

Rosmarinic acid: insights into analytical, pharmacological & pharmaceutical aspects

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Rosmarinic acid is an indigenous antioxidant molecule derived from a variety of widely available herbal plants. This molecule exhibits a diverse array of pharmacological properties. Recently, there has been an increasing interest in the importance of its effects and its possible use as a natural antioxidant in processed foods. It is a naturally-occurring hydroxylated compound that is commonly found in species. The escalating emergence of antimicrobial resistance and the detrimental repercussions resulting from the excessive utilization of antibiotics are generating a need for novel antimicrobial therapies. Researchers are currently investigating natural compounds as a promising option in this context. Medicinal plants possess a substantial quantity of rosmarinic acid. It is widely used as a spice in various cuisines and is highly regarded in traditional medicine for its healing properties. Rosemary contains significant amounts of powerful antioxidant chemicals, such as phenolic diterpenes, flavonoids, and phenolic. This review aims to analyze the relationship between the structure and activity of Rosmarinic acid, as well as its chemistry, sources, biotechnological production, and neuroprotective effect. Additionally, it will focus on the nano formulation and commercialized formulations of Rosmarinic acid.

Keywords: Rosmarinic acid. Structure activity relationship. Pharmacological activity. Formulation. Extraction.

INTRODUCTION

Polyphenols, a diverse group of compounds, can be classified based on the number of phenol rings they possess and the structural components that connect these rings. This system of classification aids in the separation of polyphenols into discrete groups, including lignans, stilbenes, phenolic acids, and flavonoids. We can learn more about the properties and purposes of these compounds by examining the arrangement and makeup of phenol rings. Moreover, there are two major processes by which polyphenols are naturally synthesized. One route is the formation of polyketides from the conjunction of activated acetate units, which cyclize to produce polyphenols. This

other process is called the shikimic acid pathway, and it is in charge of most phenolic chemical production. These pathways provide a vital part in the synthesis of polyphenols, which are present in a wide range of plants and contribute to their unique chemical compositions and biological properties (Cutrim, Cortez, 2018).

Foods derived from plants are high in polyphenols, which are molecules with intricate structures. These polyphenols are usually classified as phenolic acids and phenolic alcohols because they consist of a phenolic ring. Polyphenols can be further divided into phenolic acids, flavonoids, stilbens, phenolic alcohols, and lignans based on the strength of the phenolic ring. These bioactive substances fight against degenerative diseases that cause chronic illness, which is important for human health protection. Many plant-based foods, including tea, coffee, red wine, vegetables, fruits, and herbs, naturally contain them (Prabhu *et al.*, 2021). Rather than depending on synthetic supplements, it is advised to ingest polyphenols from these natural

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sources, as they may have less negative effects on health. Polyphenols are categorized according to their chemical structures, biological roles, origins, and distribution in nature (Belščak-Cvitanović *et al.*, 2018). Diverse pharmacological activities of polyphenols include anti-aging, improved immunity, neuroprotective, anti-cancer, cardio protective, anti-tumor, lowering of blood pressure, anti-diabetic, anti-microbial, UV protection, and skin disease prevention (Abbas *et al.*, 2017).

Numerous plants, such as *Rosmarinus officinalis*, *Prunella vulgaris*, *Melissa officinalis*, *Coleus spp.*, *Salvia officinalis*, mint, in addition to sage, are rich sources of rosmarinic acid (RA), a polyphenolic molecule (Colica *et al.*, 2018). Remarkably, it is not restricted to higher taxa but is also present in lower plant groupings like gymnosperms, ferns, and hornworts. The discovery, derivatives, distribution among the plant kingdom, chemical synthesis, biosynthesis, biotechnological production, and broad spectrum of biological activity of RA have all been the subject of much research (Stansbury *et al.*, 2012; Elansary *et al.*, 2020).

Caffeic acid (3,4-dihydroxycinnamic acid) and 3,4-dihydroxyphenyllactic acid are combined through an esterification process to create RA. This leads to the synthesis of this chemical (Stansbury *et al.*, 2012; G. Kim *et al.*, 2015).

It is present in many plant families and is an ester of 3, 4-dihydroxyphenyllactic acid and caffeic acid. Numerous biological activities are displayed by RA, such as antiviral, antibacterial, anti-inflammatory, and antioxidant qualities. When it was isolated and given the name *Rosmarinus officinalis* in 1958, it was first discovered. Eight enzymes have been identified as being involved in the production of RA, which uses the amino acids l-phenylalanine and l-tyrosine. Furthermore, plant cell cultures can be used to manufacture RA in large quantities by biotechnological techniques; two well-known examples of this are *Coleus blumei* and *Salvia officinalis*. Anti-inflammatory, antimutagenic, antibacterial, antiviral, antioxidant, hepatoprotective, antimicrobial, and anti-nociceptive properties are among the compound's biological actions (Qi *et al.* 2017; Petersen, 2013).

Some of the specific benefits of RA include its antioxidant properties, which help combat oxidative stress and reduce cell damage; its anti-inflammatory effects, which can help reduce inflammation in the body; its potential to support the immune system; its neuroprotective effects, (Wang *et al.* 2021) which may protect nerve cells and support brain health; its antimicrobial properties, which can help fight off certain bacteria and fungi; and its potential to provide relief from allergies and allergy symptoms (Elansary *et al.*, 2020; Wang *et al.* 2021)). It has been discovered that RA has anti-inflammatory properties in a number of illnesses, such as atopic dermatitis, colitis, and arthritis. NF-kB and STAT3 activation are inhibited in order to achieve these results. Furthermore, RA has the ability to inhibit myringosclerosis and demonstrates hepatoprotective properties against extra hepatic cholestasis. Moreover, it has been investigated for its potential therapeutic benefits in the treatment of collagen-induced arthritis, mast cell suppression, and periodontitis (Luo *et al.* 2020a).

The data mentioned above provides a quick overview of the research conducted thus far on rosmarinic acid, taking into account its pharmacological activity and the various natural sources of rosmarinic acid that aid in supplementing our diets with RA.

In this study, we aim to integrate the comparative analysis of rosmarinic acid phytoconstituents, including the structure-activity relationship that aids in identifying the most effective moiety with good therapeutic values, analytical techniques to determine the optimal yield-maximizing strategy, pharmacological activity and its mechanism of action, pharmaceutical approach to create rosmarinic acid nano formulations that are commercially available, and ongoing rosmarinic acid research.

STRUCTURE ACTIVITY RELATIONSHIP

The term structure-activity relationship (SAR) represents a relationship between biological activity and molecular structure of the molecule. The term “structure” is that the specific structural property of an atom, group of atoms or sub-molecule is responsible

for some particular physio-chemical property; activity can refer to many different properties (e.g. toxicity, antioxidant or enzyme inhibitory). The analysis of structure activity relationships has played a vital role within the design of drugs, and helped in understanding the action of drugs at a molecular level. For a few chemicals, it's possible to create an analogy between SAR and pharmacophore. A pharmacophore is that the “the molecular framework that carries the properties of the many ligands through which they are reciprocally recognized by receptor”(Christopoulos *et al.*, 2014). A pharmacophore may or might not be coincident with the molecular structure, but it is an abstraction from the molecular structure that is consistent with observed activity; the various possible positions of the pharmacophore together with the steric and electronic features affecting the activity make up the SAR of the molecule. It is often useful to split the SAR of a compound by its effect on various biological activities, and in mode of drug delivery, action or metabolism (Akhtar *et al.*, 2017). The analysis of RA amides' SAR revealed that compounds with tertiary amine functions on an alkyl chain have a stronger affinity with the α -glucosidase active site. These compounds exhibited a higher affinity compared to other compounds without this structural feature. The most potent amides were those with this structural feature. Additionally, the best inhibitor, compound A1, demonstrated effective antioxidant properties, indicating the potential of RA amides as dual-role therapeutic agents for diabetes mellitus treatment (Cardullo *et al.*, 2019).

The structure of RA is characterized by two aromatic rings, each containing two hydroxyl groups. This unique structure enables RA to effectively scavenge free radicals, specifically superoxide anions and hydroxyl radicals. As a result, RA exhibits strong antioxidant properties, which contribute to its effectiveness in various biological activities, including anti-inflammatory, antibacterial, antiallergic, and antiviral activities (Babaei *et al.*, 2020).

Phenolic compounds are a group of substances that have an aromatic ring with one or more hydroxyl substituents. These compounds possess a wide range of health beneficial effects which include antioxidative, anti-inflammatory, antimicrobial, and anticancer properties (Swisłocka *et al.*, 2019). Further substitution at compound A2 results in Compound A3 & A4 shows a good inhibition against the growth of *L. donavani* for the treatment of Leishamanial activity at a concentration of 50 μ M and 25 μ M (Hassan *et al.*, 2023).

The detailed are depicted in the Figure 1.

PHARMACOLOGICAL ACTIVITY

Hepatoprotective

The term “hepatoprotective” describes materials or compounds that have the capacity to shield the liver against harm or damage brought on by a variety of things, including medications, poisons, infections, or other detrimental impacts. These hepatoprotective drugs aim to protect the liver from damage and prevent liver diseases by reducing damage to the liver or promoting its healing. They are often studied in preclinical and clinical settings for their potential to maintain liver health. In addition to various methods of action, hepatoprotective medications reduce inflammation, oxidative stress, and apoptosis in liver cells. Additionally, they may promote liver regeneration, strengthen detoxification processes, and stop fibrosis from developing. Moreover, certain hepatoprotective drugs change specific enzymes or signalling pathways linked to liver illness in order to maintain liver function and prevent further damage (Farghali *et al.*, 2016). The Hepatoprotective activities are mentioned in the Figure 2.

The study investigated the potential of RA to prevent liver damage caused by carbon tetrachloride (CCl₄) and understand its hepatoprotective mechanism. In the in vitro investigation, RA was pretreated on rat liver hepatocytes, while in the in vivo study, mice were given RA orally for 28 days. The results showed that oral administration of RA significantly reduced

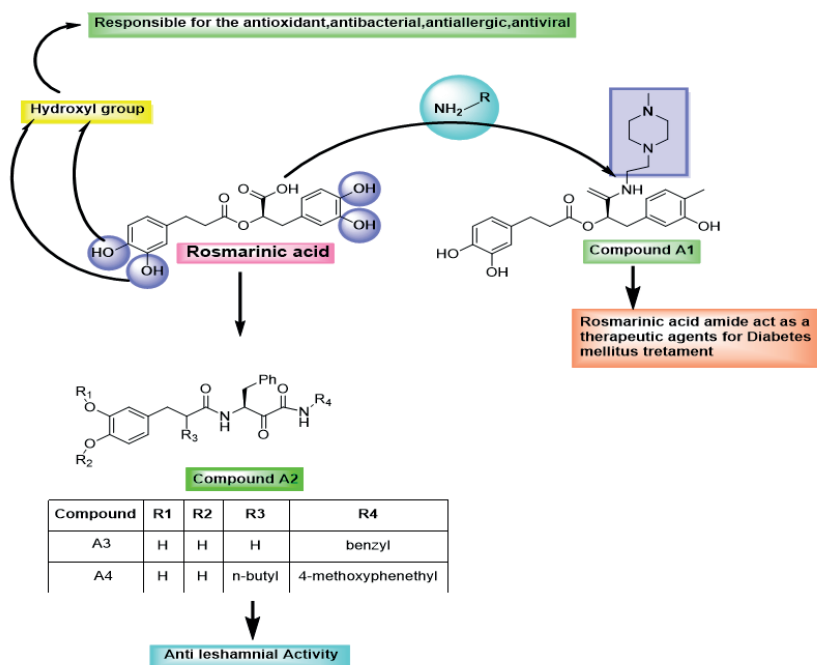


FIGURE 1 - Structure Activity Relationship of Rosmarinic Acid.

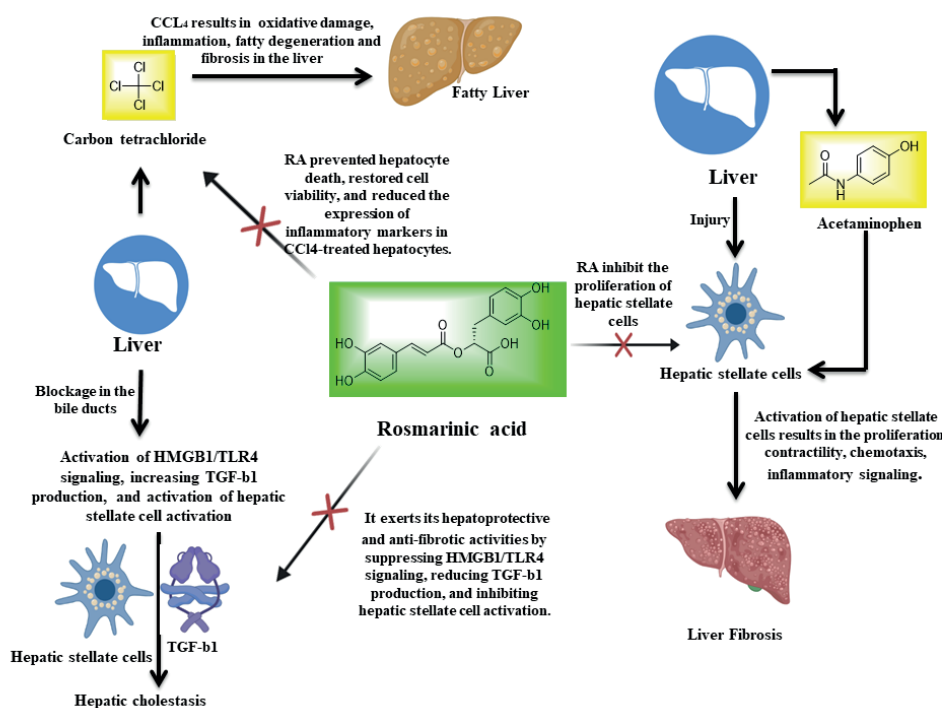


FIGURE 2 - Mode of action of Rosmarinic acid in the management of hepatoprotection.

liver damage indicators caused by CCl_4 in mice. RA also increased the expression of certain proteins then heightened the levels of hepatic glutathione, superoxide dismutase, and catalase. Histopathological analysis confirmed that RA reduced liver tissue damage caused by CCl_4 . In the *in vitro* investigation, RA prevented hepatocyte death, restored cell viability, and reduced the expression of inflammatory markers in CCl_4 -treated hepatocytes. RA pretreatment also reduced the rise of mitochondrial membrane potential in CCl_4 -treated hepatocytes (Lu *et al.*, 2022).

Further research on RA therapy revealed that this compound had hepatoprotective qualities by mitigating oxidative stress and acetaminophen-induced hepatic injury. Research has demonstrated that RA can shield liver cells from harm and maintain their ideal performance. Moreover, it inhibits the proliferation of hepatic stellate cells, which is implicated in liver fibrosis. Because it reduces the activation and proliferation of these cells, RA aids in the prevention of fibrosis. Furthermore, RA influences several signaling pathways that are critical for liver function, such as NF- κ B signalling, Nrf2 antioxidant system, and TGF- β 1 signalling. By modifying these pathways, it helps to regulate inflammation, fibrosis, and liver regeneration (Elufioye, Habtemariam, 2019).

Furthermore, RA hepatoprotective qualities against rats' extra hepatic cholestasis were investigated in another study. Adult male Sprague-Dawley rats were induced to develop extra hepatic cholestasis via common bile duct ligation (BDL). The divided rats were divided into five experimental groups, each consisting of eight rats. The sham group had an abdominal incision made without ligation, saline solution, and 20 mg/kg of RA. Two different dosages of RA (5 and 20 mg/kg) were administered to the BDL rats in addition to a saline solution. The treatments were administered orally, once day, for four weeks. After operation, all the rats were put to sleep and killed after three weeks in order to do more analysis. Molecular, biochemical, and histological analysis. The study evaluated the effects of RA on liver histology, serum biochemical markers, oxidative stress, inflammation, and fibrosis in the liver. Myeloperoxidase (MPO) activity measures, Western blot analysis, and

assessments of NF- κ B and AP-1 DNA binding activity were among the molecular analyses. The enzyme-linked immunosorbent assay (ELISA) was employed to measure the levels of TNF- α , IL-1 β , and IL-6 in serum specimens (Lin *et al.*, 2017).

Antioxidant activity

RA is a substance with a number of advantageous properties. It can stabilize cell membranes and has strong antioxidant properties. It guards against primary DNA damage, oxidative liposome damage, and chemically induced chromosomal breakage. In addition, RA has neuroprotective qualities, shields skin from UVB rays, and lessens the damage that doxorubicin does to DNA. It has actions that improve cognition and can inhibit activated T cells from producing cytokines. Furthermore, RA contains anti-angiogenic properties and appears to be promising in preventing Alzheimer's disease. All things considered, RA has a variety of effects and possible uses in medicine and nutrition (Khojasteh *et al.*, 2020). RA demonstrates its antioxidant capabilities by effectively managing the equilibrium between prooxidant and antioxidant activities. Within biological systems, it efficiently scavenges free radicals and shields against oxidative harm. Additionally, this compound showcases its antioxidant effects by inhibiting enzymatic browning in both vegetables and fruits. By achieving a delicate balance between generating and eliminating H_2O_2 and free radicals, RA ultimately exhibits a notable net antioxidant characteristic (Muñoz-Muñoz *et al.*, 2013).

Cancer

Cancer is a medical disorder characterized by the uncontrolled growth and spread of abnormal cells in the body. There are many different kinds of cancer, including anatomically related pan-gastrointestinal, pan-gynecological, pan-kidney, and pan-squamous tumours. Furthermore, stemness traits can be used to categorise malignancies, which may help guide future treatment development methods. Cancer is typically treated using a variety of therapeutic approaches, which vary depending on the kind and stage of the disease. Treatment options may encompass surgery, chemotherapy, radiation

therapy, targeted therapy, immunotherapy, hormone therapy, or stem cell transplantation. Treatments are being more specifically designed to target specific molecular abnormalities that tumours across various tissue types share, thanks to developments in molecular and genomic research. This individualized strategy seeks to lessen negative effects and enhance therapeutic results (Hoadley *et al.*, 2018). The anticancer activity of the is depicted in the Figure 3.

Herbal medicines are commonly utilized as an alternative therapy by cancer patients because they are believed to have little adverse effects on healthy cells. Herbal drugs inhibit important processes in cancer cells, such as nuclear factor- κ B signaling and protein tyrosine kinase pathways. Scientifically validated herbal treatments can aid in the prevention or relief of cancer symptoms and the adverse effects of treatment. Additional research on medicinal herbs, particularly in

India, demonstrates the potential of different chemical components for cancer treatment (Chandra, 2018).

RA and its derivatives have shown promising effectiveness in treating various forms of cancer, including colon, breast, liver, and stomach malignancies. The anticancer effects of RA impact cellular pathways, including as the death receptor and mitochondrial pathways, leading to the initiation of apoptosis, also referred to as programmed cell death. Suppression of cellular growth arrest and replication. The suppression of angiogenesis, which refers to the formation of new blood vessels, as well as the reduction of inflammation, modification of epigenetic mechanisms, including the regulation of gene expression (Ijaz *et al.*, 2023). Studies in the paper show that RA treatment greatly slows the growth of certain cancer cells, like MDA-MB-231 and A549 cells, without affecting the health of cells that are not

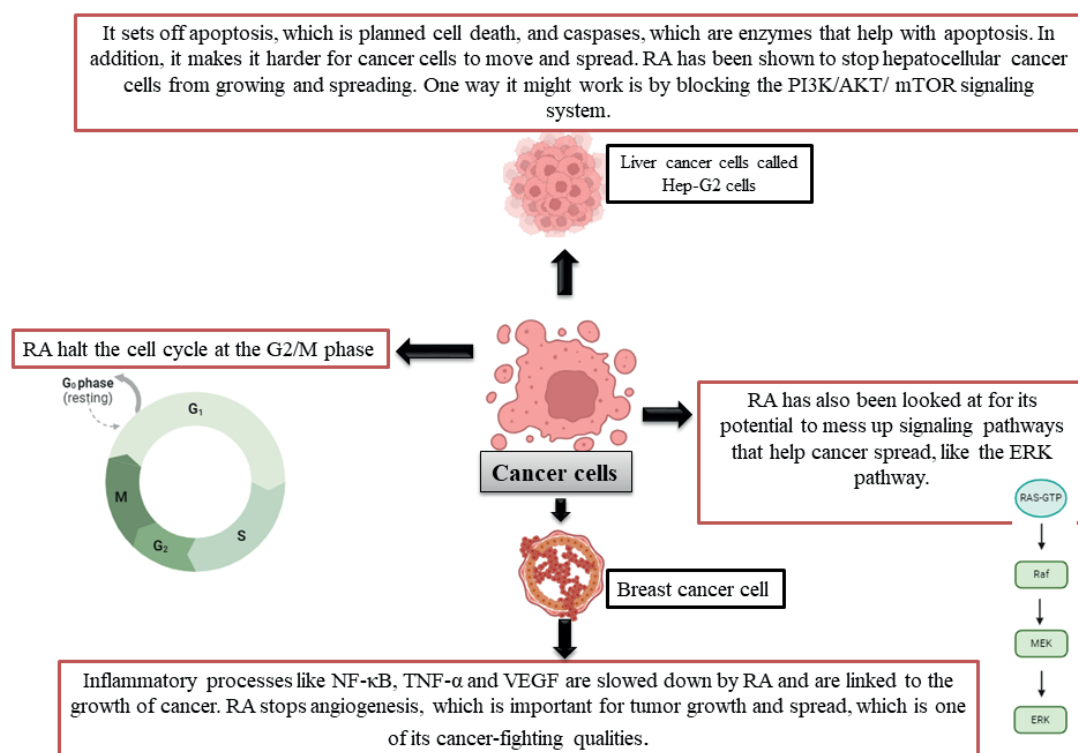


FIGURE 3 - Mechanism of action of anticancer activity of Rosmarinic acid.

cancer. RA has also been shown to cause cancer cells to die in a way that depends on the amount. This shows that RA might be able to target cancer cells only and leave healthy cells alone. RA has also been looked at for its potential to mess up signaling pathways that help cancer spread, like the ERK pathway. RA might be able to stop the growth of different types of cancer by targeting important factors like ERK and COX-2. The report also says that RA stops MARK4, a protein that helps cancer cells grow and avoid apoptosis. RA's ability to stop MARK4 from working opens up a new way to treat cancer (Anwar *et al.*, 2020). Furthermore, RA has shown potential as a cancer treatment, especially when it comes to oral cancer cells from people. It has been shown to stop cancer cells from multiplying, bring about death, halt the cell cycle at the G2/M phase, and make it harder for them to move. RA has been shown to cause oxidative stress in the endoplasmic reticulum, which makes its anticancer effects stronger. Researchers have found that RA could be used to make stronger medicines that are meant to treat mouth cancer. In the model used to treat the disease, RA was given to cells from people with mouth cancer. As part of this treatment model, the cancer cells' ability to move, their distribution by cell cycle phase, their ability to die, their ability to live, and their rate of proliferation were all looked at. To see what effect RA has on oral cancer cells, we used techniques such as CCK-8 tests, DAPI staining, flow cytometry, transwell migration experiments, and Western blotting to look at protein expression levels. The study's goal was to find out how RA works against human mouth cancer cells in order to find out if it might have anticancer properties (Luo *et al.*, 2020b). According to another study, RA may help fight cancer, especially liver cancer cells called Hep-G2 cells. It sets off apoptosis, which is planned cell death, and caspases, which are enzymes that help with apoptosis. In addition, it makes it harder for cancer cells to move and spread. In tests done on Hep-G2 cells, adding 28 μM of RA raises the number of cells that have died from 5.8% to 24.68%. This chemical also changes the

production of proteins related to apoptosis, like Bax and Bcl-2. It reduces the ability of liver cancer cells to survive by encouraging apoptotic cell death, raising Bax levels, and lowering Bcl-2 levels. Additionally, RA has been shown to stop hepatocellular cancer cells from growing and spreading. One way it might work is by blocking the PI3K/AKT/mTOR signaling system (Chen, Su, 2020). In an alternate investigation, the Solid Ehrlich carcinoma (SEC) mouse model was used to look like breast cancer. High malignancy and fast proliferation are two things that the SEC model has in common with inflammatory breast cancer in people. The SEC model was used to look at the chemo-preventive and therapeutic effects of RA on breast cancer, both by itself and when combined with paclitaxel (PTX). How the action works: RA helps kill cancer cells, lowers inflammation, and grows new blood vessels to treat breast cancer. By increasing the amounts of the apoptotic markers P53 and Caspase-3 in addition decreasing the Bcl2/Bax ratio, RA helps cells die in a planned way. Inflammatory processes like NF- κ B, TNF- α and VEGF are slowed down by RA and are linked to the growth of cancer. RA stops angiogenesis, which is important for tumor growth and spread, which is one of its cancer-fighting qualities (Mahmoud *et al.*, 2021). Also, RA has been shown to fight cancer by targeting several pathways in stomach carcinoma cells. It stops cells from taking in glucose, making lactate, and expressing the transcription factor HIF-1 α . RA also stops the action of cytokines and microRNAs that cause inflammation, which could stop the Warburg effect in stomach cancer cells. The IL-6/STAT3 pathway and miR-155 have been named as possible RA targets for controlling the Warburg effect. In vitro experiments were done with MKN45 human gastric cancer cells, and in vivo tests were done by implanting the cells under the skin of BALB/c-nu mice. In vitro, different amounts of RA were added to cells to test how well they took in glucose and made lactate. To study lactate production and glucose uptake in living things, RA was injected into the celiac veins of mice that had tumors. The research showed that RA

stops stomach cancer cells from taking in glucose, making lactate, and expressing HIF-1 α . It also lowers pro inflammatory cytokines and microRNAs that are linked to inflammation, which suggests that it might be useful for treating the Warburg effect in stomach cancer. Overall, these results show that RA might be able to stop cancer from spreading by changing important processes involved in how cancer cells work and how inflammation is caused (Han *et al.*, 2015).

Inflammation

Inflammation is a normal way for the body to fight off germs that are trying to get inside. If it's not handled, it can lead to long-term illnesses. It has redness, growth, pain, heat, and the ability to do things. A further choice is herbal medicine, which has few to no side effects. People with a lot of different kinds of illnesses, like pain and inflammation, use herbal medicines. People are urged to use herbal therapy because it is easy to get, cheap, and has few side effects (Arome *et al.*, 2014). According to a study, RA compounds derived from *Rosmarinus officinalis* have potential for treating inflammation and pain. More specifically, the acetyl ester form of RA exhibits significant anti-inflammatory properties, indicating that it is a useful anti-inflammatory drug. Additionally, the research reveals that the acetyl derivative of RA acts as an analgesic via a peripherally mediated mechanism, providing insight into how it works to reduce inflammation. The Carrageenin-induced paw edema paradigm in rats is used in the study to assess inflammation. This entails injecting a saline solution into the left paw and carrageenin into the right. The inflammatory response is subsequently evaluated by measuring the foot volume using a Plethysmometer model 7140 every hour for five hours. The third hour is the time when edema peaks, making it possible to assess the anti-inflammatory properties of the substances. Overall, the study offers insightful information about the prospective applications of *Rosmarinus officinalis* derivatives

called RA in pain and inflammatory management. It clarifies the acetyl ester derivative's mode of action in treating inflammation and emphasizes its potent anti-inflammatory properties (Lucarini *et al.*, 2013).

In an alternate study, it was discovered that RA had anti-inflammatory properties via covalently attaching to activated C3b and inhibiting complement C3-convertase. Moreover, it opposes the activation of cyclooxygenase-2 (COX-2), a gene implicated in inflammation, which is dependent on activator protein-1. In addition, RA possesses the capacity to decrease pro-inflammatory cytokines such as IL-6 and TNF-alpha, indicating its potential application in the treatment of inflammatory disorders (Colica *et al.*, 2018). The study examined the anti-inflammatory effects of *Rosmarinus officinalis* along with its active compound, RA, in a rat model of sciatic nerve neuropathic pain induced by chronic constriction damage. It was shown that RA significantly decreased the spinal cord's nitric oxide (NO) production as well as the levels of inflammatory markers like MMP2 and IL-1b. These results suggested that RA may have neuroprotective properties and a reduction in inflammatory reactions. The study demonstrated the benefits of RA and *Rosmarinus officinalis* for treating neuropathic pain (Rahbardar *et al.*, 2017). Moreover, studies have demonstrated the anti-inflammatory qualities of RA, suggesting that it may find use in the treatment of inflammatory diseases and pain. RA demonstrated anti-inflammatory activity during both the chronic and second phases of inflammation in mouse research, and it also efficiently reduced the formation of granulomas. It was proposed that one of RA's anti-inflammatory properties is its inhibition of prostaglandin formation. Additionally, RA has shown both peripheral and central antinociceptive actions, suggesting that it may be used as a painkiller. Moreover, it has been discovered that RA reduces pain and inflammation in mice, demonstrating anti-inflammatory qualities in models of both acute and chronic inflammation. These results demonstrate how RA can treat inflammatory disorders and pain (Boonyarikpunchai, Sukrong, Towiwat, 2014).

Anti-diabetic

Diabetes mellitus (DM) is a chronic condition marked by elevated blood sugar levels brought on by either inadequate insulin synthesis or inefficient insulin utilization by the organism. Diabetes causes the body to have trouble controlling blood sugar levels, which can result in a number of problems. The pancreas releases the hormone insulin, which facilitates the uptake of glucose by cells for energy production. This mechanism is compromised in diabetes, leading to increased blood sugar levels. Certain herbal remedies, such as aloes, black seeds, neem, myrrh, helteet, harmel, and fenugreek, have been used in alternative and traditional medicine to treat diabetes. These herbs may help with blood sugar regulation, enhance insulin sensitivity, and have antioxidant benefits. It is advisable to use caution, though, as many herbs can be harmful at excessive quantities or interact negatively with other treatments. It's imperative to speak with medical professionals before beginning any natural diabetic remedies (Alsanad *et al.*, 2018). In accordance in a study, RA works in several ways, such as by reducing insulin resistance, promoting the uptake of glucose by muscle cells, and inhibiting the enzymes that break down sugar. This is accomplished by blocking the enzymes α -amylase and α -glucosidase, increasing the uptake of glucose in muscle cells through the AMPK pathway, and adjusting the sodium-glucose co transport protein. Therefore, in people with diabetes, RA may be able to successfully control blood glucose levels, enhance insulin sensitivity, and shield against oxidative stress and pancreatic β -cell malfunction (Ngo, Lau, Chua, 2018).

Likewise, the paper investigates how diabetes affects the activity of acetylcholinesterase (AChE) in diabetic rats and the possible medical uses of RA to prevent cognitive decline. It draws attention to the relationship that exists between oxidative stress, AChE activity, and diabetic cognitive impairment. The results of the study show that RA therapy can stop the rise in lipid peroxidation and AChE activity in diabetic rats, suggesting that RA is a promising natural substance for

neuroprotection in diabetic settings. Over the course of three weeks, a dose of 10 mg/kg body weight of RA has been given to the rats with diabetes (Mushtaq *et al.*, 2014).

In a mouse model of type 2 diabetes mellitus (T2DM), the effects of RA on neurogenesis and neuroprotection were investigated. The administration of RA to diabetic mice was reported to improve memory impairments and increase cognitive function. Following RA treatment, gene expression analysis revealed heightened concentrations of the neuronal markers Ki67, DCX, and NeuN, indicating a neuroprotective action against T2DM-related neuronal damage. According to the results, RA may prove to be a useful medicinal substance for treating diabetes-related hyperglycemia and reducing dementia. Furthermore, it was discovered that RA treatment lowered the fasting blood glucose levels in animals with diabetes (Ali, Zahid, 2020).

Furthermore, the examination of different investigation into the impact of the polyphenol RA on the metabolism of carbohydrates in rats with experimental diabetes mellitus caused by a high-fat diet. The use of RA produced a number of advantageous effects. First of all, it resulted in lower blood glucose, urea, and uric acid, creatinine, and glycosylated hemoglobin levels. Furthermore, administering RA raised plasma insulin levels. Additionally, the study discovered that RA dramatically lowered AST and HOMA-IR readings, suggesting that it may be useful in the management of insulin resistance and hyperglycemia. Overall, the results point to RA's potential therapeutic function in the management of diabetes and its ability to improve metabolic problems linked to the disease (Jayanthi, Subramanian, 2014). The study investigated the impact of RA on diabetic rats induced by streptozotocin and fed a diet high in fat. There were multiple benefits from the administration of RA. First, it resulted in higher levels of plasma insulin and lower blood glucose. The injection of RA also increased the amount of glycogen in the liver and muscular tissues. The study also discovered that RA has effects similar to metformin by restoring abnormal amounts of tissue and plasma glycoprotein

components. All things considered, the research points to RA as a possible diabetes management medication (Ramalingam, Karuppiah, Thirupathi, 2020).

In addition, RA was investigated for possible protective benefits against oxidative stress in the kidney and liver of rats that had been given streptozotocin-induced diabetes. In diabetic rats, RA showed promise in lowering oxidative stress indicators, preserving enzyme activity, and preventing lipid peroxidation. These results imply that RA may be a viable option for the treatment and prevention of pathological problems in diabetic models. Rats with diabetes produced by streptozotocin served as the study's model. By stopping lipid peroxidation, preserving enzyme activity, and lowering oxidative stress indicators, the results showed that RA had protective effects against oxidative stress in the liver and kidney of diabetic rats. The prevention of lipid peroxidation in the liver and kidney of diabetic rats, the maintenance of enzyme activity levels (such as SOD and CAT) in diabetic rats, the decrease of oxidative stress markers, and the restoration of antioxidant levels in diabetic rats were among the outcomes of the anti-diabetic study employing RA (Mushtaq *et al.*, 2015).

Moreover, in diabetic rats, RA regulates PEPCK and GLUT4 expression, which in turn affects hyperglycemia and insulin sensitivity. The article highlights the possible therapeutic effects of RA in enhancing insulin resistance and glucose homeostasis while delving into its chemical structure and biological production. After administering RA therapy, the researchers observed a significant decrease in insulin and plasma glucose levels in rat models of diabetes type 2-like and type 1-like generated by streptozotocin and high-fat diet, respectively. RA may be utilized to treat diabetes-related problems, according to these studies. Improved glucose homeostasis and insulin sensitivity in diabetes circumstances are the results of RA's modulation of the expression of important enzymes involved in this metabolism. RA's capacity to improve insulin resistance and hyperglycemia is also aided by its antioxidant qualities (Runtuwene *et al.*, 2016).

Besides, the rats in the study were given oral RA

after being given streptozocin to induce diabetes in them. Ten weeks following the treatment of streptozocin to diabetic rats, the effects of RA on vascular function and structure were assessed using electron microscopy, real-time PCR analysis, and functional tests. The findings demonstrated that RA protected the ultrastructure and function of the aorta endothelium against damage brought on by diabetes, indicating the possibility of therapeutic benefits for the management of vascular problems associated with diabetes. Moreover, the research demonstrated that diabetic rats showed decreased endothelium-dependent relaxation in the aorta along with increased expression of tumor necrosis factor, preproendothelin-1, endothelin converting enzyme-1, and interleukin-1. Using electron microscopy, structural alterations in the endothelium were seen, suggesting diabetes-related aortic dysfunction. On the other hand, RA treatment stopped these diabetes-related aortic diseases. This defense system seemed to be aided by RA anti-inflammatory and antioxidant properties (Sotnikova *et al.*, 2013).

Rats were used in the study, and injections of streptozotocin were used to cause diabetes in the diabetic and control groups. For eight weeks, RA was given orally to the diabetic groups at several doses to evaluate its effects on allodynia and hyperalgesia. The findings demonstrated that RA therapy dramatically lowered the diabetic rats' pain responses in the formalin test. When administered to rats with diabetic neuropathy, RA had anti-hyperalgesic and anti-allodynic effects. These results imply that RA may be used to treat excruciating diabetic neuropathy.

The study also showed that, in comparison to controls, diabetic rats without treatment showed appreciable hyperalgesia at both stages of the formalin test. This suggests that diabetic rats have aberrant pain processing mechanisms. Based on its impact on pain responses, RA may have an impact on the central and peripheral systems that underlie pain. Overall, the research shows that RA may be able to heal excruciating diabetic neuropathy in experimental rat models and lessen pain responses. In order to assess

RA's effectiveness in clinical trials and investigate the processes underlying its benefits, more study is required (Hasanein, Mohammad Zaheri, 2014).

The treatment Approach used in the study involved first giving rats streptozocin to induce diabetes and then giving the rodents oral RA. After a period of ten weeks following the administration of streptozocin, the effects of RA on vascular function and structure in diabetic rats were evaluated using functional investigations, electron microscopy, and real-time PCR analysis. According to the findings, RA prevented diabetes-induced damage from affecting the function and ultrastructure of the aortic endothelium, pointing to possible therapeutic advantages for the treatment of vascular problems associated with diabetes. Overexpression of interleukin-1, tumor necrosis factor, preproendothelin-1, and endothelin converting enzyme-1 was observed in the aorta of diabetic rats along with reduced endothelium-dependent relaxation. Administration of RA reduced the structural changes in the endothelium that indicated diabetes-induced aortic dysfunction. The mechanism of this protection seemed to involve the antioxidant and anti-inflammatory properties of RA (Govindaraj, Sorimuthu Pillai, 2015).

Seizure

Seizure clusters are acute episodes of deteriorating seizure control that involve a series of grouped seizures with short periods between them. Clinical definitions suggest that seizures occurring within 8 hours may originate from the same source. Seizure clusters can include different types of seizures, such as focal, generalized, tonic, atypical absence, or myoclonic seizures, which occur within specific time frames and are distinguishable from the patient's usual pattern. Statistical definitions describe seizure clustering as an increase in seizure occurrence compared to the patient's average frequency, with a threefold or fourfold increase within a 3-day period considered significant. Antiepileptic medications (AEDs) are commonly used as the first line of treatment for seizures. Rescue drugs

like benzodiazepines, like diazepam or midazolam, are frequently used to end prolonged or recurrent seizures in seizure clusters. For individuals with drug-resistant seizures, non-pharmacological therapies such as the ketogenic diet, vagus nerve stimulation, or epilepsy surgery may be taken into consideration (Jafarpour *et al.*, 2019).

Using a kindling paradigm in mice, the study examines the effects of RA and caffeic acid (CA) on seizures brought on by pentylenetetrazole (PTZ). By lowering free radicals, protecting against oxidative and DNA damage, and lowering the DNA damage index in the comet assay, the study shows that both RA and CA have neuroprotective properties. In the kindling epilepsy model, RA and CA show possible neuroprotective benefits, but they are not antiepileptogenic. In particular, 1 mg/kg of RA reduces free radicals and lessens the increase in reactive oxygen species (ROS) brought on by many PTZ injections. But in the PTZ-kindling scenario, RA had no effect on the behavioral assessment. Furthermore, in the comet experiment, RA at 4 mg/kg and CA at 4 mg/kg both reduce the DNA damage index. All things considered, the results show that RA has neuroprotective and antioxidant qualities when it comes to PTZ-induced seizures (Coelho *et al.* 2015).

EXTRACTION

Phytochemicals and bioactive substances that are extracted from medicinal plants or herbs are referred to as herbal medicine extraction. For the purpose of obtaining the necessary components for additional examination, characterization, and possible medical application, this extraction process includes procedures like maceration and percolation. Extraction of the active components from plant materials with optimal potency and therapeutic characteristics is the aim of herbal medication extraction. Extractions must be done quickly and carefully (Fonmboh *et al.*, 2020).

To maximize the extraction efficiency and concentration of RA in the final extract, various

extraction procedures were used, such as maceration and the use of different solvent combinations. The extraction sample was subjected to chromatographic techniques, including High-Performance Liquid Chromatography (HPLC) using specialized columns and mobile phases, to facilitate the separation and quantification of RA. To obtain the maximum concentration of RA in the crude extracts, the extraction of RA from plants was optimized under particular circumstances. These parameters included a temperature of 40°C and extraction durations of 2, 4, and 8 hours with different solvents like methanol, water, and a combination of water and methanol. The range of 5.40 to 7.99 mg/g of RA in the crude extracts emphasizes the significance of extraction parameters in optimizing RA output (Amoah *et al.*, 2016).

The study aimed to assess the effectiveness of three distinct extraction methods (HAE, UAE, and MAE) for RA extraction from *Melissa officinalis* leaves. The results showed that ultrasound-assisted extraction (UAE) was the most successful of the three techniques. The ideal parameters for ultrasonic extraction were found by the researchers to be 33 minutes, 372 W of power, and 40% ethanol as the solvent. Under these circumstances, the production of RA per gram of dried plant material was 86.3±4.1 mg. Using heat-assisted, microwave-assisted, and ultrasound-assisted extraction methods, the study sought to extract RA from *Melissa officinalis* L. leaves. Response surface approach, which takes into account factors including duration, temperature, the ratio of ethanol to water for heat and microwave extraction, and ultrasonic power for ultrasound extraction, was used to optimize the extraction process. The most successful procedure among those examined was ultrasonic extraction. Ultrasonic extraction produced 86.3 ± 4.1 mg of RA per gram of dried plant material under ideal circumstances as established by the optimization procedure. By contrast, the yield of extraction with microwave assistance was only 49.4 ± 2.3 mg/g, but the yield of extraction using heat assistance was 59.4 ± 2.2 mg/g. The extraction yield of the recovered residue and the amount of RA were measured using HPLC-DAD to quantify the extraction procedure (Caleja *et al.*, 2017).

One method used to extract RA from peppermint (*M. piperita*) was reflux extraction. Reflux extraction is a solid-liquid extraction technique that, at a given temperature, results in steady solvent evaporation and condensation over a predefined period of time without solvent loss. Due to its efficiency, ease of use, and low cost, this method is widely used in the herbal industry to maintain the antioxidant properties of RA. The exact extraction method used to extract the RA from peppermint needed reflux extraction with 90% methanol at 70°C for 45 minutes. This approach ensures no solvent loss, consistent results, and a quick extraction process (Aldoghachi, Noor Al-Mousawi, Shari, 2021).

To extract RA from rosemary leaves, various conditions and extraction techniques were tried. In addition to conventional techniques like maceration and percolation, they included extraction using ultrasonic assistance. The extraction methods that were employed were maceration and ultrasound-assisted extraction using either 70% ethanol, 90% ethanol, or water at pH 9. To extract the maximum amounts of ursolic acid, RA, and oleanolic acid from rosemary leaves, these techniques were used. The RA extraction settings included a material/solvent ratio of 1:15, an extraction temperature of 85°C, and extraction durations of 90, 180, and 300 minutes. The surfactant Tween 20 was present during these extractions, which were carried out at various medium pH values 7, 9, and 11 (Bernatoniene *et al.*, 2016).

Plants were treated with RA by use of a technique known as HPLC. A C18 column, a kind of stationary phase used in HPLC, produced the best separation results. To ensure the purity of RA, it was specifically created to be separated from other interfering substances using the HPLC method. A C18 column kept at 30°C, a Waters HPLC system with a diode array detector, and a mobile phase gradient of 0.085% O-phosphoric acid in water, methanol, and 2-propanol were all used in this procedure. The detection wavelength was 330 nm, the mobile phase flow rate was adjusted to 1.0 ml min⁻¹. A sample injection volume of 20 µl was used to determine the presence of RA by comparing its retention time and UV spectra to a reference standard (Shekarchi *et al.*, 2012).

ANALYTICAL STUDIES

This was found that the RA analysis method developed by GC-MS was linear, accurate, and exact. Complete separation, clear identification, and quantitative measurement of substances even at trace levels are only a few of its benefits. The technique was especially verified for trans-RA analysis, and it was shown to be useful in figuring out how much trans-RA was present in different Lamiaceae plant species. The presence of RA was determined using a variety of techniques, such as GC-MS, HPLC, and other chromatographic methods. The stability and degradation of trans-RA under various stress conditions, including exposure to light, temperature, and solvents, were the main subjects of the study. The findings demonstrated that trans-RA easily isomerized into its cis-form under specific circumstances, highlighting how crucial it is to comprehend RA's stability for both detection and analysis (Razboršek, 2011).

The analytical technique used to detect RA was LC-MS/MS (Liquid Chromatography with Tandem Mass Spectrometry). The conditions of the LC-MS/MS analysis ensured precise identification and quantification of RA in the sample. LC-MS/MS is known for its high sensitivity and specificity, making it a robust method for detecting RA in plant extracts (Ertas *et al.*, 2015).

An HPLC examination of the rosemary extract showed that 8% of the sample contained RA and about 6% carnosic acid. An Agilent 1100 series HPLC equipment with particular column and mobile phase settings was used to perform the analysis. Additionally, the extract from rosemary showed a total phenolic concentration of 162 mg GAE/g. Using an Agilent 1100 series HPLC apparatus with an autosampler, the rosemary extract was subjected to an HPLC analysis. A Hypersil ODS C18 type column with particular guard cartridge dimensions was utilized. With a mobile phase of 0.1% aqueous ortho-phosphoric acid and acetonitrile (40:60) at a flow rate of 1 ml/min and a column temperature of 27°C, the separation was carried out isocratically (Erkan, Ayranci, Ayranci, 2008).

Moreover, RA can be quantified using the HPLC method, which has a number of important procedures and parameters. A gradient elution with a two-solvent system is used in the analysis. The solvents consist of different ratios of formic acid, water, and methanol. A 1 ml/min flow rate and a 10 µl injection volume are used, and detection takes place at 280 nm, the wavelength at which phenolic chemicals such as RA have the highest absorption. The plant components' dried aerial portions are pulverized and extracted using petroleum ether, then at 40 °C, methanol/water (70:30; v) is added. Reasonable results were achieved when the procedure was used to the extracts of specific Lamiaceae species, namely *Salvia candidissima* Vahl. subsp. *candissima*, *S. sclarea* L., *S. verticillata* L. subsp. *verticillata*, and *Rosmarinus officinalis* L. When (3.67×10^{-5} M) RA was used, the repeatability findings as RSD% were 1.66, 1.17, and 1.26 for intra-day and 1.38 for inter-day. Within a broad concentration range of 1.13×10^{-5} – 5.65×10^{-4} M, a limit of linearity (LOL) was noted. Very excellent correlation was found when linearity parameters were also examined in the 5.95×10^{-6} – 7.14×10^{-5} M RA range. For inter-day, the LOD and LOQ were 1.60×10^{-6} M (Öztürk *et al.*, 2011).

Different plant extracts were found to contain varied quantities of RA. The methanol extracts of *Mentha longifolia* (59.1 mg analyte/g extract) and *Melissa officinalis* (88.3 mg analyte/g extract) had the highest amount. Because of its potential as an antioxidant, the study stressed the significance of natural sources for RA production. Accurate phytochemical and RA measurement in the examined plant species was made possible by the verified LC-MS/MS approach. The study emphasized the importance of natural sources for the synthesis of RA as well as the accuracy of the LC-MS/MS method's detection and quantification (Yilmaz, 2020).

Using a liquid chromatographic technique, RA in plant extracts was identified. This system was operated by Millennium 32 software and included a Waters Alliance 2695 separations module and a Waters 2996 variable-wavelength diode array detector. A LiChroCART RP-18

TABLE I - Comparative analytical results for rosmarinic acid and their outcomes (*continue*)

Compound	Method	Condition & Outcomes	References
Rosmarinic acid	ESI-MS 1H-NMR HPTLC	RA exhibits UV absorbance at 328 nm and ESI-MS peaks with a base peak at $m/z=161$. The HPTLC chromatogram showed a distinct peak representing 37.60% with an R_f value of 0.30.	(Tawfeeq et al., 2018)
	Extraction•	The LCMS confirmed the methanol extracts of <i>Mentha longifolia</i> and <i>Melissa officinalis</i> , with 59.1 mg and 88.3 mg analyte/g, respectively, had the highest amount.	(Yilmaz, 2020)
	LiChroCART RP-18 reversed-phase column•	The study used mobile phase: 1% glacial acetic acid (solvent A), 6% glacial acetic acid (solvent B), and 5% glacial acetic acid (solvent C) in water/acetonitrile (65:30 v/v). glacial acetic acid solutions for separation and identified RA in plant extracts by comparing retention periods and UV spectra with reference chemicals.	(Pérez-Tortosa et al., 2012)
	• HPLC	The rosemary extract underwent HPLC analysis using a mobile phase of 0.1% aqueous ortho-phosphoric acid and acetonitrile at a flow rate of 1 ml/min and column temperature of 27°C.	(Erkan, Ayranci, Ayranci, 2008)
	LC-MS/MS•	The presence of phytoconstituents was concealed in this setting by a variety of plants and their distinct extracts. The rosmarinic acid (88.3 mg analyte/g extract) was detected in the <i>M. officinalis</i> methanol extract.	(Yilmaz, 2020)
	HPLC & Spectrophotometry	The chromatograms demonstrate that the phenolic compounds were effectively isolated from one another using the HPLC method. The process of identification involved comparing the UV spectra and retention periods of reference solutions. Using calibration curves, the quantities of phenolic compounds were determined and expressed as $\mu\text{g mL}^{-1}$. $146.3 \pm 0.8 \mu\text{g mL}^{-1}$ – $1372 \pm 14 \mu\text{g mL}^{-1}$ is the rosmarinic acid. The tinctures' chromatographic profiles indicate that some rosmarinic acid breaks down into caffeic acid.	(Sik et al., 2021).

TABLE I - Comparative analytical results for rosmarinic acid and their outcomes (*continuation*)

Compound	Method	Condition & Outcomes	References
Rosmarinic acid	HPTLC	Myricetin, ellagic acid, p-coumaric acid, and rosmarinic acid were all present in the Manuka honey samples. Based on color and R _f values, the extract's rosmarinic acid, ellagic acid, p-coumaric acid, and myricetin were qualitatively identified. This suggests that the extraction technique can have a substantial impact on the kinds of chemicals that are found during analysis.	(Lawag et al., 2022)
	HPLC•	The procedure was applied to extracts of Lamiaceae species, achieving reasonable results. The repeatability and linearity were good within a broad concentration range, with excellent correlation in the 5.95×10 ⁻⁶ –7.14×10 ⁻⁵ M RA range, and inter-day values of 1.60×10 ⁻⁶ M.	(Öztürk et al., 2011)
	HPLC-ELSD	To measure rosmarinic acid in rosemary using a Zorbax SB-C18 column. The method has good repeatability, linearity, high sensitivity, and satisfactory accuracy, with limits of detection ranging from 1.3 to 8.6 µg/mL and recovery between 95.5% and 100.8%.	(Li et al., 2019)

reversed-phase column was used for the separation, and a gradient elution technique employing various mobile phases was employed. The efficiency of the HPLC approach in identifying and quantifying RA in the plant extracts was demonstrated by the identification of RA based on its retention time and UV spectra compared to reference standards. The analysis's specifics include the use of a liquid chromatograph with a Waters Alliance 2695 separations module and a Waters 2996 variable-wavelength diode array detector controlled by Millennium 32 software. Merck's reverse-phase column LiChroCART RP-18 (250 mm x 4 mm, 5 µm particle size). The following solutions are employed as the mobile phase: 1% glacial acetic acid (solvent A), 6% glacial acetic acid (solvent B), and 5% glacial acetic acid (solvent C) in water/acetonitrile (65:30 v/v).

A particular gradient elution technique applied to the separation. The identification of RA in the plant extracts was accomplished by comparing retention periods and UV spectra with reference chemicals (Pérez-Tortosa *et al.*, 2012).

The chemical formula for RA, C₁₈H₁₆O₈, was extracted as an off-white powder with a 0.5 R_f value. Through chemical analysis, the structure of RA was verified, and the molecular formula was further corroborated by data from ESI-MS, IR, and ¹H-NMR. Characteristics of RA include UV absorbance at 328 nm and ESI-MS peaks with a base peak at m/z=161. It is well-known for its biological activities, which include antibacterial, anti-inflammatory, and anti-hyperglycemic properties. The RA in the rosemary extract was detected using TLC, HPLC,

and High-Performance thin layer chromatography (HPTLC) studies. With an R_f value of 0.30, the HPTLC chromatogram displayed a distinct peak that represented 37.60% of the total extract components. Based on the reference R_f value of standard RA, this peak was determined to be RA (Tawfeeq *et al.*, 2018).

In another study, five essential phytochemicals found in rosemary are simultaneously determined using HPLC with evaporative light scattering detection (HPLC-ELSD): rosmarinic acid, carnosol, carnosic acid, oleanolic acid, and ursolic acid. These chemicals have drastically different polarity, therefore baseline separation between them was achieved by optimizing the technique. HPLC-ELSD was chosen as it can effectively assess non-volatile chemicals with weak chromophores, unlike typical HPLC-UV procedures. High sensitivity, linearity, reproducibility, and accuracy were established by the validated method, which made it an effective instrument for quality control and the analysis of rosemary and its extracts for useful components. To measure rosmarinic acid in rosemary simultaneously, HPLC-ELSD was created. The Zorbax SB-C18 column (4.6 mm $\times$ 250 mm, 5 μ m) was used for the gradient elution mode chromatographic separation, and the mobile phases used were methanol and 0.6% acetic acid. The ELSD drift tube temperature was 70 °C, and the nebulizer nitrogen gas pressure was 40 Psi. The developed method has good repeatability (with intra- and inter-day CV less than 3.1% for all analytes), acceptable linearity over the tested concentrations (with correlation coefficients from 0.991 to 0.999), high sensitivity (with limits of detection from 1.3 to 8.6 μ g/mL), and satisfactory accuracy (with recovery between 95.5% and 100.8%) (Li *et al.*, 2019).

Furthermore, the *Melissa officinalis* methanol extract contains a significant amount of rosmarinic acid, which has antioxidant properties and can be used as a preservative in food and pharmaceutical industries. The phytochemicals in the extracts were quantified using LC-MS/MS, revealing richness in fumaric acid, rosmarinic acid, quinic acid, protocatechuic aldehyde, and cosmosiin. The study

also assessed the antioxidant activity of *Astragalus membranaceus* L. extract. Certain species are abundant in acetone and water extracts, containing fumaric acid, rosmarinic acid, quinic acid, protocatechuic aldehyde, and cosmosiin. These substances can be obtained naturally from water extracts of *Alcea hohenackeri* and *Carduus pycnocephalus*, methanol extracts of *M. officinalis*, *Centaurea lycopifolia*, and *A. schizopterus* (Yilmaz, 2020).

Study on the Lamiaceae species were used in hydroethanolic tinctures, with macerations taking two hours. HPLC was used to quantify rosmarinic acid and other phenolic components. On the same day, a rosmarinic acid standard solution (500 μ g mL⁻¹) and three separate injections of tinctures were used to evaluate the repeatability of the assessment. RSD% of retention periods and extracted phenolic component quantities were used to express the precision measurements. ANOVA was used to evaluate the data in accordance with the statistical analysis to identify significant differences at a 95% confidence level ($p < 0.05$). Whereas the HPLC analysis the chromatograms demonstrate that the phenolic compounds were able to segregate from one another. The process of identification involved comparing the UV spectra and retention periods of reference solutions. Using calibration curves, the quantities of phenolic compounds were determined and expressed as μ g mL⁻¹. Rosmarinic acid (146.3 $\pm$ 0.8 μ g mL⁻¹–137.2 $\pm$ 14 μ g mL⁻¹) and caffeic acid (11.80 $\pm$ 0.33 μ g mL⁻¹–7.54 $\pm$ 0.16 μ g mL⁻¹) were the most prevalent phenolic acids (Sik *et al.*, 2021).

Another, HPTLC is used to examine RA, which is known to be present in honey. (R_f): 0.692 (plus extra values of 0.630 in certain circumstances). Acidified Manuka honey was extracted using Amberlite XAD-2, leading to qualitative detection of RA based on color and R_f values. The technology makes use of UV irradiation and fluorescence quenching to enable sensitive detection of phenolic chemicals, including RA. The kinds of chemicals found are greatly influenced by the extraction technique, underscoring the significance of methodology in phenolic analysis (Lawag *et al.*, 2022).

NANO FORMULATION

Nanotechnology is used in nano formulations to produce medicine delivery systems with improved characteristics. These formulations may enhance the target specificity, bioavailability, stability, and solubility of the drug. With benefits like enhanced pharmacokinetics, tailored drug distribution, and less toxicity, nano formulations show promise for a range of medical uses. For topical and oral administration, RA nano formulations have been produced, such as solid lipid and chitosan nanoparticles. Excellent thermal stability, prolonged release characteristics, and effective antioxidant actions without aggregation are displayed by these compositions. They have demonstrated biocompatibility, safety, and prospective uses in wound healing, ocular medication delivery systems, and diabetic retinopathy (Dewanjee *et al.*, 2020).

The study discusses the encapsulation of sage, savory, and RA into chitosan nanoparticles for drug delivery purposes. The study evaluated the size, surface charge, and morphology of the nanoparticles, as well as their encapsulation efficiency and antioxidant activity. The research highlights the potential of using natural extracts in nano formulations for enhanced drug delivery and antioxidant performance. RA, known for its antioxidant properties, has been studied for various applications, including drug delivery systems. The article mentions the release of RA from chitosan nanoparticles and its association efficiency with the particles. RA has shown potential in inhibiting lipid peroxidation and has been encapsulated into nanoparticles for controlled drug delivery. The formulation of RA into chitosan nanoparticles offers potential pharmacological benefits due to its antioxidant properties. When encapsulated in nanoparticles, RA can be used for controlled drug delivery, enhancing its bioavailability and therapeutic effects (Da Silva *et al.*, 2015).

They encapsulated RA in solid lipid nanoparticles to facilitate oral administration. The experiments conducted *in vitro* and *in vivo* demonstrated the safety of the nanoparticles that were loaded with moderate quantities

of RA. The study indicates that because of these solid nanoparticles' good safety profile and bioavailability, they may find usage in nutraceutical applications. Solid lipid nanoparticles (SLN) were used to encapsulate RA, a naturally occurring polyphenol carboxylic acid, for oral administration. Witepsol and Carnauba waxes were used in the SLN formulation as matrix to contain RA. With no cytotoxicity or genotoxicity seen *in vitro* and a favourable safety profile in rats given oral treatment, the study demonstrated the safety of the RA-loaded SLN and suggested that it may find use in nutraceutical applications (Madureira *et al.*, 2016).

The study compared the effectiveness of Ethosomes (ETHs) and Liposomes (LPs) loaded with RA for transdermal delivery. Results indicated that ETHs exhibited higher transdermal flux and enzyme inhibition compared to LPs, suggesting their superiority in delivering RA through the skin. The study evaluated the potential of ETHs as a promising delivery system for RA in skincare applications. The mechanism of action for both ETHs and LPs involves enhancing penetration by reducing intercellular lipids, increasing skin lipid fluidity, and facilitating the transmission of active substances to deeper skin layers. Due to the presence of ethanol, ETHs demonstrated higher transdermal flux, making them more effective in delivering substances through the skin. The article emphasized the anti-aging benefits of RA, which acts as a potent antioxidant by scavenging free radicals and preventing oxidative damage caused by UV rays and other external factors. RA exhibited higher antioxidant activity than vitamin E, making it a valuable ingredient in skincare products for reducing signs of aging and promoting youthful-looking skin without the associated toxicity of synthetic antioxidants (Yücel, Şeker Karatoprak, Değim, 2019).

Another article discusses the encapsulation of RA in albumin nanoparticles using the desolvation method. Physicochemical characterization techniques, such as DSC, FT-IR, and microscopic imaging, were employed to confirm the properties of the RosA/BSA formulation. The formulation maintained high drug loading efficiencies (>90% encapsulation efficiency)

TABLE II - List of rosmarinic acid nano formulations (*continue*)

Compound	Formulations	Outcomes	References
Rosmarinic acid	Solid lipid nanoparticles	formulation that is administered orally An in vitro investigation demonstrated a good safety profile for evaluating the cytotoxic effects on lymphocytes.	(Madureira et al., 2016)
	PEGylated RA-derived nanoparticles (RANPs)	RANPs show potential in colitis treatment when combined with anti-inflammatory medications, demonstrating their biocompatibility and low cytotoxicity in a mouse model.	Chung et al., 2020)
	RA-loaded nanoparticles	The Nano formulation preserves fatty and minced food quality, especially in preventing oxidation in fish meat. It's in vitro release study shows a controlled, slow-release pattern of RA.	(Ceylan et al., 2022)
	Rosmarinic Acid-Loaded Silk Fibroin Nanoparticles (RA-SFNs)	The nanoparticles showed enhanced antitumor activity against HeLa and MCF-7 cells, with rapid drug release, high encapsulation efficiency (39%), and improved cellular uptake.	(Fuster et al., 2021)
	Chitosan nanoparticles	RA, when encapsulated into nanoparticles, can inhibit lipid peroxidation and enhance drug delivery, enhancing bioavailability and therapeutic effects due to its antioxidant properties.	(Da Silva et al., 2015)
	Nanoparticle encapsulated with chitosan and poly-vinyl alcohol	The study revealed that rosemary extract nanoparticles exhibited cytotoxicity, apoptosis, and cell cycle arrest in Hep-2 cells, resulting in increased reactive oxygen species and cell death.	(Ellithy, Aly, Tarek, 2022)
	Rosemary extract-loaded PEGylated nanoliposomes	Alzheimer disease	(Shalabalija et al., 2021)

TABLE II - List of rosmarinic acid nano formulations (*continuation*)

Compound	Formulations	Outcomes	References
	Nano pocket	In vitro and in vivo tests are used in the study to evaluate the antibacterial activity and wound healing effectiveness of the formulation. The proposed formulation exhibits promise for improved wound healing and antimicrobial activities, according to the results.	(Chhabra, Chauhan, Kumar, 2020)
	Liposomal nano formulation Silk fibroin nanoparticle Gold nanoparticle	In the treatment of cancer like breast cancer, human cervical carcinoma	(Chaitanya et al., 2022)

during storage in solution or freeze-dried powder for two months. The RA /BSA formulation exhibited superior antioxidant effects compared to curcumin/BSA, showing potential for protecting retinal epithelial cells from oxidative stresses. The study suggests that the formulation of RA with albumin nanoparticles demonstrates enhanced antioxidant properties and could be a promising strategy for ocular protection against oxidative stress and potential treatment of ocular diseases (Kim *et al.*, 2019).

The study addresses the development of a novel wound healing ointment, RA-CH-G-NPOs, including rosmarinic acid, chitosan, and graphene oxide nanopockets. Based on graphene oxide sheets, chitosan adhesion, and antioxidant capabilities, the formulation has improved wound healing and antibacterial effects. The antibacterial efficacy of the RA-CH-G-NPOs formulation is enhanced by targeting cell membranes. Its several toxicity pathways, high density, monodispersity, and tiny size make it an intriguing antibacterial material with enhanced effectiveness (Chhabra, Chauhan, Kumar, 2020).

Using a PEG-containing amine, RA can be converted into PEGylated RA-derived nanoparticles

(RANPs) in a single step. These RANPs are about 63.5 ± 4.0 nm in diameter and show good colloidal stability. In preclinical models, they have demonstrated promise as a therapeutic nanomedicine for the treatment of inflammatory bowel disease (IBD). RANPs exhibit effective hydrogen peroxide scavenging and shield cells from oxidative damage. Furthermore, the anticancer effectiveness of encapsulating RA in chitosan nanoparticles has been investigated, demonstrating the adaptability of RA formulations for many therapeutic applications. There has been discussion on the synthesis and therapeutic potential of RANPs for the treatment of acute inflammatory bowel illness. In a mouse model of colitis, RANPs have demonstrated potential, particularly when combined with anti-inflammatory medications. Their low cytotoxicity shows that they are biocompatible. Conflicting interests unrelated to the research were not disclosed by the financial interest disclosures. The anti-inflammatory characteristics of RA and the use of PEGylated nanoparticles in drug delivery systems have been emphasized in references. All things considered, RANPs have potential as a medicinal nano medicine for the treatment of inflammatory illnesses,

such as inflammatory bowel disease (IBD) (Chung *et al.*, 2020).

The study examined the efficacy of Rosmarinic Acid-Loaded Silk Fibroin Nanoparticles (RA-SFNs) in transporting RA to specific cells. These nanoparticles demonstrated enhanced bioavailability, leading to increased antitumor activity against HeLa and MCF-7 cells. RA-SFNs exhibited rapid drug release, high encapsulation efficiency (39%), and improved cellular uptake, contributing to their enhanced efficacy. Overall, the study concluded that RA-SFNs showed improved bioavailability and antitumor efficacy against the tested cancer cell lines, outperforming free RA in inhibiting cell proliferation and inducing apoptosis. The findings suggest that RA-SFNs are a promising delivery system for enhancing the antitumor activity of RA in skincare applications (Fuster *et al.*, 2021).

The article discusses the development and optimization of rosemary extract-loaded PEGylated nanoliposomes for potential treatment of Alzheimer's disease. The study aimed to create stable formulations with high antioxidant capacity for efficient delivery to the brain. The nanoliposomes exhibited a mean vesicle diameter of around 120 nm with a narrow distribution. Increasing the proportion of PEG resulted in slower release of the active component. The study highlights the potential significance of these formulations in Alzheimer's treatment. The formulation and optimization of rosemary extract-loaded PEGylated nanoliposomes for potential treatment of Alzheimer's disease are the focus of the article. The goal is to create stable formulations with high antioxidant capacity for efficient delivery to the brain. The nanoliposomes exhibit a mean vesicle diameter of around 120 nm and show potential significance in Alzheimer's treatment (Shalabaliya *et al.*, 2021).

The study aimed to develop an antioxidant Nano formulation using RA to prevent oxidation in salmon. Minced salmon samples treated with RA-loaded nanoparticles showed improved antioxidant effects, resulting in lower TBA values compared to untreated samples. The Nano formulation also enhanced sensory scores and extended the shelf-life of salmon during

refrigerated storage. The article highlights the successful development of a Nano formulation using RA to enhance antioxidant effects in minced salmon samples. The study found that the RA-loaded nanoparticles effectively reduced oxidation in the salmon, leading to improved sensory quality and extended shelf-life. These findings demonstrate the potential of the Nano formulation in preserving the quality of fatty and minced food products, particularly in preventing oxidation in fish meat. Furthermore, the *in vitro* release study revealed a controlled and slow-release rate of RA from the nanoparticles over time, with a cumulative release of 93.85% by the end of 144 hours. This indicates a gradual and sustained release pattern of the active ingredient RA (Ceylan *et al.*, 2022).

The article discusses the cytotoxic effects of rosemary extract nanoparticles on Hep-2 cells, a cell line of oral squamous cell carcinoma (OSCC). The nanoparticles were encapsulated with chitosan and polyvinyl alcohol (PVA) and synthesized using the nanoprecipitation method. Transmission electron microscopy (TEM) analysis confirmed the spherical shape and size of the nanoparticles, ranging from 0.5 μm to 200 nm, with a solid and dense structure. The study found that the rosemary extract nanoparticles exhibited dose-dependent cytotoxicity, induced apoptosis, and caused cell cycle arrest in Hep-2 cells. Treatment with the nanoparticles led to an increase in intracellular reactive oxygen species (ROS), contributing to cell death. The article suggests that rosemary extract nanoparticles have the potential to mitigate the side effects of traditional chemotherapeutic agents and improve the quality of life for cancer patients. Furthermore, the study proposes that rosemary extract nanoparticles could be a promising candidate for oral cancer management, offering a new avenue for cancer treatment (Ellithy, Aly, Tarek, 2022).

Numerous liposomal nano formulations of RA have been developed for the treatment of cancer. Using film hydration, rososomes (RS) produce the RA-lipid matrix. Their polydispersity index of 0.194 and diameter of 198.9 nm suggest that they may find therapeutic use in the management of cancer. With a

TABLE III - List of marketed formulation of Rosmarinic acid (*continue*)

Product name	Brand	Manufacturer
Healthyhey Nutrition Natural Rosemary Leaf Extract 60 vegetable capsules	Healthyhey Nutrition	Healthyhey Foods LLP, 227, Building No.5-B, Mittal Industrial Estate, AK Road,, Andheri (E), Mumbai- Mumbai, Maharashtra, 400059
Organix Mantra Rosemary Oil Improves Hair Growth, Strengthens Hair & Balances Scalp Moisturizes Skin & Nourishes Rosemary Oil 15ml	Organix Mantra	ASG Mantra, Organix Mantra
Pilgrim Spanish Rosemary & Biotin Hair Growth Oil to Control Hair Fall & Strengthens Hair 100ml Rosemary essential oil for hair growth Reduces Hair Fall Strengthens Hair	Pilgrim	Naturis Cosmetics Pvt. Ltd
Mamaearth Rosemary Hair Growth Oil with Rosemary & Methi Dana for Promoting Hair Growth - 200 ml Controls Hair Fall Strengthens Hair Stimulates New Hair Growth Healthy Nourished Hair	Mamaearth	Indo Herbal Products, (A) Indo Herbal Products, Unit-2, Plot No. 28-29, Sector 1B, IIE, SIDCUL, Ranipur, Haridwar, 249403 Regd. Address: - C-84 Panchsheel Enclave, New Delhi, India – 110017 M. : 20/C/U.A/2010 (B) Indo Herbal Products, Plot No 17, Sector 12, IIE, Sidcul, Haridwar Uttarakhand, India - 249403 M. : 2/C/U.A/2023
Avimee Herbal Rosemary Hair Oil For Hair Fall, Hair Growth & Dandruff Enriched with Neem, Amla & Bhringraj Oil For Men & Women 100 ml	AVIMEE HERBAL	Avimee Herbal Pvt Ltd, Avimee Herbal Pvt Ltd, 422/2, F-Building, Pandesera GIDC, Surat, Gujarat, 394221
Soulflower Rosemary Lavender Hair Oil For Healthy Hair, Scalp Nourishment, Hair Growth - 100% Pure & Natural Undiluted Cold Pressed Oil, 120ML	Soulflower	PT Invent India Pvt Ltd, Plot no B-67, additional midc, anand nagahr, near papu canteen, ambernath (E) 421506, 022-62871234
Parachute Advanced Rosemary-enriched Coconut Hair Oil Rosemary Hair Oil Superfoods' Magic Long & Thick Hair 300 ML	Parachute Advanced	Marico Limited, Ameya plastics, Door no: 5E, SF no. 605/3-C, Moolakalipalayam, Kaduvettipalayam (PO), Coimbatore -641659, Tamil Nadu. M C-1182
Botanic Hearth 100% Pure Rosemary Oil For Hair Growth Infused With Biotin Hair Strengthening Treatment Nourishing & Volumizing With Jojoba Oil & Castor Oil Non GMO Verified Hair Oil with Applicator Scalp Massager for Hair Growth 198 ML	Botanic Hearth	Botanic Hearth, Botanic Hearth
Khadi Natural Rosemary & Henna Hair Oil, Natural Oil For Thick & Voluminous Hair Oil For Controlling Hair Fall & Premature Greying, Paraben & Mineral Oil Free, Suitable For All Hair Types, 210ml	Khadi Natural	Khadi Naturals, KHADI NATURAL HEALTHCARE 801, SHAHPURI TIRATH SINGH TOWER, C-58 COMMUNITY CENTRE, JANAKPURI, West Delhi, Delhi, 110058, helpdesk@khadinatural.com
Aravi Organic Rosemary Essential Oil for Hair Growth, Quick Hair Growth Rosemary Oil for Women and Men - 100% Pure Rosemary Leaves Oil - 100 ml	Aravi Organic	Aravi Organic, Aspiro Natural

TABLE III - List of marketed formulation of Rosmarinic acid (*continuation*)

Product name	Brand	Manufacturer
Wishcare® Rosemary Essential Oil 15 ML - 100% Pure, Undiluted & Natural	Wishcare	Wishcare, Rabiko Lifestyle Pvt Ltd
Exotic Aromas Rosemary Oil for Hair Growth 100 ML, Skin, Aromatherapy 100% Pure & Natural	Exotic Aromas	Natural Biotech Solutions, Exotic Aromas
Mystiq Living Rosemary Leaves and Rosemary Water for Hair Growth 100% Pure, Natural and Organic	Mystiq Living	Silvercloud Ventures, silvercloud Ventures
Bare Advanced Hair Growth Serum Anatomy	Bare Anatomy	Naturis Cosmetics Pvt. Ltd
Plix - the plant fix Rosemary Hair Growth Serum with 3% Redensyl, 4% anagain, 3% Baicapil, 50 ml (Pack of 1) Stimulates Hair Growth, Increase Hair Density & Thickens Hair For Men & Women	Plix - the plant fix	HCP Wellness Pvt. Ltd

particle diameter of 255 nm, silk fibroin nanoparticles coated with rosmarinic acid (RA-SFN) exhibit potential for therapeutic uses against cervical carcinoma and breast cancer cell lines. Furthermore, RA containing gold nanoparticles (AuNPs) have demonstrated promise in the treatment of breast cancer. These nanoparticles successfully integrated RA into mouse fibroblasts and human breast cancer cells, demonstrating their potential in cancer treatment (Chaitanya *et al.*, 2022).

Several commercially available formulations containing rosmarinic acid as an active ingredient are now on the market. The table III and IV below provide a list of some of these formulations.

APPLICATION

- RA can be administered topically, intranasally, via pulmonary administration, or intravenously.
- However, the primary way it enters the human body is through oral application.
- The main process of metabolism for RA occurs in the gut microflora, resulting in the formation

of simpler and more readily absorbed phenolic units (Hitl *et al.*, 2021).

- RA finds applications in multiple industries, including food preservation, cosmetics, and pharmaceuticals, owing to its wide range of health-promoting properties.
- It is employed as a food additive due to its antioxidant activity and as a potential agent for hepatoprotection.
- RA has demonstrated its ability to protect against oxidative damage in cell lines and is currently being researched for its potential in developing formulations that enhance bioavailability (Marchev *et al.*, 2021).
- RA is utilized in food supplements due to its antioxidant properties, which aid in preventing lipid peroxidation and increasing acetylcholinesterase activity in the brains of diabetic rats.
- Furthermore, it exhibits potential anti-inflammatory effects, making it advantageous for addressing inflammation in the body.

TABLE IV - Mother tincture Homeopathy

Product name	Brand	Manufacturer name
Dr. Willmar Schwabe India Rosmarinus Officinalis 30Ch 30ml	Dr. Willmar Schwabe	Dr. Willmar Schwabe India Pvt. Ltd.A -36,sector-60, Noida-201304
SBL Rosmarinus Officinalis 30 CH (30ml)	Shop homeo	As Per label
Dr Reckeweg Rosmarinus Officinalis 30 CH (11ml)	Shop homeo	As Per label
Hollywood Secrets Pure Rosemary Liquid Extract 50ml	Hollywood secrets	Hollywood Secrets
Lirene acid power Anti-wrinkle cream with rosmarinic acid 50 ml	Lirene	Lirene

- Moreover, its anti-viral activity against enterovirus infections holds promise in supporting immune health when incorporated into food supplements (Alagawany *et al.*, 2017).

CONCLUSION

A comprehensive analysis of the existing scientific literature has been conducted, with a primary emphasis on the assessment of compounds that include polyphenols, particularly plants that contain rosmarinic acid. This analysis specifically focuses on the findings of recent pharmacological research. Furthermore, other biological origins of rosmarinic acid have been cited. Studies have discovered that rosmarinic acid molecules exhibit numerous pharmacological properties, and the structure-activity relationship has also demonstrated a strong therapeutic effect. Rosmarinic acid has been extracted using various criteria. The study states that multiple experimental studies have been conducted to examine the significant biological impacts of rosmarinic acid, and ongoing research is being conducted on various nanoformulations. Despite the availability of numerous marketed formulations, Additional clinical trials are necessary due to the limited number of

studies conducted to evaluate the drug's safety and therapeutic efficacy in human subjects.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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