Therapeutic Insights into the Anti-Inflammatory Properties of *Ocimum basilicum, Rosmarinus officinalis* and *Cymbopogon citratus* Essential Oils

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Abstract

Tissue injury or stress triggers inflammation that is a protective physiological response, but persistent inflammation is a pathological condition. Synthetic anti-inflammatory drugs have adverse effects and resistance but natural plant alternatives are gaining much interest. This chapter focuses on the phytochemistry and mechanism of action of *Ocimum basilicum* (Sweet basil), *Rosmarinus officinalis* (Rosemary), and *Cymbopogon citratus* (Lemongrass) essential oils that possess anti-inflammatory effects. This chapter shows that *O. basilicum* essential oil, containing bioactive compounds of linalool, estragole and methyl eugenol inhibit pro-inflammatory cytokines, enzymes such as cyclooxygenase (COX) and lipoxygenase (LOX) and nitric oxide. The essential oil of *R. officinalis*, rich in camphor, α-pinene and 1,8-cineole has shown its great anti-inflammatory action by stopping leukocytes migration, NF-κB transcription and prostaglandin synthesis. Likewise, *C. citratus* essential oil containing flavonoids, myrcene and citral has anti-inflammatory properties by inhibiting inflammatory mediators and diminution of oxidative stress. These findings indicate the therapeutic potential of these essential oils as effective and safer alternatives for inflammatory and associated disorders.

Keywords: Anti-inflammatory properties, Essential oils, *Ocimum basilicum*, *Rosmarinus officinalis*, *Cymbopogon citratus*, Phytochemicals

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Introduction

Tissue injury, stress or infection triggers inflammation, a physiological state of adaptative condition, to recover tissue homeo-staticity. Chemical inflammatory mediators are cytokines, arachidonic acid products, histamine, chemokines and so on which exert their action through receptors in the target tissue of any organ of the living-being. They produce the cellular and vascular events that foster the exudative and emigrated leukocytes. If the inflammatory process is chronic, and if cells and plasma proteins go on producing more and more chemical mediators then they may injure the tissue and indicate some favourable signs and symptoms. In some conditions, when there are certain specific types of cells, like macrophages and lymphocytes, some stimuli tend to prolong the inflammatory process, thereby making the defense process chronic (Lawrence & Gilroy, 2007). These cells can form dangerous chemicals that toxicologically offend to the agent and body itself (Santos et al., 2013). However, unless inflammation is unresolved, self-damage is a necessary evil and anti-inflammatory agents are needed to control it (Schmid-Schönbein, 2006; Ashley et al., 2012).

Resistance to the drugs and side effects associated with chemosynthetic drugs have increasing the concern towards natural products such as medicinal herbs and plant extracts by researchers and other people in the global society (Tiwari et al., 2013; Dhama et al., 2018; Tiwari et al., 2018; Salehi et al., 2019). They are credited with both their numerous positive health impacts and their nontoxic profile in treating diseases in clinical and medical settings (Tesfahuneygn & Gebreegziabher, 2019). Moreover the scientific authentication of these herbs, metabolites and phytoconstituents have now been brought into practice and public awareness (Rahal et al., 2012; Salehi et al., 2019). The main attributable factor is their well proven and bright potential of desired beneficial health effects and therapeutic uses in human and animal health against many infections and noninfectious diseases (Dhama et al., 2015; Saeed et al., 2019). The multiple and overall positive health impact of natural plant products encompass antimicrobial action, antioxidants, immunostimulant, and protection against cancer and ailments of lifestyle diseases like hypertension, diabetes, high lipid levels, obesity, gastrointestinal disorders, chronic cough, headaches, diarrhea, constipation and kidney disorders (Islam et al., 2020). Furthermore, many herbs and plant metabolites have been established to have potential use in eradicating major as well as more recent pathogens including hepatitis B, C and E viruses, human immunodeficiency virus, herpes simplex virus, enteroviruses, Influenza A viruses, Ebola virus, dengue virus and Zika virus. Somewhat expectedly, it has also done this at times through combating antimicrobial resistance in the medicinal plants as a method of controlling nosocomial infections (Munjal et al., 2017).

Three examples of such holistic herbs are *Rosmarinus officinalis, Ocimum basilicum* and *Cymbopogon citratus* which have enormous therapeutic uses due to the presence of a wide range of pharmacologically active plant secondary metabolites (Thakur et al., 2016).

General Information of *Ocimum basilicum*

Ocimum basilicum known as sweet basil or European basil in America, basil in Europe, Badrooj, Hebak, or Rihan in the Middle East. The leaves of *O. basilicum* are known for their characteristic anise-like flavor, combined with a strong, sweet, and pungent aroma. This plant's flowers are typically purple or white in color. Basil thrives in a variety of climates, ranging from tropical and subtropical to temperate regions and is widely found in areas such as Africa, India, Pakistan, West Asia, Nepal (particularly in the Himalayan regions), Sri Lanka, Southeast Asia, and beyond. The essential oil extracted form *O. basilicum* is pale yellow to greenish-yellow liquid having its aromatic and therapeutic values (Ch et al., 2015). The Ocimum comes under the family named *Lamiaceae* and it is composed of several species of annual or perennial aromatic herbs and shrubs (Yaldiz et al., 2019). That versatility of *O. basilicum* is confirmed by the significant number of scientific publications, which investigate pharmacological, therapeutic, and biomedical potential of this plant (Balakrishnan Purushothaman et al., 2018).

Phytochemistry of Essential Oil of *Ocimum basilicum*

Ocimum basilicum has been characterized for its fixed and volatile oil components for health and medicinal uses. Various methods can be employed to extract its polyphenolic compounds, which are generally classified into two types:

1. **Traditional Techniques:** These entails techniques such as Soxhlet extraction, Shaking, Maceration and Hydro-distillation.
2. **Modern techniques** including the Microwave-assisted Extraction techniques and Supercritical Fluid Extraction with CO_2 are gaining a wide acceptance (Coelho et al., 2018).

Concerning the impact, it is declared that the choice of the extraction method and various factors such as temperature, solvent, and extraction time, do affect the chemical composition and the extracted basil compounds' quality. Altogether, the type of the initial raw material, fresh, frozen or lyophilized basil leaves employed in the extraction, also plays an important role in defining the efficiency of the extracted phenolic compounds (Filip et al., 2017). Basil essential oil is composed of 25.44% of the oxygenated monoterpenes, 38.39 % of the hydrocarbons and 17.43% of phenylpropanoids. The yield profile of the residue sequentially extracted through molecular distillation of the crude essential oil consist of some compounds namely methyl eugenol, linoleic acid and estragole in the percentage of 11.35%, 11.40% and 17.06% respectively. On the other hand, the distillate fraction mainly consisted of α-cadinol (16.24%), α-bergamotene (11.92%) and methyl eugenol (16.96%) (Li et al., 2017).

New researchers have identified other accompanying bioactive constituents in *O. basilicum*, namely belonging to the group of salvianolic acid (Prinsi et al., 2019). For example, simple phenolic acids including salvianic acid A and 4-hydroxybenzoic acid were found, especially in flowers of basil. Flavonoid and polyphenol content assessed from the aqueous extract reveals that different fractions possess good quantity of flavonoids and polyphenols like Luteolin (5.94 %) and Apigenin- O-glucoside (7.53%). There were also found other polyphenolic compounds, namely, caftaric acid containing 9.39% and rosmarinic acid containing 15.76% (Ibrahim et al., 2020). Besides, the main compounds of the mixture which showed significant anti-inflammatory activity includes linoleic acid, eugenol, α-cadinol, linalool, methyl cinnamate, estragole, α-bergamotene and methyl eugenol. Such phytochemicals reinforce each other making basil a valuable herb in the treatment of inflammation related diseases (Dhama et al., 2023).

General Information of *Rosmarinus officinalis*

Rosmarinus officinalis commonly called Rosemary in English, Romero in Spanish and Alecrim in Portuguese, is an aromatic evergreen shrub from the *Lamiaceae* family that was formerly known as Labiatae (Centeno, 2002; Begum et al., 2013). This aromatic shrub is native to the Mediterranean region and can cultivate as tall as 2 meters, with perennial, linear leaves that remain vibrant year-round. The plant topographies upright, brown branches that are occasionally flat, supporting dark-green leaves. Its small flowers are arranged in clusters along the branch axils and presents a corolla that varies in color from pale white to soft pink. The androecium is categorized by two flagrantly visible lateral stamens, each with filaments that has a characteristic small lateral tooth. Rosemary thrives in well-drained, dry to moderately moist soils, making it unsuitable for anaerobic or waterlogged conditions. It reveals moderate tolerance to salinity, which complements its ability to flourish in Mediterranean climates. The plant naturally flowers between May and June in these regions, with fruiting occurring during the spring and summer months (Ribeiro-Santos et al., 2015).

Phytochemistry of Essential Oil of *Rosmarinus officinalis*

The extracted oil of *Rosmarinus officinalis* has immense commercial importance because of its applications for diverse sectors which include the food and pharmaceutical industries (Kfoury et al., 2015). This oil is classically a colorless or pale-yellow liquid that has an intense burning odour which can immediately be distinguished. It makes up approximately 1 to 2.5 percent of the plant's gross weight. Nevertheless, its contents may vary from time to time in response to various aspects such as climate, plant part used, place and method of extraction (Tawfeeq et al., 2016; Mouahid et al., 2017). These are the bioactive compounds found in the oil; myrcene, bornyl acetate, borneol, camphene, α-terpineol, 1,8-cineole, α-pinene, camphor, limonene, β-pinene and β-caryophyllene (Chávez-González et al., 2016). Of these, camphor, α-pinene and 1,8-cineole stand out to be primarily effective in their anti-inflammatory properties (Bajalan et al., 2017).

General Information of *Cymbopogon citratus*

Lemongrass is a perennial herb that has its origin from tropical Asia (Francisco et al., 2011) and is mostly a native of the South and Central America region (Oladeji et al., 2019). Its common names are lemongrass or citronella. The species Cymbopogon comes from Greek and it has been derived from "kymbe-pogon" which translates to "boat-beard" that refers to the flower spikes of the plant The "citratus" coming from Latin means lemon-scented leaves (Shah et al., 2011). This plant is of *Gramineae* family and has over 55 species of it. Three species that spread most widely belong to *Cymbopogon citratums* or West Indian grass, *Cymbopogon pendulus* or Jammu grass and *Cymbopogon flexuosus* or Malabar grass (Kouame et al., 2016). Lemongrass has long and narrow bright green slender leaves with a pointed apex, lanceolate in shape, widths of 1.3–2.5 cm and length of about 0.9 cm. Lustrous, bluish green in color, they proclame a citrus fragrance when crushed because the leaves of the tree contain neral, citral and geranial aldehyde (Tajidin et al., 2012).

Phytochemistry of Essential Oil of *Cymbopogon citratus*

The leaves of *Cymbopogon citratus* contain substantial quantities of the volatile oil (Brügger et al., 2019), which consists of geraniol, citronellol, citral (a mixture of terpenoids and geranial), myrcene and α-oxobisabolene. Concentration of these compounds varies depending on the plant species and origin of the plant part used. For example, East Indian lemongrass contains 10-13% of essential oil while West Indian lemongrass contains 12-15% of the essential oil (Ranitha et al., 2014). Citral is mainly responsible for lending the plant the specific taste it has (Costa et al., 2013). Other components present in the oil in lesser concentration include Eudesma-7 (11) -en-4-ol (5.3%), Decanal (0.25%), Neointermediol (7.2%), Methyheptenone (1.2%), Selina 6-en-4-ol (27.8%), α-cadinol (8.2%) and Naphthalene (0.79%) (Farhang et al., 2013). Some of the more recent analysis have also identified β-eudesmol (45%), cubebol (4.7%), elemol (41%), and humulene (4%) (Halabi & Sheikh, 2014), accompanied by other compounds such as β-eudesmol (45%), cubebol (4.7%), citral acetate, citral diethylacetal and citronella. (Nanon et al., 2014). As highlighted in the literature, the *C. citratus* contains essential oil and flavonoids, which are claimed to be the main responsible for the therapeutic and pharmacological activities (Roriz et al., 2014).

Anti-inflammatory Activity

Ocimum basilicum

The best known anti-inflammatory compounds of sweet basil include methyl eugenol, methyl cinnamate, estragole, α-bergamotene, α-cadinol and linoleic acid (Shiwakoti et al., 2017). All these effects are thought to emanate from the suppression on the pro-inflammatory mediators and the stimulation of anti-inflammatory cytokines (Güez et al., 2017). In this in vitro assessment study, the plant extract has been noted to suppress the release of nitric oxide (NO) and inducible nitric oxide synthase (iNOS)(Li et al., 2017). The ethanolic extract of *O. basilicum* leaf callus and leaf also showed in vitro anti-inflammatory activity on LPS stimulated RAW 264.7 macrophage cells by reducing NO production (Aye et al., 2019). In addition, the *O. basalicum* used in this study exerted anti-inflammatory properties through the suppression of the lipoxygenase enzyme activity. Furthermore, the crude methanolic extract of *O. basilicum* associated with anti-inflammatory property by suppressing the expression of pro-inflammatory cytokines which are crucial to activate Janus kinase-Signal transducer and activator of transcription (JAK-STAT) pathway (figure 1) which in turn is responsible for induction of inflammation. This extract also suppresses the expression of adjuvants including TNF-α, IL-2 and IL-1β and other inflammatory molecules including NO and iNOS. In an acute inflammatory model using turpentine oil in rats, the ant-inflammatory effects of *O. basilicum* tincture were evidenced by decrease in total leukocyte count, monocyte percentage, and increased circulating phagocytic activity. However, the anti-inflammatory effect of the tincture was weaker than that of the drug diclofenac that was used for the experiment (Benedec et al., 2007).

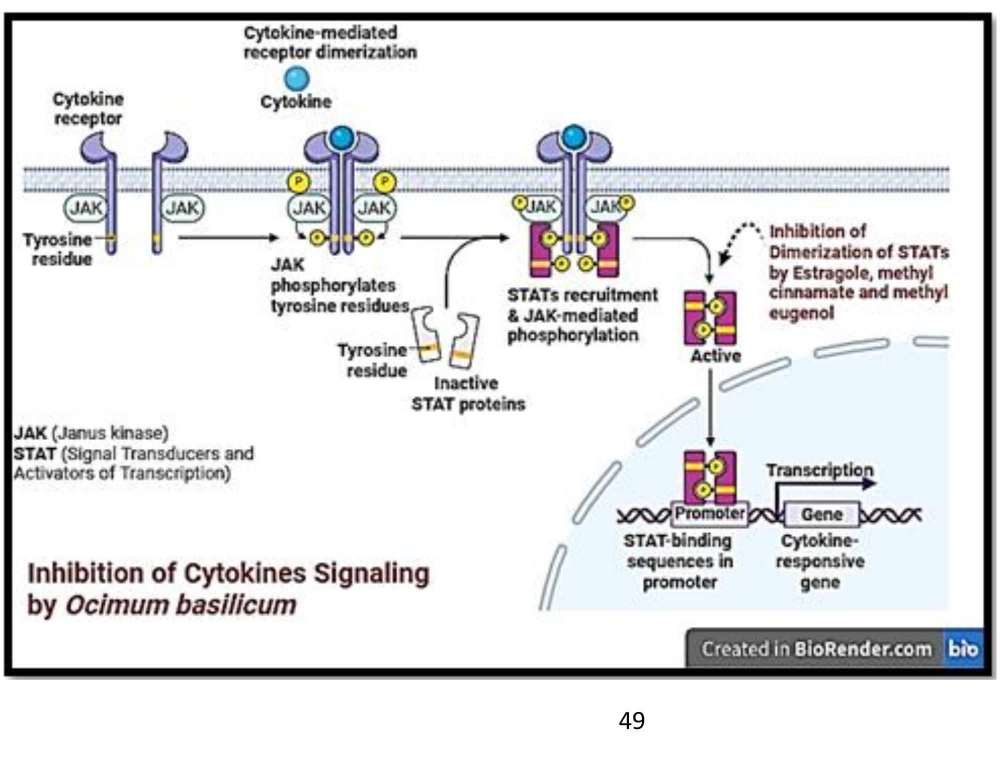

Fig. 1: Suppression of Cytokine signaling and synthesis of pro-inflammatory cytokines including TNF-α, IL-6 and IL-β, and the related cytokine gene.

Recent studies to understand the effect of *O. basilicum* extract on in-vitro cultures demonstrated that the extract dose not significantly alter the levels of pro-inflammatory cytokines including IL-6 and TNF-α even when exposed at different concentrations. Conversely, a dose related increase in both IL-4 and IL-10 was evident especially when comparing it with ibuprofen. On the other hand, the extract of *O. basilicum* reduced the production of IL-6. The extract also appeared to decrease COX-2 expression, which also has an anti-inflammatory effect. (Mueller et al., 2010).

The potential of *O. basilicum* to give a new dimension to the understanding of the healing properties and the development of anti-inflammatory drugs has been successfully grasped by its essential oil. In mice, a conjugated complex of β-cyclodextrin with the plant's essential oil increased its potent anti-inflammatory properties for the treatment of both, acute and chronic inflammations. The research findings regarding *O. basilicum* essential oil and its major constituent estragole support its application as a therapeutic agent. The study claimed that both the essential oil and estragole had a role in lowering inflammation where the authors found the essential oil to be especially effective in chronic and acute inflammation (Rodrigues et al., 2016).

(Aye et al., 2019) highlighted the potential health benefits of the bioactive compounds that can minimize and stop the pathological inflammation. These elicitor compounds include β -aminobutyric acid, jasmonic acid and arachidonic acid which increase the level of anthocyanin in *O. basilicum*. Using these elicitors, the scientists gained the ability to enhance the anti-inflammatory potential of anthocyanins which was shown by the increased effectiveness of lipoxygenase and cyclooxygenase enzymes inhibition. Analyzing other plant hormones, it was observed that endogenous phytohormone jasmonic acid which is necessary for the plant growth and development, led to the enhancement of essential oil content of *O. basilicum*. As a result, it could be assumed that the elevation of the essential oil production is associated with higher anti-inflammatory activity due to the level of linalool, limonene and eugenol included into it (Szymanowska et al., 2015). Further, reduction in acute phase response in bone marrow and modulation in phagocytic functions are considered to be the reason for anti-inflammatory properties of *O. basilicum* (Benedec et al., 2007).

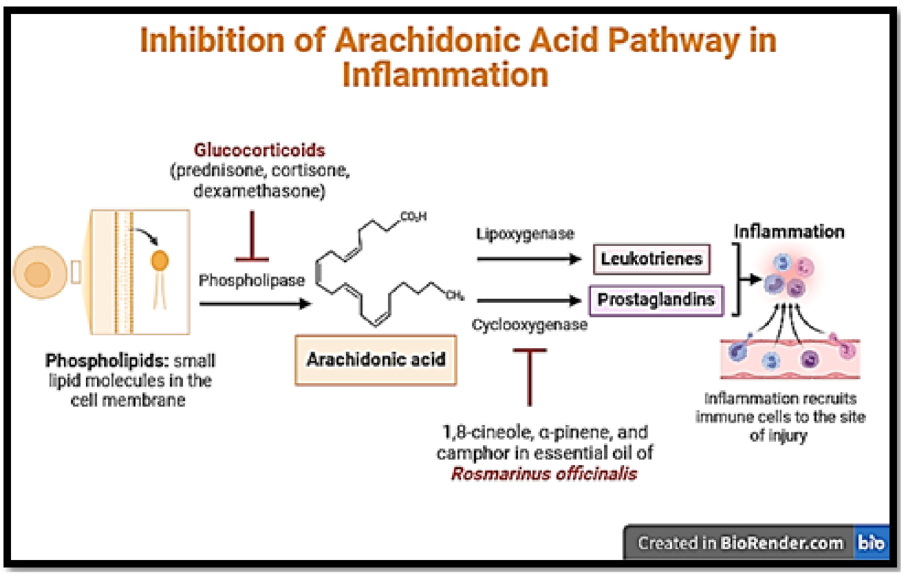

Fig. 2: 1,8-Cineol, α-pinene, and camphor inhibiting the activity of lipoxygenase (LOX) and cyclooxygenase (COXs)

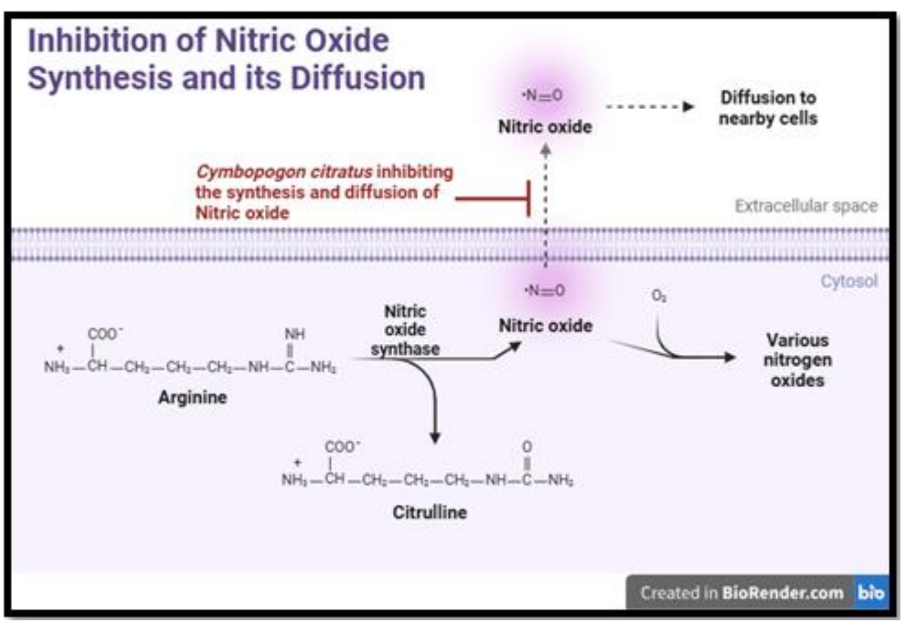

Fig. 3: Inhibition of Nitric Oxide (NO) synthesis by Phytochemicals of *Cymbopogon citratus*

Rosmarinus officinalis

The essential oil of *R. officinalis* possesses all those biological activities that make this plant to stand out as it has anti-inflammatory activity. However, one cannot deny the possibility that other monoterpenes such as those other than 1,8-cineole, α-pinene and camphor may equally possess inflammation-inhibiting properties of rosemary. Such monoterpenes include limonene, and myrcene (Rufino et al., 2015). (Faria et al., 2011) investigated the impact of R. officinalis essential oil in rats in doses of 100 – 1000 mg/kg employing croton oil induced ear edema, carrageenan induced paw oedema and cotton pellet granuloma poultice induced formation of granulomatous tissue as the models to study inflammation. The ED50 value of paw edema test was found to be 300mg/kg while a magnificent decrease in edema formation was observed in a dose dependent manner comparable to that of Indomethacin positive control used at 10 mg/kg of P. O. Ear inflammation reduction was observed, the level of granulomatous tissue formation being decreased by 59%, compared with the negative control group (0.5 ml saline solution, p.o.), while the ear oedema degree was reduced by 77%. Remarkably, the given intervention with *R. officinalis* essential oil revealed 64% less gastric damage than the indomethacin group did. This finding is predominantly considerable as NSAIDs usage for longer periods is associated with the development of gastric injuries and peptic ulcers (Drini, 2017). Likewise, adverse effects of steroidal anti-inflammatory drugs are time-bound, they include immunosuppression, hypertension, delay in the process of wound healing, growth failure in children, thinning of bones or osteoporosis, and several metabolic disorders (Rhen & Cidlowski, 2005). Hence, there is increasing call for novel anti-inflammatory agents. Additionally, (Takaki et al., 2008) investigated the anti-inflammatory effect of the *R. officinalis* essential oil, in doses of 250 and 500 mg/kg, by measuring exudative fluid and leukocyte infiltration in a carrageenan-induced pleurisy model and by determining the effects on paw edema in rats. The treatment with this essential oil reduced the development of edema one to four hours following the carrageenan treatment when compared to the animals in the control group. Additionally, the content of pleural inflammatory exudates was reduced, as well as the amount of migrated cells.

(Borges et al., 2018) further suggested another mode of action in the arachidonic acid cascade, that experimentally camphor present in the essential oil of *R. officinalis* possesses considerable binding with critical anti-inflammatory targets like COX-2 as shown in figure 2, which has been confirmed by molecular docking analysis (Mekonnen et al., 2016) further strengthened a beneficial effect of *R. officinalis* essential oil as perceived through in vivo and in vitro inflammatory mediators – leukocyte migration, chemotaxis. They proposed that the terpenes present in the oil might aid in preventing the NF-κB transcription that could be credited to the plant inflammation fighting properties. This agrees with their findings on inhibition of NF-κB a transcription factor responsible for chemokines formation and adhesion molecules important in chemotaxis and movement of leukocytes.

Cymbopogon citratus

Inflammation is now a tremendous global health concern and is linked to severe diseases like cancer (Colotta et al., 2009). Natural products which are usually used in traditional medicine practices have been proven to be useful when it comes to inflammation. In fact, lemongrass was used in different parts of African and Asia to treat inflammation-related ailments. Citral that can be extracted from *C. citratus* has proved to possess a great influence on the inflammatory mediators, and it is often used in topical formulations like ointments, and creams for inflammation. Research has shown that it can suppress TNF-α induced neutrophil adhesion having a concentration of 0.1% (de Cássia da Silveira e Sá et al., 2013), and to overwhelm iNOS, nitric oxide (NO) production, and other pathways triggered by lipopolysaccharide (LPS). Citral is also an antagonist of NF-κB receptors, decreases the activity of COX-2 up to 60-70% and affects peroxisome proliferator-activated receptor alpha (PPAR-α). Its anti-inflammatory effects extend both orally and topically, achieving a suppression rate of 80-90% in tissue inflammation (Boukhatem et al., 2014). Several compounds isolated from *C. citratus*, including citral, epoxyestragole and 6,7-epoxycitral have been shown to inhibit the synthesis and secretion of nitric oxide (NO) as shown in figure 3. These compounds, which involve a connection of sugar and aglycone moieties, have established additional anti-inflammatory effects, including plummeting postoperative pain by lowering pain mediator expression (Nishijima et al., 2014).

Conclusion

Inflammation is an important mechanism that is useful in healing and in general body health but when it becomes chronic or uncontrolled it becomes damaging. Evaluating inflammation and bringing specific changes at the molecular level necessary for therapeutic impact means applying safe and sustainable methods. Some plants that have encouraged the use as sources of anti-inflammatory drugs rather than synthetic drugs are *Ocimum basilicum*, *Rosmarinus officinalis* and *Cymbopogon citratus* since they are endowed with rich phytochemicals hence low on side effects. For instance, basil contains methyl eugenol and linoleic acid; rosemary contains camphor and 1,8-cineole; and lemongrass contains citral that have excellent qualities to control inflammation. These compounds operate in several ways, for example by suppressing inflammatory routes/mechanisms and pro-inflammatory signaling molecules, and by up-regulating anti-inflammatory molecules.This ability to modulate several pathways at once is particularly relevant to their potential application for both the treatment of acute and chronic inflammation.

Indeed, the results obtained provide basis for use of these natural remedies as a part of complementary medicine, as an effective tool against inflammation instead of using drugs. Thus, using the current knowledge of the biological activity of these plants it is possible to create more comprehensive and, possibly, more efficient therapies, diminish the usage of synthetic drugs, and decrease potential dangers, connected with side effects or emergence of drug resistance. This reaffirms the global call to tap into nature as a natural resource bank in coming up with the modern healthcare solutions.

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