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REVIEW ARTICLE

A Review on the Pharmacological Activity of *Monarda fistulosa* L.

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A B S T R A C T

Monarda fistulosa L. is a plant with numerous benefits often used in traditional medication. This review paper aims to elaborate on the phytochemicals of *M. fistulosa* that contribute to its pharmacological activity. Numerous studies have found that the bioactive compounds of *M. fistulosa,* including carvacrol, thymol, thymoquinone, flavonoid, α-pinene, caryophyllene oxide, limonene, and geraniol, possess several pharmacological activities, including antimicrobial, antidiabetic, anticancer, anti-inflammatory, antioxidants, and immunomodulatory properties. Although preceding evidence shows that *M. fistulosa* potentially be applied in many areas, further clinical studies still have to be conducted to assess the effects of this plant.

K E Y W O R D S

Monarda fistulosa; pharmacological activity; phytochemicals; review

H I G H L I G H T S

- Carvacrol and thymol act as antimicrobial agents against numerous gram positive and negative bacteria.
- Thymoquinone has antidiabetic properties that decreases ROS and increases COX-2 inhibition.
- Carvacrol, thymol, and thymoquinone are also antioxidant, anti-inflammatory, anticancer, and immunomodulatory.
- Other anti-inflammatory compounds include camphene, borneol, p-cymene, and γ-terpinene.
- Geraniol can modulate the immune systems by inhibiting the Kv1.3 ion channel

INTRODUCTION

The practice of utilizing and consuming medicinal plants emerged thousands of years ago in rural communities across the globe and still plays a significant role in shaping modern healthcare (Fitzgerald et al., 2020). In the more recent centuries, an increase in scientific research, development, innovation, and analysis can be observed amongst herbal substances (Thomford et al., 2018). This paved the way for synthetic drug discovery, recommencing interest and higher rates of patient acceptance and administration of herbal substances as complementary and alternative medicine (CAM) (Fitzgerald et al., 2020; Sánchez et al., 2020). Herbal medicines contributed as derivatives for most of the entire synthetic drugs in existence. They acted as the first-line therapeutic approach in developing countries alone, with a percentage of 80% for both cases (Bauer & Brönstrup, 2014). Meanwhile, a majority of the global population (87.5%) relied on herbal medicine to treat their health conditions (Sánchez et al., 2020). Increased speed, capacity, and reliability of research

and analysis toward the safety, efficacy, and quality of herbal medicines can be highly associated with the advancement of analytical instrumentations and techniques in the modern day (Fitzgerald et al., 2020).

The Monarda fistulosa (*M. fistulosa*) plant is commonly known as bee balm or wild bergamot. It is a perennial herb that belongs to the mint (*Lamiaceae*) family and blooms during the summer. The plant is native to North America and is found in the relatively dry soils of prairies, thickets, and glades, typically in calcareous soils ("*Monarda fistulosa*", 2019). The plant is distributed in Southeastern Canada and the Midwestern United States, and several varieties of the species are known. The plant requires a partial shade to full sun exposure and a dry to medium level of moisture with good air circulation ("*Monarda fistulosa*", 2019). The species *M. fistulosa* has been used for a long time as traditional medicine, which includes the treatment of various digestive diseases, possessing several therapeutic properties (e.g., antimicrobial, anthelmintic, antioxidant, and anti-inflammatory) (Shanaida et al., 2021). The plant's leaves can be consumed raw and cooked ("*Monarda fistulosa* - L", n.d.). The aerial part of *M. fistulosa*, consisting of stems, leaves, and flowers, is used to extract essential oils (EOs), which possess a wide range of functions in addition to traditional medicine, such as a flavoring agent (Selvamuthukumaran & Pathak, 2018).

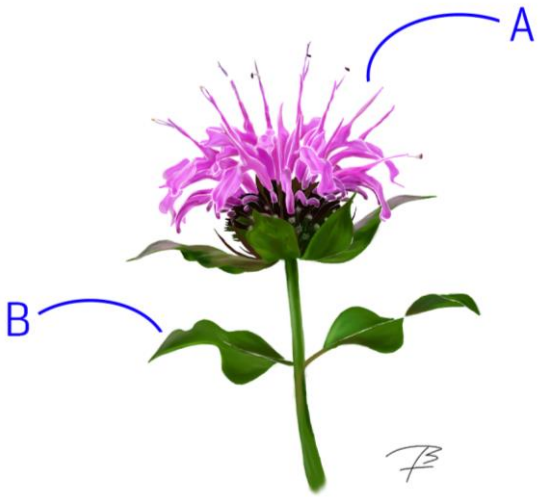

Figure 1. *M. fistulosa* illustration

The overall appearance of *M. fistulosa* can be seen in the illustration in Fig 1. The corolla of the plant has a color that ranges from lavender to soft pink. The plant grows in upright stem clumps with a height ranging from 2' - 4' tall and a width ranging from 2' - 3' ft wide ("*Monarda fistulosa*", n.d.). The plant possesses somewhat hairy square stems that are branched. Moreover, it is spread by rhizomes and seeds. Leaf blades of *M. fistulosa* have a deep green color with an ovate to lanceolate shape. Leaves of *M. fistulosa* have two leaves per node arranged opposite from each other. The flowers are terminal and solitary, with two stamens surpassing the upper lip. The shape of the flower is tubular and has a lobe shorter than the tubes (Anderson, n.d.). The flowers are in a clustered head-like cyme arranged like a ring (Dingwell, n.d.).

M. fistulosa EOs have different percentages of compounds depending on the part of the plant that is used for extraction. According to Ghosh et al. (2020), *M. fistulosa* EOs contains monoterpenes and oxygenated monoterpenes; the EOs sourced from the flower (A) contain 43.1% and 54.8%, whereas EOs sourced from the leaf (B) contain 21.1% and 77.7% of each compound, respectively (Figure 1). The components of *M. fistulosa* have shown a number of pharmacological activities with therapeutic potential in several studies. However, despite the promising therapeutic benefits of *M. fistulosa,* research studies of this plant are still limited on a clinical level. Hence, this review interprets the pharmacological activities of *M. fistulosa* bioactive compounds from both *in vivo* and *in vitro* studies.

METHODS

The literature search for the review on the pharmacological effects of *M. fistulosa* was conducted using the following databases: Google Scholar, PubMed, and SpringerLink. The keywords used during the search are a combination of search terms including: "(*Monarda fistulosa* OR Wild bergamot)" AND "pharmacological property)". Literature discussing the pharmacological properties of specific compounds of *M. fistulosa* was collected with a combination of search terms including: "(*Monarda fistulosa* chemical constituent)" AND "(pharmacological property)".

Collected works of literature were then selected based on original research articles and patents regarding the pharmacological properties of *Monarda fistulosa* extract published in 2014-2021. In addition, the selected literature was all written in the English language. Data collected include the pharmacological properties of *M. fistulosa* EOs along with each chemical component of *M. fistulosa*. Information about the mechanism underlying the properties of each compound was also extracted.

PHYTOCHEMICAL/PHARMACOLOGICAL EFFECTS OF *MONARDA FISTULOSA* L.

M. fistulosa contains a few bioactive compounds responsible for various pharmacological activities, including antimicrobial, antidiabetic, antioxidant, anti-inflammatory, anticancer, and immunomodulatory (Table 1). Several *in vivo* and *in vitro* studies have been performed to assess the properties of these compounds.

Table 1. The pharmacological activity of *M. fistulosa,* their bioactive compounds, and *in vitro* and *in vivo* studies.

Pharmacological Activity	Bioactive Compound	Type of Study	Concentration/Dosage Used	Dosage Used (Human dose)	Reference
Antimicrobial	Carvacrol, thymol	*In vitro: Exorista lavarum* eggs	Thymol: 0.02148 µg/mL	As it is	Dindo et al. (2020)
Antidiabetic	Thymoquinone	*In vivo:* Wistar rats	50 mg/kg for 12 weeks through intraperitoneal (IP) injection	8.06 mg/kg for 12 weeks through intraperitoneal (IP) injection	Atta et al. (2018)
			35 mg/kg for 5 weeks through oral administration	5.645 mg/kg for 5 weeks through oral administration	El-Shemi et al. (2017)
Antioxidant	Carvacrol, flavonoid, thymol, thymoquinone	*In vitro:* DPPH free radical scavenging assay	Thymol: 1000 µg/mL Carvacrol: 1000 µg/mL	As it is	Yildiz et al. (2020)
Anti-inflammatory	α-pinene, carvacrol, caryophyllene oxide, limonene, thymol, thymoquinone, and other monoterpenes in *M. fistulosa*	*In vivo:* Mice	Thymoquinone: 5 mg/kg and 25 mg/kg through oral administration	Thymoquinone: 0.4 mg/kg and 2.03 mg/kg through oral administration	Hossen et al. (2017)
			p-cymene: 25 mg/kg, 50 mg/kg, and 100 mg/kg through IP or subcutaneous (s.c.) injection	p-cymene: 2.03 mg/kg, 4.065 mg/kg, and 8.13 mg/kg through IP or subcutaneous (s.c.) injection	Santana et al. (2015)
			Carvacrol: 20 mg/kg, 40 mg/kg, and 80 mg/kg via intragastric gavage	Carvacrol: 1.626 mg/kg, 3.25 mg/kg, and 6.504 mg/kg via intragastric gavage	Kara et al. (2015)

			B-caryophyllene: 200 µg/mice		Brito et al. (2019)
Anticancer	Carvacrol, thymol, thymoquinone	*In vitro:* HCT-116 cells (human colorectal carcinoma cell lines)	Thymol: 3.76-22.533 µg/mL	As it is	Li et al. (2017)
Immunomodulatory	Carvacrol, geraniol, thymol, thymoquinone	*In vitro:* Human neutrophils	Thymol: 56 µg/mL Red thyme: 47 µg/mL	As it is	Péréz-Rosés et al. (2015)

Antimicrobial properties

Numerous European countries have been cultivating *M. fistulosa* as an aromatic plant. Studies have shown that *M. fistulosa* is rich in biologically active substances, making it a great source of EOs (Dindo et al., 2020). Furthermore, studies have proven that the EOs and hydrolates from *M. fistulosa* exhibit high activity against pathogenic microorganisms and can be used in humans and plants (Dindo et al., 2020). Some of the major constituents found in *M. fistulosa*, such as carvacrol, γ-terpinene, geraniol, rosmarinic acid, and thymol, as well as the presence of thymol and carvacrol in the EOs of *M. fistulosa*, enable the extract to pose antibacterial and antihelmintic properties (Mattarelli et al., 2017). According to Dindo et al. (2020), thymol is the bioactive compound that exhibited one of the strongest antimicrobial activities when incorporated with 0.5% (w/w) of *M. fistulosa* hydrolate containing 0.143 mM (0.0022%) of thymol into an *artificial medium placed with E. larvarum eggs* for *in vitro analysis.*

Thymol is one of the phenolic compounds classified as monoterpene, a carvacrol isomer known for its antimicrobial properties (Dhifi et al., 2016; Memar et al., 2017). On top of that, thymol is also a p-cymene derivative compound reported to have both anti-inflammatory and antimicrobial activities (Memar et al., 2017).

Another major component in the EOs of *M. fistulosa* is carvacrol, a monoterpene with antimicrobial properties as its biological properties (Memar et al., 2017). According to Memar et al. (2017), carvacrol has both hydrophobic and hydrophilic properties related to its aromatic ring of carvacrol and its phenolic group, respectively. Additionally, numerous studies have reported the properties associated with carvacrol, such as antibacterial, antifungal, and antiprotozoal properties, similar to thymol (Memar et al., 2017).

In regard to their antibacterial properties, Dhifi et al. (2015) has found that both thymol and carvacrol are able to pose their effects against a wide variety of bacteria, including Gram-negative bacteria such as *Escherichia coli* and *Salmonella enterica*, as well as Gram-positive bacteria such as *Lactobacillus sakei* and *Staphylococcus aureus.*

In order to exert those effects, some researchers reported that thymol acts as an antibacterial agent against bacteria such as *Staphylococcus aureus* and *Escherichia coli* by targeting bacterial cell walls. The disturbance of the lipids in the bacterial plasma membrane due to EOs changes its membrane permeability and subsequently leads to the leakage of cell content and cell death (Memar et al., 2017). Furthermore, the disintegration of the entire cell membrane further impacts the homeostasis of pH and inorganic ions in the bacteria, culminating in bacterial lysis as the end outcome (Memar et al., 2017).

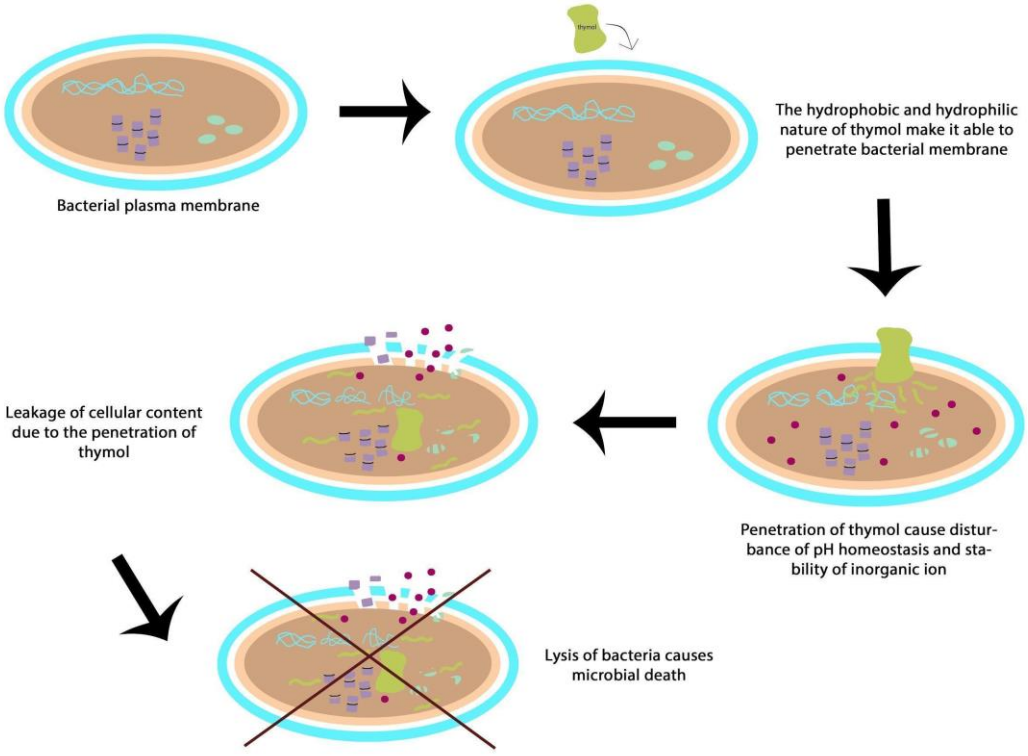

Figure 2. Mechanism of thymol as an antibacterial agent

Consequently, the antibacterial properties induced by carvacrol and thymol are dependent on their ability to penetrate, depolarize, and interfere with the cytoplasmic membrane of a bacteria. Several studies on the antifungal activities of thymol and carvacrol stated their efficacy in inhibiting *Penicillium digitatum* and *Penicillium italicum*. However, it was concluded that thymol has higher antifungal efficacy than carvacrol (Memar et al., 2017). Thymol alone or with carvacrol can alter the fluidity of the cell membrane and its permeability (Memar et al., 2017). This compound can also alter the cell membrane permeability in fungi, such as *Candida albicans*, by affecting the synthesis of β-glucan, which composes the cell wall polysaccharides and inhibits the growth of cells, leading to cell death (Memar et al., 2017).

Several experimental studies have been done on quantifying the antimicrobial activity of *M. fistulosa*. Mattarelli et al. (2017) used a microdilution procedure to test the antimicrobial activity with both pathogenic and non-pathogenic strains of fungi and bacteria as microorganisms. After the incubation process, it was shown that the MIC (minimum inhibitory concentration) and MLC (minimum lethal concentration) were at the lowest concentration, meaning that there was no growth with the death of around 99.9% of the microorganisms. Their result shows that the MIC of EOs varies between 0.25% to 1%, Eos exhibiting a more sensitive effect towards human pathogenic microorganism than non-pathogenic microorganisms.

One source states that *M. fistulosa* EOs containing trans-ocimene, geraniol, and linalool display a high antibacterial activity against *Pseudomonas putida*. It was believed that the lipid composition on the surface of bacterial membranes determines the antibacterial effect of the EOs. The active compounds might be able to penetrate the cells causing a binding site inside the cell that is important for the antibacterial activity to interact with active compounds of the plant (Shutava & Shutava, 2018).

Antidiabetic properties

Constituent(s) found in *M. fistulosa* also demonstrate a promising therapeutic potential against Diabetes mellitus (DM). Pharmacological treatments involving the administration of conventional antidiabetic drugs is the most common route for alleviating the severity of the disease (Zsombok & Derbenev,

2016). Nonetheless, various therapeutic effects possessed by herbal medicine can be a viable alternative if sufficient research and innovation are performed.

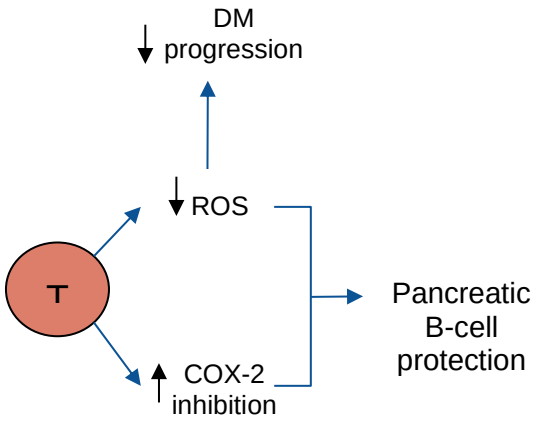

Figure 3. Thymoquinone antidiabetic mechanism

Thymoquinone (TQ), which is a significant bioactive compound of *M. fistulosa* that decreases Reactive Oxygen Species (ROS) and increases Cyclooxygenase-2 (COX-2) inhibition (Chen et al., 2018); hence, possessing antidiabetic properties that enable protection of the pancreatic β-cells from injury or exhaustion (Fouad & Alwadani, 2015). The antidiabetic potential of TQ is linked with the antioxidant and anti-inflammatory activity possessed by *M. fistulosa*. Increased levels of ROS is apparent in the progression of DM, which is generally characterized by the formation of oxidative stress, auto-oxidation of glucose, modification of antioxidant enzymes, lipid peroxides production, non-enzymatic protein glycosylation, as well as impaired glutathione metabolism (Farkhondeh et al., 2017). Furthermore, the overproduction of ROS as a result of increased levels of free radicals initiates several inflammatory signaling pathways (Hussain et al., 2016). Inflammation by pro-inflammatory genes may deteriorate the local cells, leading to type 2 diabetes and β-cell apoptosis (Ježek et al., 2019).

A study conducted by Atta et al. (2018) showcases the protective potential of TQ against oxidative damage from streptozotocin (STZ) induced diabetic cardiomyopathy, a complication that disrupts the structure and function of the myocardium. STZ is a nitrosourea alkylating agent and possesses selective toxicity to the glucose transporter 2 (GLUT2) of pancreatic β-cells due to its similarity in structure to glucose (Papich, 2016). Experimental research utilizing STZ is only limited to *in vivo* testing using rodents to exhibit resistance in human β-cells due to impaired uptake. This is done to quickly induce diabetic effects as desired (Al-awar et al., 2016). Nevertheless, STZ-induced diabetic male Wistar rats experienced improvements in cardiac function after the administration of TQ through intraperitoneal injection with a dose of 50 mg/kg for 12 weeks (Atta et al., 2018). This is achieved through the downregulation of nitric oxide synthase (NOS) expression (Abd-Elbaset et al., 2016) and upregulation of vascular endothelial growth factors, erythropoietin growth factors, and associated transcription factors such as nuclear factor erythroid 2–related factor 2 (Nrf2) (Shi et al., 2018), as well as high-density lipoprotein (HDL) levels (Prabhakar et al., 2015). In addition, the antioxidant and anti-inflammatory activity of cardiac tissue is also greatly improved through the upregulation of superoxide dismutase (SOD), the inhibition of inflammatory proteins such as interleukin-6 (IL-6) (Atta et al., 2018), and the normalization of troponin I and creatine kinase (CK) which function as plasma cardiac markers (Nawale et al., 2017).

The exact mechanism of TQ action in relieving blood glucose concentration levels in STZ-induced diabetic rats has yet to be discovered. However, this can be hypothesized by the protective and regenerative effects of TQ on pancreatic β-cells (El-Shemi et al., 2017). Other findings by El-Shemi et al. (2017) include a

sharp decrease in serum glucose concentration, an increase in the serum insulin concentration after shorter fasting periods, as well as a decrease in glycated hemoglobin levels (HbA1c) after daily oral administration of 35 mg/kg TQ daily for five weeks in STZ-induced diabetic rats. TQ administration and its implications were also tested on pregnant female rats since offspring of gestational diabetic mothers are seven times more likely to experience health problems later in life than offspring of non-diabetic mothers (Badr et al., 2013). Administration of TQ in pregnant and lactating diabetic mice mothers improved T cell responses in the offspring and reduced diabetic complications (de Cássia da Silveira e Sá et al., 2013). This was followed by a significant increase in the mean body weight, IL-2 levels, as well as T cell proliferation of neonates (Badr et al., 2013).

Although *Nigella sativa* oil was the species highlighted in most of the TQ-related findings obtained, due to insufficient research data on *M. fistulosa*, these results are still relevant to the scope of this review. Depending on the extraction method, TQ makes up one of the significant bioactive components found on the aerial parts of *M. fistulosa* EOs, accounting for approximately 20-32% (Igor et al., 2020). Despite being generally recognized as "safe" when administered to experimental animals (Goyal et al., 2017), the clinical evaluation of TQ remains limited, with no known clinical assessment for its antidiabetic effects in human patients until now. Taking into account the differences between human β-cells and other mammalian β-cells will be an important consideration for researchers to advance in future clinical trials regarding the therapeutic effects of TQ found in *M. fistulosa* in human diabetic patients.

Antioxidant properties

Another property that *M. fistulosa* possesses is an antioxidant activity that prevents the conversion of ROS into free radicals and induces its conversion to non-radical species. In addition, antioxidants were also found to be able to break the auto-oxidative chain reaction caused by ROS. Hence, a lower level of localized oxygen can be obtained (Oroian & Escriche, 2015).

The essentiality of antioxidants has been increased due to the danger of free radicals that can be obtained from some exogenous sources, such as ozone radiation, pesticides, and pollutants, as well as from endogenous sources such as normal metabolic processes (Phaniendra et al., 2015). According to Lourenço et al. (2019), an increase in the concentration of ROS in the body may cause damage to several molecules such as proteins, lipids, RNA, and DNA, as well as increase the risk of developing diseases including cancer, arthritis, atherosclerosis, and diabetes.

Based on their sources, antioxidants can be divided into two groups which are natural and synthetic antioxidants. The natural ones are usually present in plants such as herbs, spices, fruits, and vegetables. At the same time, synthetic antioxidants were firstly made to replace natural antioxidants due to their higher stability, low cost, and wide availability (Lourenço et al., 2019). However, some studies found that synthetic antioxidants have some safety issues, such as causing skin allergies, gastrointestinal tract issues, and increasing cancer development risk if consumed long term. In addition, synthetic antioxidants may cause DNA damage and induce premature senescence (Kornienko et al., 2019). Since then, people have started to go back to natural antioxidants, and the studies regarding this have also increased (Lourenço et al., 2019). One of the potential natural sources of antioxidants is the EOs made from the aerial parts of *M. fistulosa*.

Based on the study by Shanaida et al. (2018), some active compounds in the EOs of *M. fistulosa* have a role in its antioxidant activity, such as TQ, flavonoids, thymol, and carvacrol. According to Diaz-Vivancos et al. (2015), when TQ is incubated *in vitro* with methylglyoxal or glucose, it could inhibit the glycation of superoxide dismutase (SOD), as well as maintain its antioxidant activity. In order to express these effects, TQ works by scavenging the carbon-centered and hydroxyl radicals and conducting a non-enzymatic reaction involving glutathione which reduces the mechanisms of ROS-facilitated stress (Armutcu et al., 2018). However, the electron reduction of TQ with the help of enzymes such as microsomal NADPH cytochrome

P450 reductase and NAD(P)H quinone acceptor oxidoreductases can result in the synthesis of thymohydroquinone which expresses a strong antioxidant effect (Diaz-Vivancos et al., 2015).

Aside from the reactions discussed above, TQ can also undergo a non-enzymatic reduction which will result in glutathionylated-dihydrothymoquinone (GS-TQ) with the help of glutathione (GSH) (Darakhshan et al., 2015). After being compared to the original form, reduced TQ, GS-TQ, and dihydro TQ was found to have a greater ability to scavenge the radicals, which means they have greater antioxidant activity. This might happen due to the TQ non-enzymatic intracellular activation that depends on the presence of NADPH, NADH, and GSH to coordinate the antioxidant contents inside the cells. Therefore, when those metabolites are produced, they can imitate the works of SOD and GSH as endogenous antioxidants to remove the free radicals from the body so that peroxidation of lipids can be prevented and help with the recovery of some oxidative stresses by reacting with both hemoglobin and myoglobin (Armutcu et al., 2018).

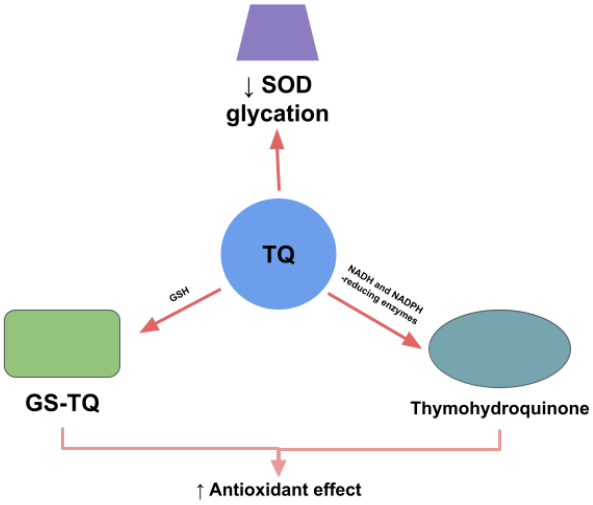

Figure 4. Thymoquinone antioxidant mechanism

The second compound is flavonoids which work as antioxidants by doing several activities such as radical scavenging, inhibition of xanthine oxidase (XO), and lipid peroxidation. Flavonoids can directly scavenge the free radicals by stabilizing the ROS due to the reaction between the hydroxyl (-OH) group of the flavonoids with the radicals, which causes the inactivation of the radical. Additionally, the presence of the -OH group in the structure of flavonoids also leads to the second mechanism of this compound as an antioxidant by inhibiting XOs that are originally formed from xanthine dehydrogenases during ischaemic conditions and known to be reactive towards oxygen molecules which can release superoxide free radicals (de Araújo et al., 2017).

Another mechanism is effectively preventing lipid peroxidation, which is responsible for diseases like diabetes, hepatotoxicity, and atherosclerosis, to name a few (Panche et al., 2016). Based on Kurkin et al. (2020), the herb of *M. fistulosa* contains several types of flavonoids such as monardoside (Apigenin 5-O-Rutinoside), isorhoifolin (Apigenin 7-O-Rutinoside), linarin (Acacetin 7-O-Rutinoside), and didymin (Isosakuranetin 7-O-Rutinoside), in which monardoside and isorhoifolin are classified as apigenin while linarin is classified as methoxyflavone (Semwal et al., 2019). However, all three of the listed compounds above are classified as flavanones which do not really show a function as antioxidants (Panche et al., 2016). The last compound is didymin which is classified as isosakuranetin. According to Barreca et al. (2017), isosakuranetin is one of the flavanone glycosides that can scavenge free radicals, thus making them function as antioxidants (Panche et al., 2016).

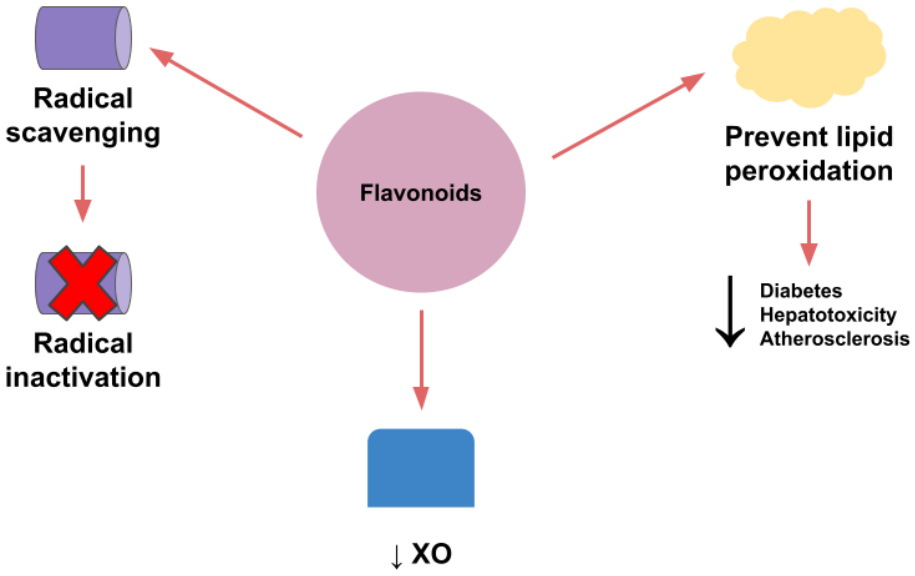

Figure 5. Flavonoids antioxidant mechanism

Another active compound of *M. fistulosa* with an antioxidant property is thymol and its isomer, carvacrol (Shanaida et al., 2018). Based on Markovska et al. (2020), phenol consisting of thymol and carvacrol comprises about 67-86% of the active compounds of Monarda Eos. The thymol contained in the Eos made from the *M. fistulosa* plants harvested from the Novokakhovske Research farm of NAAS, located in Ireland, ranges between 70.44% and 78.28%, which makes it a predominant component of those Eos. Meanwhile, carvacrol was found to be approximately 3.58% to 3.84% of Eos. Additionally, methyl carvacrol is also found to constitute about 4.80% to 4.89% of the Eos (Markovska et al., 2020). However, the composition of active compounds depends on the genotype and the cultivation place (Grzeszczuk et al., 2020).

In order to obtain information regarding the antioxidant activities of thymol and carvacrol, several tests can be conducted. The first one is the DPPH test which will monitor the effectiveness of the compounds against free radicals (Grzeszczuk et al., 2020). According to the study by Yildiz et al. (2020), thymol shows 25.0% of DPPH antiradical activity and carvacrol shows 18.3% activity at 1000 ppm concentration. These results show that thymol and carvacrol can scavenge the DPPH radicals by donating hydrogen, which will quench the singlet and triplet oxygen, decompose peroxides, and neutralize radicals. The second test is the linoleic acid test. In this test, thymol and carvacrol show the highest antioxidant activities compared to some commercial antioxidants such as BHT, BHA, and α-tocopherol. The last test, which is the ferric reducing power test, shows that commercial antioxidants have stronger reducing power than natural ones. However, thymol has stronger antioxidant properties than carvacrol (Yildiz et al., 2020). Other than their direct function as antioxidants, thymol and carvacrol also take part in obtaining TQ and thymohydroquinone. The higher the amount of thymol and carvacrol, the higher the TQ and thymohydroquinone can be obtained (Markovska et al., 2020).

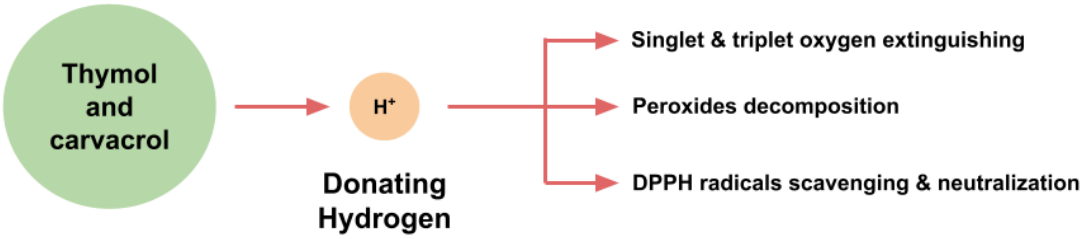

Figure 6. Thymol and carvacrol antioxidant mechanism

Anti-inflammatory properties

M. fistulosa has been known to have anti-inflammatory properties due to its high TQ content and has long been cultivated for TQ extraction (Ghosh et al., 2020; Kurkin, 2020; Rohlfsen, 2017). The extract of *M. fistulosa* is also used as a replacement for NSAIDs in treating neuropathic pain, with the amount preferably being more than 0.5% by weight of the total composition (McLellan, 2017).

According to a study conducted by Hossen et al. (2017), TQ was demonstrated to reduce gastritis by up to 98% in a mouse model when given orally at a dose of 5 and 25 mg/kg. This reduction rate was higher when compared to the result shown by the positive control ranitidine at a dose of 40 mg/kg, which is a standard drug with anti-ulcer activity. This reaction might be possible due to the ability of TQ to prevent the recruitment of neutrophils that can cause inflammation when being used in the pre-treatment process. In the same study, it was also found that TQ was able to inhibit luciferase activity, p65, and c-Fos translocation in LPS-treated RAW264.7 cells, inhibit the phosphorylation of IκBα, IKKα/β, AKT, and PDK1, and suppress interleukin-1 receptor-associated kinase 1 (IRAK1)-linked activator protein (AP)-1/nuclear factor kappa B (NF-κB) pathways (Hossen et al., 2017).

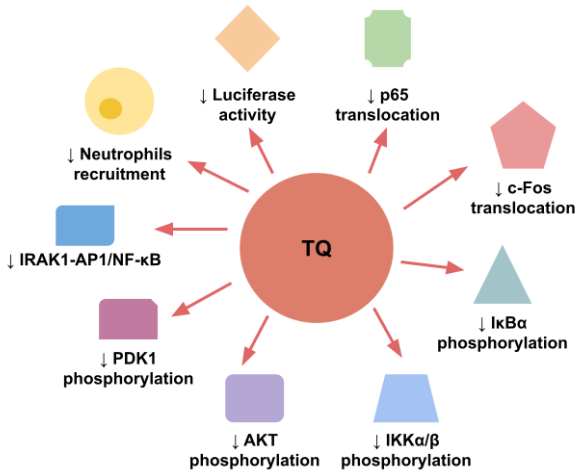

Figure 7. Thymoquinone anti-inflammatory mechanism and effects

Another study by Zuzarte et al. (2018) found that the synergy of camphene and borneol showed effective anti-inflammatory activity by inhibiting the production of nitric oxide (NO) *in vitro* at a concentration of 0.16 µL/mL. At higher concentrations (0.32 µL/mL), the inhibition of two key pro-inflammatory proteins, COX-2 and iNOS, was successfully achieved.

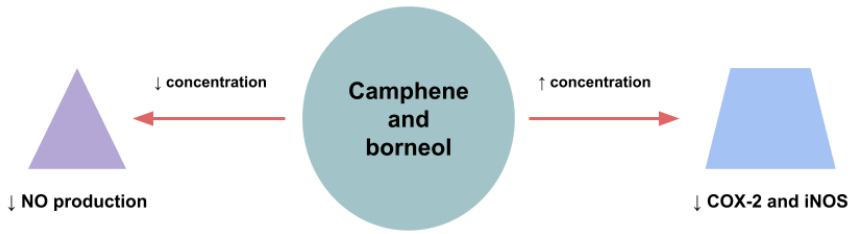

Figure 8. Camphene and borneol mechanism and effects

Santana et al. (2015) evaluated p-cymene induced with several pro-inflammatory substances independently (e.g., carrageenan, TNF-α, PGE_2, and dopamine) and found that it lowered the severity of hyperalgesia. In addition, there was also a decrease in the total leukocyte migration, neutrophils, and TNF-α

production *in vivo* at doses of 25 mg/kg, 50 mg/kg, and 100 mg/kg, and all doses (25, 50, and 100 mg/kg), respectively through IP or subcutaneous (s.c.) injection.

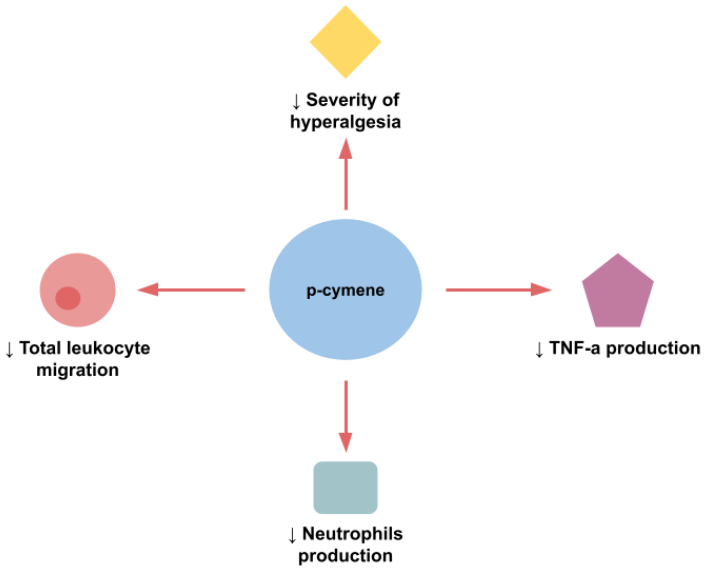

Figure 9. p-cymene anti-inflammatory mechanism and effects

A study conducted on mice showed that the mice sample pre-treated with 25 mg/kg or 50 mg/kg γ-terpinene showed a significant reduction of carrageenan-induced paw edema. Similar effects were still observed with the treatment of γ-terpinene after administration of bradykinin, PGE_2, and histamine. The results also show that the migration of neutrophils, as well as the production of TNF-α and IL-1β, are decreased in comparison to the control (Ramalho et al., 2015).

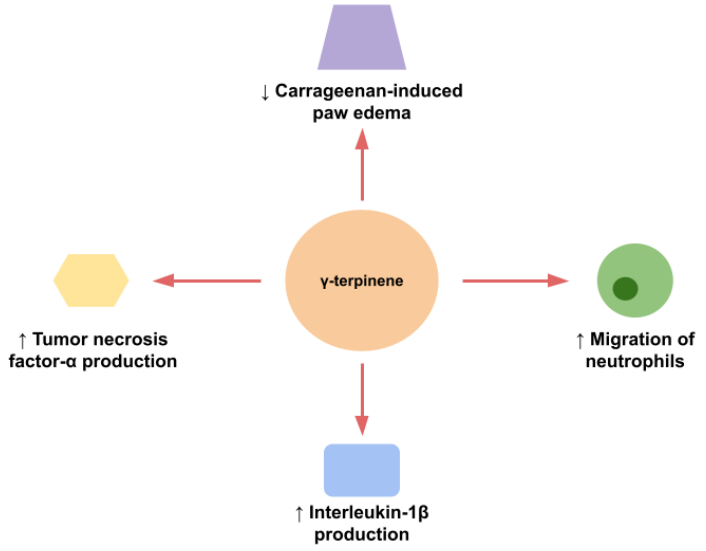

Figure 10. γ-terpinene anti-inflammatory mechanism and effects

Kara et al. (2015) evaluated carvacrol's anti-inflammatory activity using an enzyme-linked immunosorbent assay technique with IL-6 and TNF-α as the pro-inflammatory markers. In addition, the levels of malondialdehyde, NO and arginase were also measured. Their results show that treatment with different doses of carvacrol (20, 40, and 80 mg/kg) via intragastric gavage reduced the levels of pro-inflammatory cytokines, malondialdehyde, NO, and arginase in accordance with the doses. This indicates that carvacrol has dose-dependent anti-inflammatory properties.

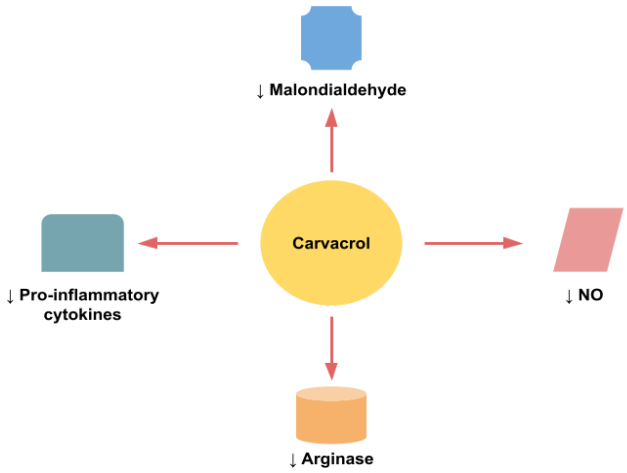

Figure 11. Carvacrol anti-inflammatory mechanism and effects

Moreover, the anti-inflammatory activity of thymol was proven by a study on mouse models with inflammatory skin diseases and imiquimod-induced psoriasis (Pivetta et al., 2018). Anti-inflammatory activity, marked by edema inhibition and ear thickness increase inhibition, is better observed in 50% w/w of thymol-nanostructured lipid carriers (NLCs) compared to free-thymol (without NLCs).

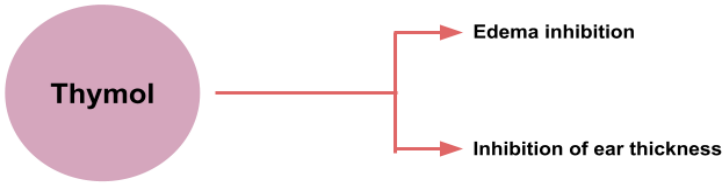

Figure 12. Thymol anti-inflammatory mechanism and effects

Brito et al. (2019) investigated the anti-inflammatory activity of β-caryophyllene in combination with docosahexaenoic acid by injecting it into mice in a Cg-induced peritonitis model at a dose of 200 µL/mice. The results show that the treated group has lower neutrophil migration, bacterial load, and leukocyte count. According to their study, the combination of β-caryophyllene and docosahexaenoic acid is able to suppress inflammation in both carrageenan-induced peritonitis and sepsis induced by *Staphylococcus aureus* intraperitoneal inoculation.

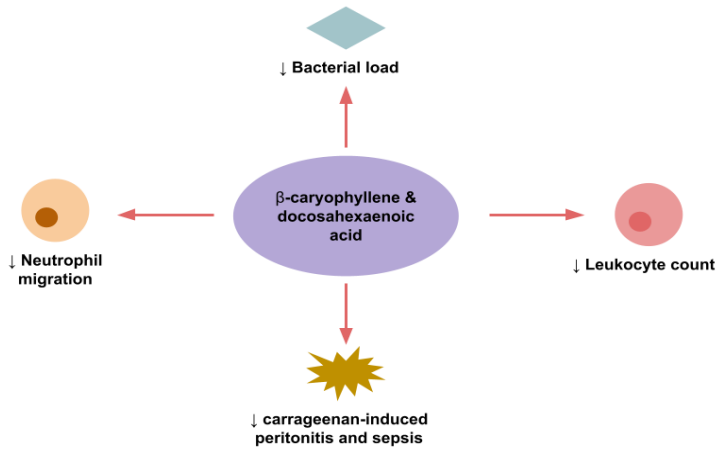

Figure 13. β-caryophyllene and docosahexaenoic acid anti-inflammatory mechanism and effects

Anticancer properties

Cancer is a disease that occurs due to an imbalance between the level of cell proliferation and its apoptosis. Thus, inducing apoptosis of those cells is considered to be one of the most promising treatments against cancer (Bhakkiyalakshmi et al., 2016). Alaufi et al. (2017) found that TQ exhibits cytotoxic activity against oral cancer cell lines (UM-SCC-14C) by upregulating apoptotic genes (caspase-9 and p53) expression and downregulating anti-apoptotic genes (Bcl-2) expression which induces the apoptosis of oral squamous cell carcinoma (OSCC) cells. Additionally, their study found a milder cytotoxic activity of TQ against normal oral epithelial cells (OEC), making it a candidate for head and neck cancer therapy. Darakhshan et al. (2015) mentioned that TQ has promising anticancer, anti-mutagenic, anti-neoplastic, antiproliferative, and anti-neoplastic activity in miscellaneous tumor models. They claimed that TQ was the most promising candidate for chemotherapy toxicity reduction and anti-tumor potential enhancement. However, it is important to note that TQ still lacks clinical data (Barkat et al., 2020).

As TQ is indicated to possess anticancer activity, several research studies show that TQ performs this anticancer activity through different mechanisms, in particular disrupting the structure of DNA as well as affecting signaling molecules or pathways of carcinogenicity (Khan et al., 2017). Khan et al. (2017) stated that TQ roles as anticancer are diverse depending on the cancer cell types. They also stated that the important mechanism of TQ as an anticancer is that TQ induces apoptosis in cancer cells, as inducing apoptosis is considered the best way to target cancer cells. The apoptosis is induced by producing ROS (Khan et al., 2017). Besides generating ROS that induces apoptosis, TQ is also able to induce apoptosis by DNA damage, as TQ disrupts the structure of DNA (Khan et al., 2017). TQ specifically targets the cellular copper in the chromatin, which is related to DNA base guanine, which will ultimately cause oxidative DNA breakage cell death (Khan et al., 2017). One more important mechanism in inducing cell death by TQ is through targeting carcinogenic signalling pathways (Khan et al., 2017). Some of the few examples have been studied, such as in prostate cancer, TQ works by downregulating the expression of androgen receptors and cell proliferation regulators (E2F-1) (Khan et al., 2017).

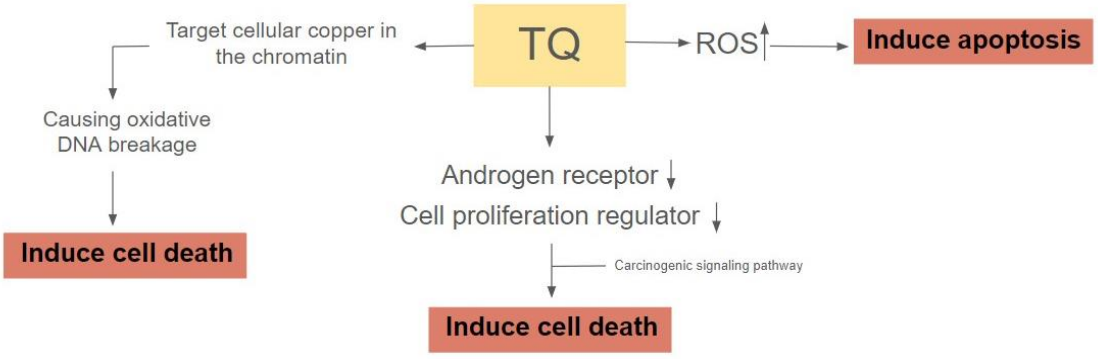

Figure 14. Thymoquinone anticancer mechanism

Another bioactive compound that also exhibits anticancer activity is carvacrol. Based on the study conducted by Bhakkiyalakshmi et al. (2016), carvacrol can induce the apoptosis of HL-60 (human acute promyelocytic leukaemia cell lines) and Jurkat cells (immortalized human T lymphocyte cell lines) which can work against leukaemia through the mitochondria-mediated pathway which involve the caspase-3. The results of the MTT assay show that carvacrol induces cytotoxicity in HL-60 and Jurkat cells in a dose-dependent manner. Therefore, carvacrol can efficiently block cancer cell proliferation *in vitro* without attacking the normal human peripheral blood mononuclear cells. Furthermore, the observations show a decrease in the level of anti-apoptotic Bcl-2, which is reported to prevent apoptosis and maintain the mitochondrial membrane potential stability. Reduction in Bcl-2 expression will disrupt the mitochondrial

membrane potential, resulting in the release of cytochrome c (Cyto-c). Furthermore, it will activate and increase the number of cleaved caspase-3, indicating the apoptotic state of the cell. However, further clinical trials are still needed (Bhakkiyalakshmi et al., 2016).

Thymol, which also possesses anticancer activity, is able to undergo several mechanisms that lead to the apoptotic and antiproliferative effects of cancer cells. Research conducted by Li et al. (2017) showcases the effects of thymol on inhibiting bladder cancer cell proliferation through ROS induction. The administration of 25–150 µM Thymol in HCT-116 cells (human colorectal carcinoma cell lines) caused severe DNA damage, increasing oxidative stress levels, as well as the upregulation of caspase-3, poly ADP-ribose polymerase 1 (PARP-1), and Bax/Bc-2-c-Jun N-terminal kinase pathway (p-JNK), leading to the release of cytochrome c from the mitochondria (Islam et al., 2018; Li et al., 2017). Several pathways involving the apoptosis of cancer cells can also be illustrated through either the mitogen-activated protein kinase (MAPK) pathway or p-JNK pathway after caspase-3 upregulation, followed by the cleavage of PARP-1 (Yue & López, 2020). In normal mammalian cells, thymol promotes cell survival by reducing T-cell activation and proliferation, resulting in antiproliferative and anti-inflammatory effects through the downregulation of interferon-gamma (IFN-γ), IL-2 (Gholijani & Amirghofran, 2016), and NF-κB expression (Gholijani et al., 2015; Islam et al., 2018). Despite thymol being claimed to exhibit a promising potential as a plant-derived chemotherapeutic agent, further clinical testing is recommended to advance this novel approach (Li et al., 2017).

Immunomodulatory properties

Some components of *M. fistulosa* contribute to their immunomodulatory and immunosuppressive properties. Thymol and carvacrol are the main constituents of *M. fistulosa* that exhibit immunomodulatory effects by inhibiting the maturation of dendritic cells (DCs) and are also capable of inhibiting the allogeneic and mitogenic response of T cells, as well as secretion of IL-4 and IFN-γ cytokines (Amirghofran et al., 2016). Thymol has a strong inhibitory effect on phagocytosis. Likewise, carvacrol also has an inhibitory effect on phagocytosis but at a mild level (Pérez-Rosés et al., 2015). *In vitro* studies by Pérez-Rosés et al. (2015) which administered 56 µg/ml of thymol and 47 µg/ml of red thyme on human neutrophils, showed inhibition of phagocytic activity. The phagocytic inhibitory values induced by thymol and red thyme were 72% and 38%, respectively. Both compounds also increase hypersensitivity reaction, decrease heterophils/lymphocyte ratio, and total IgG anti-sheep red blood cell (SRBC) titers (Salehi et al., 2018). In bronchial asthma, serum IgE levels decreased after carvacrol treatment (Ezz-Eldin et al., 2020).

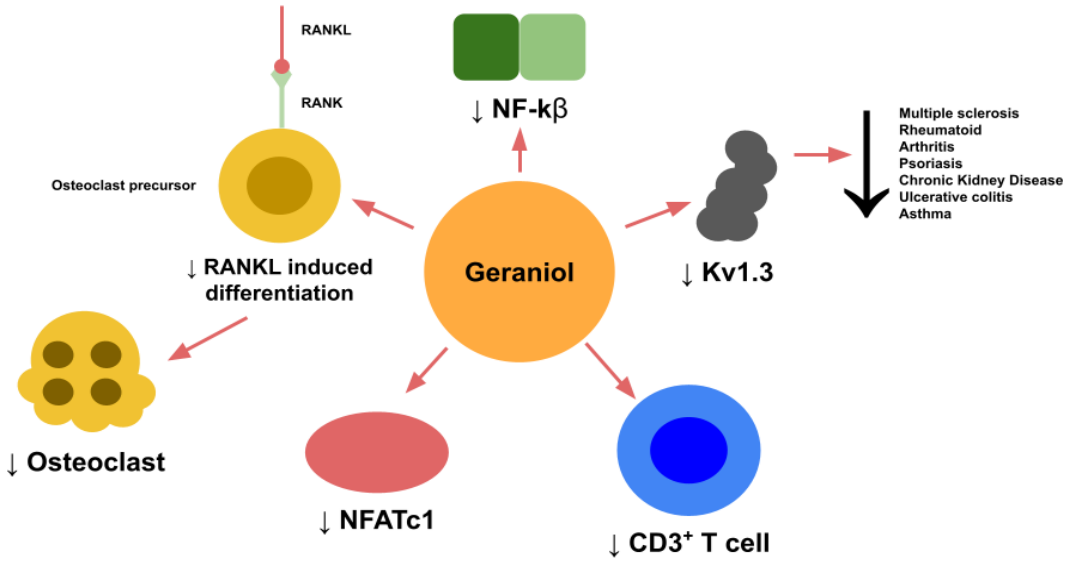

Figure 15. Immunomodulatory mechanism of geraniol

Geraniol is one of the major constituents of *M. fistulosa*, which exhibits immunomodulatory properties after being tested both *in vivo* and *in vitro* for immunosuppressive function. The result shows it has an inhibitory effect on the Kv1.3 ion channel, which is a confirmed drug target for chronic and autoimmune disease and exhibits a strong immunosuppressive activity on human $CD3^+$ T cells (Ye et al., 2019). Evidence shows that geraniol suppresses NF-κB, downregulates Nuclear Factor of Activated T Cells 1 (NFATc1), and osteoclastogenic gene expression in Nuclear Factor κβ-Ligand receptor activator (RANKL) induced osteoclast differentiation model, which leads to inhibition of osteoclastogenesis (Deepak et al., 2017).

Shaterzadeh-Yazdi et al. (2018) found that TQ content in *M. fistulosa* displayed an immunomodulatory effect in both cellular and humoral immunity. Their study shows that TQ could improve immune performance and reduce oxidative stress, which increases the toxicity of imidacloprid. Furthermore, TQ is observed to reverse the decreased reproduction of T cell and IL-2 levels and improves both Thymus homing and T cell circulation in the offspring of diabetic mother rats (Shaterzadeh-Yazdi et al., 2018). In mixed lymphocyte cultures, TQ was shown to decrease cytokine production, such as IL-8 and IL-1 beta, as well as inhibit IL-6 production (Darakhshan et al., 2015; Gholamnezhad et al., 2015).

CONCLUSION

As reviewed in this paper, *M. fistulosa* contains several phytochemicals that have some role in its pharmacological activities, including carvacrol, thymol, thymoquinone, flavonoids, α-pinene, caryophyllene oxide, limonene, and geraniol. Some of the major compounds, including thymol, carvacrol, and TQ, play a wide variety of roles in their pharmacological activity. Previous research has revealed some of the pharmacological activity of *M. fistulosa,* including antimicrobial, antidiabetic, antioxidant, anti-inflammatory, anticancer, and immunomodulatory properties. In relation to its pharmacological activities, the authors of this paper believe that *M. fistulosa* has the potential to be utilized as a medicinal herb. However, due to the lack of clinical data in this regard, further clinical studies are suggested to unravel the possibilities of applying *M. fistulosa* in the medical field.

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