemi-Moghaddam et al., 2018). Lo Presti et al. (2005) have evaluated the physical action of microwave beams on *R. officinalis* L. and shown a series of advantages for their proposed technique involving cost-effectiveness, need to water in sample pre-treatment, reduced isolation time, and high quality of the obtained essential oil distillate. A similar study on *R. officinalis* L. used MAHD and obtained similar yields at a reduced extraction duration of 15.5 min in comparison to UAE, HD, and SFE, which took 15 min, 30 min, and 1 hour, respectively (Moradi et al., 2018).

A literature survey displays that maceration and Soxhlet extraction are the most common conventional extraction processes to obtain non-volatile compounds (Azwanida, 2015). The use of maceration and Soxhlet extraction has been reduced in the industrial setting due to several disadvantages viz., long extraction time, thermal degradation of thermolabile compounds, high energy and solvent consumption, extract contamination, and loss of solutes (Azwanida, 2015). Several new extraction techniques, MAE, UAE, PLE, and SFE, are currently being investigated to obtain bioactive extracts from *R. officinalis* L. These techniques minimize the use of organic solvents and remarkably prevent the decomposition of natural substances (Lešnik et al., 2021) (Fig. 5). UAE and MAE are among the most popular modern techniques that boosted the extraction processes (Ali et al., 2019). A study on the extraction of *R. officinalis* L. leaves using MAE probably favors the selective extraction of phenolic compounds, and the relevant extract with the higher total phenolic compound showed the best antioxidant activity (Pontillo et al., 2021). A selective recovery of carnosic acid and rosmarinic acid from *R. officinalis* L. using MAE and UAE in ethanol and acetone increased phenol yield up to three times in comparison to more traditional solid-liquid extraction. UAE in *n*-hexane gave up ~13% of the dried extract as the carnosic acid content (Bellumori et al., 2016). Bellumori et al. (2016) subjected the leaves of *R. officinalis* L. To the extraction using MAE and UAE and different solvents and found that hexane was more selective for carnosic acid and other terpenoids. In another study, water to acetone was used with maceration and exhibited that acetone favored carnosic acid extraction from *R. officinalis* L. (Oliveira Gde et al., 2016). Zhu et al. (2023) worked on the green MAE technique using the biodegradable,

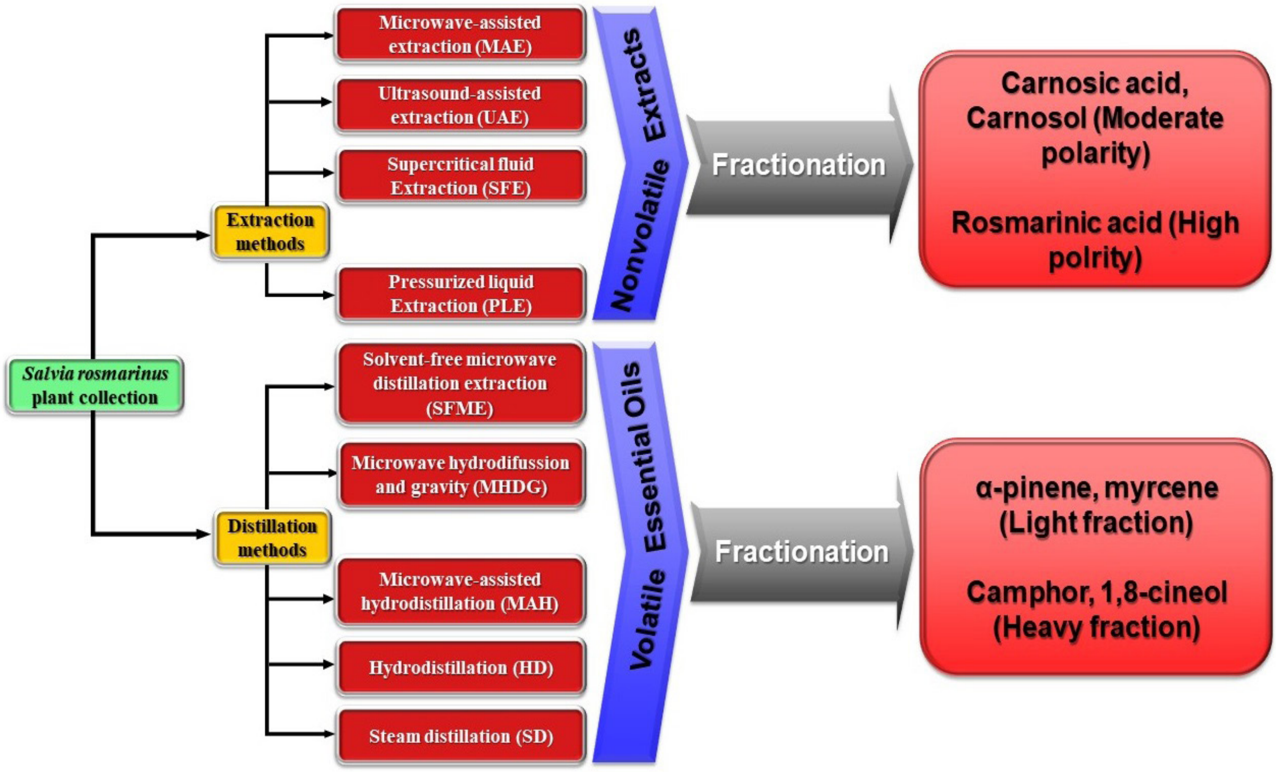

Fig. 5. The overview of the processing to collect bioactive compounds from *R. officinalis* L.

less-toxic, and non-flammable solvent polyethylene glycol (PEGs) for the extraction of carnosic acid and rosmarinic acid from *R. officinalis* L. leaves and concluded that PEG concentration and microwave irradiation time has significantly affected the extraction of carnosic acid. In a very recent work, chemometrics-enhanced high-performance liquid chromatography with photodiode-array detector (HPLC-DAD) method was used for the simultaneous determination of rosmarinic acid, carnosol, and carnosic acid post-UAE of *R. officinalis* L. (Xie et al., 2023). Paloukopoulou and Karioti (2022) also developed an extraction protocol based on UAE and acetone which ensures the chemical stability of the target diterpenoids and is found as a suitable analytical tool for the determination of carnosic acid in a large number of plant tissues of both *R. officinalis* L. and *S. officinalis* L.

PLE is the commonly used method for the extraction of bioactive phenolic compounds from *R. officinalis* L. Herrero et al. (2010) have shown that PLE, at high temperatures (200 °C) using water and ethanol, is capable of efficiently extracting antioxidants of diverse polarities, such as carnosic and rosmarinic acid from *R. officinalis* L. with high yield and antioxidative activity after 20 min. The same study also denoted that PLE using ethanol is more effective for less polar carnosic acid, and carnosol, while PLE with water is more suitable for high-to-medium polar compounds including rosmarinic acid, chlorogenic acid, and caffeic acid. Using PLE, the initial composition of rosmarinic acid, carnosic acid, and carnosol have been determined in rosemary leaves. In accordance with this study, PLE gave rise to better results compared to conventional Soxhlet extraction (CSE) and no significant difference between the two procedures in terms of extraction was found, but PLE proved as a green and environmentally friendly technique (Hirondart et al., 2020). Vázquez et al. (2013) reported that the yields of carnosic acid and carnosol in PLE extracts using hexane were doubled when compared to SFE extracts using pure supercritical CO_2. In the extraction of *R. officinalis* L. essential oil by hydrodistillation, the most abundant compounds were camphor and 1,8-cineole, while in the SFE-based extract carnosic acid was found in nine times higher concentration as compared to the EO (Lešnik et al., 2021). In other endeavors for the development of a two-step sequential SFE process to obtain *R. officinalis* L. extracts mainly consisting of carnosic acid and carnosol, it has also been reported that SFE with CO_2 and ethanol as the modifier is effective for extracting these moderately polar compounds but not for highly polar compounds such as rosmarinic, chlorogenic, and caffeic acid (Herrero et al., 2010; Sánchez-Camargo et al., 2014). Vieira et al. (2022), have successfully utilized deep natural eutectic systems (NADESs) as solvent for extracting bioactive compounds such as rosmarinic acid, carnosol, carnosic acid, and caffeic acid from *R. officinalis* L. Accordingly, the extraction procedure using UAE with heat and stirring (HS) concluded that NADESs stabilize bioactive compounds for long periods; the antioxidant activity of carnosic acid decreased only 25% after 3 months, while the carnosic acid extracted in the methanol was degraded in 15 days.

2.4. Biotechnological enhancement in carnosic acid production

Several studies have reported the biosynthesis pathway of carnosic acid but, studies on the biotechnological/nanotechnological-based strategies for high-level production of carnosic acid are still very few. Wei et al. (2022) established a biosynthetic pathway in *Saccharomyces cerevisiae* to produce carnosic acid efficiently. Since the traditional plant extraction results in low yields of carnosic acid as diterpenoids are often present in low quantities, a specific carnosic acid-producing strain was developed by integrating the cytochrome P450 enzymes and cytochrome P450 reductase encoding genes. The co-expression of CYP76AH1 and SmCPR t28SpCytb5 fusion proteins, along with the overexpression of various catalases to detoxify hydrogen peroxide gives a higher concentration of carnosic acid. Hadi Soltanabad et al. (2020) have assessed the effects of silver nanoparticle (AgNP) treatment on the secondary metabolism and carnosic acid production of *R. officinalis* L. species. According to the findings of this report, AgNPs increased carnosic acid production for 12 days by more than 11%, as compared to the control plants. This report also suggested that a nanotechnological approach like using AgNP acted as an elicitor and enhanced carnosic acid accumulation, effectively. Nanotechnology can develop novel nanosized particles that have antimicrobial abilities *i.e.*, gold, silver, etc. nanoparticles, which can also be used as carriers for targeted drug delivery (Fatima et al., 2021). A study on *Rauwolfia serpentina* L. aqueous leaf extract encapsulated with gold nanoparticles found that nanoformulation enhanced the antibacterial, antioxidant, and anti-cancer activity of *R. serpentina* extracts (Alshahrani et al., 2021), and such kind of nanoformulation of *R. officinalis* L. extracts can be used as a potent source for the synthesis of antioxidant drugs.

3. Concluding remarks and future perspective

The current review provides an overview of carnosic acid and carnosol obtained from *R. officinalis* L. as well as their observed biological effects ranging from antioxidative, anti-inflammatory, anticarcinogenic, antimicrobial effects, etc. Moreover, modern extraction and distillation techniques for the efficient isolation and characterization of carnosic acid from *R. officinalis* L. extracts are discussed. The previous studies also notified that the quantity and composition of secondary metabolites in *R. officinalis* L. are highly affected by several internal and external factors. Pharmacologically active compounds like carnosic acid, carnosol, and rosmarinic acid are found in the non-volatile extracts, whereas the volatile essential oil mainly contains 1,8-cineole, α-pinene, and camphor. The SFE can give a higher yield of moderately polar carnosic acid and carnosol with organic modifiers such as ethanol.

This review article also summarizes the recent findings concerning the successful applications of biologically active compounds such as carnosic acid and carnosol obtained from *R. officinalis* L. in the pharmaceutical industries, as well as the implications of modern methods to achieve the highest yield of these bioactive compounds which can be proposed as natural drugs and further be investigated for preclinical and clinical studies against a large number of persistent diseases and pathological conditions. *R. officinalis* L. can also be widely used for developing herbal drugs for the treatment and prevention of cancers, infectious diseases, depression, Alzheimer's, and Parkinson's diseases. This study, along with other future studies, can give new insights into the future investigation on this valuable medicinal plant.

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Conceptualization and literature search were performed by Dhananjay Singh and Nishu Mittal. The first draft of the manuscript was prepared by Dhananjay Singh. Nishu Mittal and Mohd Haris Siddiqui critically analyzed and gave suggestions to finalize the manuscript. All authors read and approved the final manuscript.

Conflict of interest

All the authors declare that there is no conflict of interest.

References

Afshar, M., Najafian, S., Radi, M., 2022a. The effect of harvest time on the natural product of *Rosmarinus officinalis* L. from South Iran (Fars Province). Nat. Prod. Res. 36(10), 2637-2642.

Afshar, M., Najafian, S., Radi, M., 2022b. Seasonal variation on the major bioactive compounds: Total phenolic and flavonoids contents, and antioxidant activity of rosemary from Shiraz. Nat. Prod. Res. 36(16), 4287-4292.

Ali, A., Chua, B.L., Chow, Y.H., 2019. An insight into the extraction and fractionation technologies of the essential oils and bioactive compounds in *Rosmarinus officinalis* L.: Past, present and future. Trends Anal. Chem. 118, 338-351.

AlKahtane, A.A., Ghanem, E., Bungau, S.G., Alarifi, S., Ali, D., AlBasher, G., Alkahtani, S., Aleya, L., Abdel-Daim, M.M., 2020. Carnosic acid alleviates chlorpyrifos-induced oxidative stress and inflammation in mice cerebral and ocular tissues. Environ. Sci. Pollut. Res. 27(11), 11663-11670.

Alshahrani, M.Y., Rafi, Z., Alabdallah, N.M., Shoaib, A., Ahmad, I., Asiri, M., Zaman, G.S., Wahab, S., Saeed, M., Khan, S., 2021. A comparative antibacterial, antioxidant, and antineoplastic potential of *Rauwolfia serpentina* (L.) leaf extract with its biologically synthesized gold nanoparticles (R-AuNPs). Plants 10(11), 2278.

Anacarso, I., Sabia, C., de Niederhäusern, S., Iseppi, R., Condò, C., Bondi, M., Messi, P., 2019. *In vitro* evaluation of the amoebicidal activity of rosemary (*Rosmarinus officinalis* L.) and cloves (*Syzygium aromaticum* L. Merr. & Perry) essential oils against *Acanthamoeba polyphaga* trophozoites. Nat. Prod. Res. 33(4), 606-611.

Andrade, J.M., Faustino, C., Garcia, C., Ladeiras, D., Reis, C.P., Rijo, P., 2018. *Rosmarinus officinalis* L.: An update review of its phytochemistry and biological activity. Future Sci. OA 4(4), Fso283.

Argüelles, A., Sánchez-Fresneda, R., Martínez-Mármol, E., Lozano, J.A., Solano, F., Argüelles, J.C., 2021. A specific mixture of propolis and carnosic acid triggers a strong fungicidal action against *Cryptococcus neoformans*. Antibiotics (Basel) 10(11), doi: org/10.3390/antibiotics10111395.

Arranz, E., Mes, J., Wichers, H.J., Jaime, L., Mendiola, J.A., Reglero, G., Santoyo, S., 2015. Anti-inflammatory activity of the basolateral fraction of Caco-2 cells exposed to a rosemary supercritical extract. J. Funct. Foods 13, 384-390.

Aziz, E., Batool, R., Akhtar, W., Shahzad, T., Malik, A., Shah, M.A., Iqbal, S., Rauf, A., Zengin, G., Bouyahya, A., Rebezov, M., Dutta, N., Khan, M.U., Khayrullin, M., Babaeva, M., Goncharov, A., Shariati, M.A., Thiruvengadam, M., 2022. Rosemary species: A review of phytochemicals, bioactivities and industrial applications. S. Afr. J. Bot. 151, 3-18.

Azmir, J., Zaidul, I.S.M., Rahman, M.M., Sharif, K.M., Mohamed, A., Sahena, F., Jahurul, M.H.A., Ghafoor, K., Norulaini, N.A.N., Omar, A.K.M., 2013. Techniques for extraction of bioactive compounds from plant materials: A review. J. Food Eng. 117(4), 426-436.

Azwanida, N.N., 2015. A review on the extraction methods use in medicinal plants, principle, strength and limitation. Med. Aromat. Plants 4, 1-6.

Bai, N., He, K., Roller, M., Lai, C.S., Shao, X., Pan, M.H., Ho, C.T., 2010. Flavonoids and phenolic compounds from *Rosmarinus officinalis*. J. Agric. Food Chem. 58(9), 5363-5367.

Bakirel, T., Bakirel, U., Keleş, O.U., Ulgen, S.G., Yardibi, H., 2008. *In vivo* assessment of antidiabetic and antioxidant activities of rosemary (*Rosmarinus officinalis*) in alloxan-diabetic rabbits. J. Ethnopharmacol. 116(1), 64-73.

Barni, M.V., Carlini, M.J., Cafferata, E.G., Puricelli, L., Moreno, S., 2012. Carnosic acid inhibits the proliferation and migration capacity of human colorectal cancer cells. Oncol. Rep. 27(4), 1041-1048.

Bekut, M., Brkić, S., Kladar, N., Dragović, G., Gavarić, N., Božin, B., 2018. Potential of selected Lamiaceae plants in anti (retro) viral therapy. Pharmacol. Res. 133, 301-314.

Bellumori, M., Innocenti, M., Binello, A., Boffa, L., Mulinacci, N., Cravotto, G., 2016. Selective recovery of rosmarinic and carnosic acids from rosemary leaves under ultrasound-and microwave-assisted extraction procedures. C. R. Chim. 19(6), 699-706.

Benincá, J.P., Dalmarco, J.B., Pizzolatti, M.G., Fröde, T.S., 2011. Analysis of the anti-inflammatory properties of *Rosmarinus officinalis* L. in mice. Food Chem. 124(2), 468-475.

Birtić, S., Dussort, P., Pierre, F.-X., Bily, A.C., Roller, M., 2015. Carnosic acid. Phytochemistry 115, 9-19.

Boudiar, T., Lozano-Sánchez, J., Harfi, B., del Mar Contreras, M., Segura-Carretero, A., 2019. Phytochemical characterization of bioactive compounds composition of *Rosmarinus eriocalyx* by RP-HPLC-ESI-QTOF-MS. Nat. Prod. Res. 33(15), 2208-2214.

Boutekedjiret, C., Bentahar, F., Belabbes, R., Bessiere, J.M., 2003. Extraction of rosemary essential oil by steam distillation and hydrodistillation. Flavour Fragrance J. 18(6), 481-484.

Božić, D., Papaefthimiou, D., Brückner, K., De Vos, R.C., Tsoleridis, C.A., Katsarou, D., Papanikolaou, A., Pateraki, I., Chatzopoulou, F.M., Dimitriadou, E., 2015. Towards elucidating carnosic acid biosynthesis in Lamiaceae: Functional characterization of the three first steps of the pathway in *Salvia fruticosa* and *Rosmarinus officinalis*. PLoS One 10(5), e0124106.

Bozin, B., Mimica-Dukic, N., Samojlik, I., Jovin, E., 2007. Antimicrobial and antioxidant properties of rosemary and sage (*Rosmarinus officinalis* L. and *Salvia officinalis* L., Lamiaceae) essential oils. J. Agric. Food Chem. 55(19), 7879-7885.

Brückner, K., Božić, D., Manzano, D., Papaefthimiou, D., Pateraki, I., Scheler, U., Ferrer, A., de Vos, R.C., Kanellis, A.K., Tissier, A., 2014. Characterization of two genes for the biosynthesis of abietane-type diterpenes in rosemary (*Rosmarinus officinalis*) glandular trichomes. Phytochemistry 101, 52-64.

Çakılcıoğlu, U., Türkoğlu, İ., 2007. Plants used for hemorrhoid treatment in Elazığ central district. Acta Hortic. 826, 89-96.

Calvo, M.I., Akerreta, S., Cavero, R.Y., 2011. Pharmaceutical ethnobotany in the riverside of Navarra (Iberian Peninsula). J. Ethnopharmacol. 135(1), 22-33.

Chan, E.W.C., Wong, S.K., Chan, H.T., 2022. An overview of the chemistry and anticancer properties of rosemary extract and its diterpenes. J. HerbMed Pharmacol. 11(1), 10-19.

Chemat, F., Abert-Vian, M., Fabiano-Tixier, A.S., Strube, J., Uhlenbrock, L., Gunjevic, V., Cravotto, G., 2019. Green extraction of natural products. Origins, current status, and future challenges. Trends Anal. Chem. 118, 248-263.

Cheung, S., Tai, J., 2007. Anti-proliferative and antioxidant properties of rosemary *Rosmarinus officinalis*. Oncol. Rep. 17(6), 1525-1531.

Choi, S.H., Jang, G.W., Choi, S.I., Jung, T.D., Cho, B.Y., Sim, W.S., Han, X., Lee, J.S., Kim, D.Y., Kim, D.B., Lee, O.H., 2019. Development and validation of an analytical method for carnosol, carnosic acid and rosmarinic acid in food matrices and evaluation of the antioxidant activity of rosemary extract as a food additive. Antioxidants (Basel) 8(3).

Conde-Hernández, L.A., Espinosa-Victoria, J.R., Trejo, A.G., Guerrero-Beltrán, J.Á., 2017. CO_2-supercritical extraction, hydrodistillation and steam distillation of essential oil of rosemary (*Rosmarinus officinalis*). J. Food

Eng. 200, 81-86.
Crozier, R.W.E., Yousef, M., Coish, J.M., Fajardo, V.A., Tsiani, E., MacNeil, A.J., 2023. Carnosic acid inhibits secretion of allergic inflammatory mediators in IgE-activated mast cells via direct regulation of Syk activation. J. Biol. Chem. 299(4), 102867.
Cushnie, T.P., Lamb, A.J., 2005. Antimicrobial activity of flavonoids. Int. J. Antimicrob. Agents 26(5), 343-356.
de Oliveira, M.R., 2016. The dietary components carnosic acid and carnosol as neuroprotective agents: A mechanistic view. Mol. Neurobiol. 53(9), 6155-6168.
de Oliveira, M.R., de Souza, I.C.C., Fürstenau, C.R., 2018. Carnosic acid induces anti-inflammatory effects in paraquat-treated sh-sy5y cells through a mechanism involving a crosstalk between the Nrf2/HO-1 axis and NF-κB. Mol. Neurobiol. 55(1), 890-897.
de Oliveira, M.R., Peres, A., Ferreira, G.C., Schuck, P.F., Bosco, S.M.D., 2016. Carnosic acid affords mitochondrial protection in chlorpyrifos-treated Sh-Sy5y cells. Neurotoxic. Res. 30, 367-379.
Del Campo, J., Amiot, M.J., Nguyen-The, C., 2000. Antimicrobial effect of rosemary extracts. J. Food Prot. 63(10), 1359-1368.
Donmez, D.B., Kacar, S., Bagci, R., Sahinturk, V., 2020. Protective effect of carnosic acid on acrylamide-induced liver toxicity in rats: Mechanistic approach over Nrf2-Keap1 pathway. J. Biochem. Mol. Toxicol. 34(9), e22524.
Dörrie, J., Sapala, K., Zunino, S.J., 2001. Carnosol-induced apoptosis and downregulation of Bcl-2 in B-lineage leukemia cells. Cancer Lett. 170 1, 33-39.
Dorta, E., Lobo, M.G., González, M., 2013. Optimization of factors affecting extraction of antioxidants from mango seed. Food Bioprocess Technol. 6(4), 1067-1081.
Du, C., Li, Z., Zhang, J., Yin, N., Tang, L., Li, J., Sun, J., Yu, X., Chen, W., Xiao, H., Wu, X., Chen, X., 2023. The protective effect of carnosic acid on dextran sulfate sodium-induced colitis based on metabolomics and gut microbiota analysis. Food Sci. Hum. Wellness 12(4), 1212-1223.
Fatima, F., Siddiqui, S., Khan, W.A., 2021. Nanoparticles as novel emerging therapeutic antibacterial agents in the antibiotics resistant era. Biol. Trace Elem. Res. 199(7), 2552-2564.
Ghasemzadeh Rahbardar, M., Hemadeh, B., Razavi, B.M., Eisvand, F., Hosseinzadeh, H., 2022. Effect of carnosic acid on acrylamide induced neurotoxicity: *In vivo* and *in vitro* experiments. Drug Chem. Toxicol. 45(4), 1528-1535.
González-Vallinas, M., Molina, S., Vicente, G., de la Cueva, A., Vargas, T., Santoyo, S., García-Risco, M.R., Fornari, T., Reglero, G., Ramírez de Molina, A., 2013. Antitumor effect of 5-fluorouracil is enhanced by rosemary extract in both drug sensitive and resistant colon cancer cells. Pharmacol. Res. 72, 61-68.
González-Vallinas, M., Reglero, G., Ramírez de Molina, A., 2015. Rosemary (*Rosmarinus officinalis* L.) extract as a potential complementary agent in anticancer therapy. Nutr. Cancer 67(8), 1221-1229.
Guellouma, F.Z., Boussoussa, H., Khachba, I., Yousfi, M., Ziane Khoudja, I., Bourahla, I., 2023. *Rosmarinus officinalis* essential oils' eradication of beta-lactamase and multidrug resistant clinical bacterial pathogens from hospital settings. Nat. Prod. Res. 1-11, doi: org/10.1080/14786419.2023.2201884.
Hadi Soltanabad, M., Bagherieh-Najjar, M.B., Mianabadi, M., 2020. Carnosic acid content increased by silver nanoparticle treatment in rosemary (*Rosmarinus officinalis* L.). Appl. Biochem. Biotechnol. 191(2), 482-495.
Halmschlag, C.B., Carneiro de Melo Moura, C., Brambach, F., Siregar, I.Z., Gailing, O., 2022. Molecular and morphological survey of Lamiaceae species in converted landscapes in Sumatra. PLoS One 17(12), e0277749.
Harley, R.M., 2012. Checklist and key of genera and species of the Lamiaceae of the Brazilian Amazon. Rodriguésia 63, 129-144.
Hasei, S., Yamamotoya, T., Nakatsu, Y., Ohata, Y., Itoga, S., Nonaka, Y., Matsunaga, Y., Sakoda, H., Fujishiro, M., Kushiyama, A., Asano, T., 2021. Carnosic acid and carnosol activate ampk, suppress expressions of gluconeogenic and lipogenic genes, and inhibit proliferation of HepG2 cells. Int. J. Mol. Sci. 22(8), 4040.
Hashemi-Moghaddam, H., Mohammadhosseini, M., Azizi, Z., 2018. Impact of amine- and phenyl-functionalized magnetic nanoparticles impacts on microwave-assisted extraction of essential oils from root of *Berberis integerrima* Bunge. J. Appl. Res. Med. Aromat. Plants 10, 1-8.
Hashemi-Moghaddam, H., Mohammadhosseini, M., Basiri, M., 2015. Optimization of microwave assisted hydrodistillation on chemical compositions of the essential oils from the aerial parts of *Thymus pubescens* and comparison with conventional hydrodistllation. J. Essent. Oil-Bear. Plants 18(4), 884-893.
Hashemi-Moghaddam, H., Mohammadhosseini, M., Salar, M., 2014. Chemical composition of the essential oils from the hulls of *Pistacia vera* L. by using magnetic nanoparticle-assisted microwave (MW) distillation: Comparison with routine MW and conventional hydrodistillation. Anal. Methods 6, 2572-2579.
Hernández, M.D., Sotomayor, J.A., Hernández, Á., Jordán, M.J., 2016. Chapter 77-Rosemary (*Rosmarinus officinalis* L.) Oils, in: Preedy, V.R. (Ed.), Essential oils in Food Preservation, Flavor and Safety. Academic Press, San Diego, pp. 677-688.
Herrero, M., Plaza, M., Cifuentes, A., Ibáñez, E., 2010. Green processes for the extraction of bioactives from Rosemary: Chemical and functional characterization via ultra-performance liquid chromatography-tandem mass spectrometry and *in-vitro* assays. J. Chromatogr. A 1217(16), 2512-2520.
Hill, R.A., Connolly, J.D., 2013. Triterpenoids. Nat. Prod. Rep. 30(7), 1028-1065.
Hirondart, M., Rombaut, N., Fabiano-Tixier, A.S., Bily, A., Chemat, F., 2020. Comparison between pressurized liquid extraction and conventional soxhlet extraction for rosemary antioxidants, yield, composition, and environmental footprint. Foods 9(5), 584.
Hosokawa, I., Hosokawa, Y., Ozaki, K., Matsuo, T., 2020. Carnosic acid inhibits inflammatory cytokines production in human periodontal ligament cells. Immunopharmacol. Immunotoxicol. 42(4), 373-378.
Hossain, M.B., Barry-Ryan, C., Martin-Diana, A.B., Brunton, N.P., 2011. Optimisation of accelerated solvent extraction of antioxidant compounds from rosemary

(*Rosmarinus officinalis* L.), marjoram (*Origanum majorana* L.) and oregano (*Origanum vulgare* L.) using response surface methodology. Food Chem. 126(1), 339-346.

Hou, C.-W., Lin, Y.-T., Chen, Y.-L., Wang, Y.-H., Chou, J.-L., Ping, L.-Y., Jeng, K.-C., 2012. Neuroprotective effects of carnosic acid on neuronal cells under ischemic and hypoxic stress. Nutr. Neurosci. 15(6), 257-263.

Huang, M.T., Ho, C.T., Wang, Z.Y., Ferraro, T., Lou, Y.R., Stauber, K., Ma, W., Georgiadis, C., Laskin, J.D., Conney, A.H., 1994. Inhibition of skin tumorigenesis by rosemary and its constituents carnosol and ursolic acid. Cancer Res. 54(3), 701-708.

Iseppi, R., Sabia, C., de Niederhäusern, S., Pellati, F., Benvenuti, S., Tardugno, R., Bondi, M., Messi, P., 2019. Antibacterial activity of *Rosmarinus officinalis* L. and *Thymus vulgaris* L. essential oils and their combination against food-borne pathogens and spoilage bacteria in ready-to-eat vegetables. Nat. Prod. Res. 33(24), 3568-3572.

Jesus, E.G.d., Souza, F.F.d., Andrade, J.V., Andrade e Silva, M.L., Cunha, W.R., Ramos, R.C., Campos, O.S., Santos, J.A.N., Santos, M.F.C., 2023. *In silico* and *in vitro* elastase inhibition assessment assays of rosmarinic acid natural product from *Rosmarinus officinalis* Linn. Nat. Prod. Res. 1-6, doi: org/10.1080/14786419.2023.2196077.

Kar, S., Palit, S., Ball, W.B., Das, P.K., 2012. Carnosic acid modulates Akt/IKK/NF-κB signaling by PP2A and induces intrinsic and extrinsic pathway mediated apoptosis in human prostate carcinoma PC-3 cells. Apoptosis 17(7), 735-747.

Karagianni, K., Pettas, S., Kanata, E., Lioulia, E., Thune, K., Schmitz, M., Tsamesidis, I., Lymperaki, E., Xanthopoulos, K., Sklaviadis, T., Dafou, D., 2022. Carnosic acid and carnosol display antioxidant and anti-prion properties in *in vitro* and cell-free models of prion diseases. Antioxidants 11(4), 726.

Lefebvre, T., Destandau, E., Lesellier, E., 2021. Selective extraction of bioactive compounds from plants using recent extraction techniques: A review. J. Chromatogr. A. 1635, 461770.

Lešnik, S., Furlan, V., Bren, U., 2021. Rosemary (*Rosmarinus officinalis* L.): extraction techniques, analytical methods and health-promoting biological effects. Phytochem. Rev. 1-56.

Lin, G., Li, N., Li, D., Chen, L., Deng, H., Wang, S., Tang, J., Ouyang, W., 2023. Carnosic acid inhibits NLRP3 inflammasome activation by targeting both priming and assembly steps. Int. Immunopharmacol. 116, 109819.

Lo Presti, M., Ragusa, S., Trozzi, A., Dugo, P., Visinoni, F., Fazio, A., Dugo, G., Mondello, L., 2005. A comparison between different techniques for the isolation of rosemary essential oil. J. Sep. Sci. 28(3), 273-280.

Lo, A.H., Liang, Y.C., Lin-Shiau, S.Y., Ho, C.T., Lin, J.K., 2002. Carnosol, an antioxidant in rosemary, suppresses inducible nitric oxide synthase through down-regulating nuclear factor-kappaB in mouse macrophages. Carcinogenesis 23(6), 983-991.

Lopes, R.P., Parreira, L.A., Venancio, A.N., Santos, M.F.C., Menini, L., 2023. Chemical characterization and evaluation of acaricidal potential of rosemary essential oil and its main compound α-pinene on the two-spotted spider mite, *Tetranychus urticae*. Nat. Prod. Res. 37(17), 2940-2944.

Loussouarn, M., Krieger-Liszkay, A., Svilar, L., Bily, A., Birtić, S., Havaux, M., 2017. Carnosic acid and carnosol, two major antioxidants of rosemary, act through different mechanisms. Plant Physiol. 175(3), 1381-1394

Medini, H., Manongiu, B., Aicha, N., Chekir-Ghedira, L., Harzalla-Skhiri, F., Khouja, M.L., 2013. Chemical and antibacterial polymorphism of *Juniperus oxycedrus* ssp. *oxycedrus* and *Juniperus oxycedrus* ssp. *macrocarpa* (Cupressaceae) leaf essential oils from Tunisia. J. Chem. 2013, 389252.

Mirza, F.J., Zahid, S., Holsinger, R.M.D., 2023. Neuroprotective effects of carnosic acid: Insight into its mechanisms of action. Molecules 28(5), 2306.

Mohammadhosseini, M., 2017. Essential oils extracted using microwave-assisted hydrodistillation from aerial parts of eleven *Artemisia* species: Chemical compositions and diversities in different geographical regions of Iran. Rec. Nat. Prod. 11(2), 114-129.

Moradi, S., Fazlali, A., Hamedi, H., 2018. Microwave-assisted hydro-distillation of essential oil from rosemary: Comparison with traditional distillation. Avicenna J. Med. Biotechnol. 10(1), 22-28.

Moreno, S., Scheyer, T., Romano, C.S., Vojnov, A.A., 2006. Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. Free Radic. Res. 40(2), 223-231.

Nakagawa, S., Hillebrand, G.G., Nunez, G., 2020. *Rosmarinus officinalis* L. (rosemary) extracts containing carnosic acid and carnosol are potent quorum sensing inhibitors of *Staphylococcus aureus* virulence. Antibiotics 9(4), 149.

Oliveira Gde, A., de Oliveira, A.E., da Conceição, E.C., Leles, M.I., 2016. Multiresponse optimization of an extraction procedure of carnosol and rosmarinic and carnosic acids from rosemary. Food Chem. 211, 465-473.

Oreopoulou, A., Choulitoudi, E., Tsimogiannis, D., Oreopoulou, V., 2021. Six common herbs with distinctive bioactive, antioxidant components. A review of their separation techniques. Molecules 26(10), 2920.

Oualdi, I., Diass, K., Azizi, S.-e., Dalli, M., Touzani, R., Gseyra, N., Yousfi, E.B., 2023. *Rosmarinus officinalis* essential oils from Morocco: New advances on extraction, GC/MS analysis, and antioxidant activity. Nat. Prod. Res. 37(12), 2003-2008.

Paloukopoulou, C., Karioti, A., 2022. A validated method for the determination of carnosic acid and carnosol in the fresh foliage of *Salvia rosmarinus* and *Salvia officinalis* from Greece. Plants 11(22), 3106.

Paniwnyk, L., Cai, H., Albu, S., Mason, T.J., Cole, R., 2009. The enhancement and scale up of the extraction of anti-oxidants from *Rosmarinus officinalis* using ultrasound. Ultrason. Sonochem. 16(2), 287-292.

Pérez-Fons, L., Garzón, M.T., Micol, V., 2010. Relationship between the antioxidant capacity and effect of rosemary (*Rosmarinus officinalis* L.) polyphenols on membrane phospholipid order. J. Agric. Food Chem. 58(1), 161-171.

Pieracci, Y., Ciccarelli, D., Giovanelli, S., Pistelli, L., Flamini, G., Cervelli, C., Mancianti, F., Nardoni, S., Bertelloni, F., Ebani, V.V., 2021. Antimicrobial activity and composition of five *Rosmarinus* (Now *Salvia spp.*

and varieties) essential oils. Antibiotics (Basel) 10(9), doi: org/10.3390%2Fantibiotics10091090.

Pizani, R.S., Viganó, J., de Souza Mesquita, L.M., Contieri, L.S., Sanches, V.L., Chaves, J.O., Souza, M.C., da Silva, L.C., Rostagno, M.A., 2022. Beyond aroma: A review on advanced extraction processes from rosemary (*Rosmarinus officinalis*) and sage (*Salvia officinalis*) to produce phenolic acids and diterpenes. Trends Food Sci. Technol. 127, 245-262.

Poeckel, D., Greiner, C., Verhoff, M., Rau, O., Tausch, L., Hörnig, C., Steinhilber, D., Schubert-Zsilavecz, M., Werz, O., 2008. Carnosic acid and carnosol potently inhibit human 5-lipoxygenase and suppress pro-inflammatory responses of stimulated human polymorphonuclear leukocytes. Biochem. Pharmacol. 76(1), 91-97.

Pontillo, A.R.N., Papakosta-Tsigkri, L., Lymperopoulou, T., Mamma, D., Kekos, D., Detsi, A., 2021. Conventional and enzyme-assisted extraction of rosemary leaves (*Rosmarinus officinalis* L.): Toward a greener approach to high added-value extracts. Appl. Sci. 11(8), 3724.

Ramos da Silva, L.R., Ferreira, O.O., Cruz, J.N., de Jesus Pereira Franco, C., Oliveira Dos Anjos, T., Cascaes, M.M., Almeida da Costa, W., Helena de Aguiar Andrade, E., Santana de Oliveira, M., 2021. Lamiaceae essential oils, phytochemical profile, antioxidant, and biological activities. Evid. Based Complement. Alternat. Med. 2021, 6748052.

Rasul, M.G., 2018. Conventional extraction methods use in medicinal plants, their advantages and disadvantages. Int. J. Basic Sci. Appl. Comput. 2, 10-14.

Razavi, B.M., Abazari, A.R., Rameshrad, M., Hosseinzadeh, H., 2020. Carnosic acid prevented olanzapine-induced metabolic disorders through AMPK activation. Mol. Biol. Rep. 47(10), 7583-7592.

Richheimer, S.L., Bernart, M.W., King, G.A., Kent, M.C., Beiley, D.T., 1996. Antioxidant activity of lipid-soluble phenolic diterpenes from rosemary. J. Am. Oil Chem. Soc. 73, 507-514.

Sánchez-Camargo, A.d.P., Valdés, A., Sullini, G., García-Cañas, V., Cifuentes, A., Ibáñez, E., Herrero, M., 2014. Two-step sequential supercritical fluid extracts from rosemary with enhanced anti-proliferative activity. J. Funct. Foods 11, 293-303.

Santomauro, F., Sacco, C., Donato, R., Bellumori, M., Innocenti, M., Mulinacci, N., 2018. The antimicrobial effects of three phenolic extracts from *Rosmarinus officinalis* L., *Vitis vinifera* L. and *Polygonum cuspidatum* L. on food pathogens. Nat. Prod. Res. 32(22), 2639-2645.

Selvamuthukumaran, M., Shi, J., 2017. Recent advances in extraction of antioxidants from plant by-products processing industries. Food Qual. Saf. 1, 61-81.

Singletary, K., MacDonald, C., Wallig, M., 1996. Inhibition by rosemary and carnosol of 7,12-dimethylbenz[a] anthracene (DMBA)-induced rat mammary tumorigenesis and *in vivo* DMBA-DNA adduct formation. Cancer Lett. 104(1), 43-48.

Song, H.-M., Li, X., Liu, Y.-Y., Lu, W.-P., Cui, Z.-H., Zhou, L., Yao, D., Zhang, H.-M., 2018. Carnosic acid protects mice from high-fat diet-induced NAFLD by regulating MARCKS. Int. J. Mol. Med. 42(1), 193-207.

Sui, X., Liu, T., Ma, C., Yang, L., Zu, Y., Zhang, L., Wang, H., 2012. Microwave irradiation to pretreat rosemary (*Rosmarinus officinalis* L.) for maintaining antioxidant content during storage and to extract essential oil simultaneously. Food Chem. 131(4), 1399-1405.

Tai, J., Cheung, S., Wu, M., Hasman, D., 2012. Antiproliferation effect of rosemary (*Rosmarinus officinalis*) on human ovarian cancer cells *in vitro*. Phytomedicine 19(5), 436-443.

Takaki, I., Bersani-Amado, L.E., Vendruscolo, A., Sartoretto, S.M., Diniz, S.P., Bersani-Amado, C.A., Cuman, R.K., 2008. Anti-inflammatory and antinociceptive effects of *Rosmarinus officinalis* L. essential oil in experimental animal models. J. Med. Food 11(4), 741-746.

Tarasevičienė, Ž., Vitkauskaitė, M., Paulauskienė, A., Černiauskienė, J., 2023. Wild stinging nettle (*Urtica dioica* L.) leaves and roots chemical composition and phenols extraction. Plants 12(2), 309.

Theoduloz, C., Pertino, M.W., Rodríguez, J.A., Schmeda-Hirschmann, G., 2011. Gastroprotective effect and cytotoxicity of carnosic acid derivatives. Planta Med. 77(9), 882-887.

Tsai, C.W., Lin, C.Y., Wang, Y.J., 2011. Carnosic acid induces the NAD(P)H: quinone oxidoreductase 1 expression in rat clone 9 cells through the p38/nuclear factor erythroid-2 related factor 2 pathway. J. Nutr. 141(12), 2119-2125.

Tsai, Y.-F., Yang, S.-C., Hsu, Y.-H., Chen, C.-Y., Chen, P.-J., Syu, Y.-T., Lin, C.-H., Hwang, T.-L., 2023. Carnosic acid inhibits reactive oxygen species-dependent neutrophil extracellular trap formation and ameliorates acute respiratory distress syndrome. Life Sci. 321, 121334.

Tudu, C.K., Dutta, T., Ghorai, M., Biswas, P., Samanta, D., Oleksak, P., Jha, N.K., Kumar, M., Radha, Prćków, J., Pérez de la Lastra, J.M., Dey, A., 2022. Traditional uses, phytochemistry, pharmacology and toxicology of garlic (*Allium sativum*), a storehouse of diverse phytochemicals: A review of research from the last decade focusing on health and nutritional implications. Front. Nutr. 9, doi: org/10.3389/fnut.2022.929554.

Vázquez, E., García-Risco, M.R., Jaime, L., Reglero, G., Fornari, T., 2013. Simultaneous extraction of rosemary and spinach leaves and its effect on the antioxidant activity of products. J. Supercrit. Fluids 82, 138-145.

Vázquez, N.M., Fiorilli, G., Cáceres Guido, P.A., Moreno, S., 2016. Carnosic acid acts synergistically with gentamicin in killing methicillin-resistant *Staphylococcus aureus* clinical isolates. Phytomedicine 23(12), 1337-1343.

Vieira, C., Rebocho, S., Craveiro, R., Paiva, A., Duarte, A.R.C., 2022. Selective extraction and stabilization of bioactive compounds from rosemary leaves using a biphasic NADES. Front. Chem. 10, doi: org/10.3389/fchem.2022.954835.

Visentín, A., Cismondi, M., Maestri, D., 2011. Supercritical CO_2 fractionation of rosemary ethanolic oleoresins as a method to improve carnosic acid recovery. Innov. Food Sci. Emerg. Technol. 12(2), 142-145.

Wang, H., Wang, J., Liu, Y., Ji, Y., Guo, Y., Zhao, J., 2019. Interaction mechanism of carnosic acid against glycosidase (α-amylase and α-glucosidase). Int. J. Biol. Macromol. 138, 846-853.

Wang, L.-C., Wei, W.-H., Zhang, X.-W., Liu, D., Zeng, K.-W., Tu, P.-F., 2018. An integrated proteomics and bioinformatics approach reveals the anti-inflammatory mechanism of carnosic acid. Front. Pharmacol. 9, doi: org/10.3389/fphar.2018.00370.

Wei, P., Zhang, C., Bian, X., Lu, W., 2022. Metabolic engineering of *Saccharomyces cerevisiae* for heterologous carnosic acid production. Front. Bioeng. Biotechnol. 10, doi: org/10.3389/fbioe.2022.916605.

WFO-2023: *Rosmarinus* L. Published on the Internet;http://www.worldfloraonline.org/taxon/wfo-4000033499. Accessed on: 29 Jul 2023.

Wu, C.-R., Tsai, C.-W., Chang, S.-W., Lin, C.-Y., Huang, L.-C., 2015. Carnosic acid protects against 6-hydroxydopamine-induced neurotoxicity in *in vivo* and *in vitro* model of Parkinson's disease: Involvement of antioxidative enzymes induction. Chem.-Biol. Interact. 225, 40-46.

Xie, L., Li, Z., Li, H., Sun, J., Liu, X., Tang, J., Lin, X., Xu, L., Zhu, Y., Liu, Z., Wang, T., 2023. Fast quantitative determination of principal phenolic anti-oxidants in rosemary using ultrasound-assisted extraction and chemometrics-enhanced HPLC-DAD method. Food Anal. Methods 16(2), 386-400.

Xie, Z., Zhong, L., Wu, Y., Wan, X., Yang, H., Xu, X., Li, P., 2018. Carnosic acid improves diabetic nephropathy by activating Nrf2/ARE and inhibition of NF-κB pathway. Phytomedicine 47, 161-173.

Yesil-Celiktas, O., Sevimli, C., Bedir, E., Vardar-Sukan, F., 2010. Inhibitory effects of rosemary extracts, carnosic acid and rosmarinic acid on the growth of various human cancer cell lines. Plant Foods Hum. Nutr. 65(2), 158-163.

Younes, M., Aggett, P., Aguilar, F., Crebelli, R., Dusemund, B., Filipič, M., Frutos, M.J., Galtier, P., Gott, D., Gundert-Remy, U., Kuhnle, G.G., Lambré, C., Lillegaard, I.T., Moldeus, P., Mortensen, A., Oskarsson, A., Stankovic, I., Waalkens-Berendsen, I., Woutersen, R.A., Wright, M., Boon, P., Lindtner, O., Tlustos, C., Tard, A., Leblanc, J.C., 2018. Refined exposure assessment of extracts of rosemary (E 392) from its use as food additive. EFSA J. 16(8), e05373.

Yu, M.H., Choi, J.H., Chae, I.G., Im, H.G., Yang, S.A., More, K., Lee, I.S., Lee, J., 2013. Suppression of LPS-induced inflammatory activities by *Rosmarinus officinalis* L. Food Chem. 136(2), 1047-1054.

Zappalà, A., Vicario, N., Calabrese, G., Turnaturi, R., Pasquinucci, L., Montenegro, L., Spadaro, A., Parenti, R., Parenti, C., 2021. Neuroprotective effects of *Rosmarinus officinalis* L. extract in oxygen glucose deprivation (OGD)-injured human neural-like cells. Nat. Prod. Res. 35(4), 669-675.

Zhang, Y., Adelakun, T.A., Qu, L., Li, X., Li, J., Han, L., Wang, T., 2014. New terpenoid glycosides obtained from *Rosmarinus officinalis* L. aerial parts. Fitoterapia 99, 78-85.

Zhu, C., Fan, Y., Bai, X., 2023. A green and effective polyethylene glycols-based microwave-assisted extraction of carnosic and rosmarinic acids from *Rosmarinus officinalis* leaves. Foods 12(9), 1761.