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Review Article

PHYTOCHEMICAL PROFILE AND NEUROPROTECTIVE POTENTIAL OF *ROSMARINUS OFFICINALIS* L. FROM NILGIRIS IN ALZHEIMER'S DISEASE MANAGEMENT

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ABSTRACT

The current review discusses the possibility of using *Rosmarinus officinalis* L. (rosemary) from the Nilgiris district as a plant that can be used as a biomarker for the quantification and treatment of Alzheimer's disease (AD). Specific geographic and climatic conditions, prevalent in the Nilgiris, make such cultivations unique and replete with high levels of rosmarinus acid, carnosic acid, luteolin, and 1,8-cineole. These compounds exhibit multimodal neuroprotective effects against AD pathology through antioxidant and anti-inflammatory actions, amyloid-β (Aβ) and tau pathology modification, and enhancement of cholinergic function. The pre-clinical studies concerning the treatment of AD were quite important to its consequences on neuroprotection, cognitive improvement, inhibition of Aβ aggregation, and reduction of tau hyperphosphorylation. This paper also highlights the possibility that such compounds could be used as biomarkers to quantify AD because they can be measured in biological fluids and can have associations related to the mechanisms behind AD. However, challenges ahead are standardization of rosemary extracts improvement in their bioavailability, along with penetration across the blood–brain barrier, and all this in well-designed clinical trials. Future research would include the development of standardized cultivation and processing protocols to improve the bioavailability of the extract; large-scale clinical trials on AD patients; and a combination of the therapy with existing AD drugs, not forgetting personalized approaches to medicine.

Keywords: *Rosmarinus officinalis* L., Alzheimer's disease, Neuroprotection, Phytochemicals, Biomarkers, Cognitive enhancement.

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INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder that develops progressively and is characterized by basic features of cognitive decline, loss of memory, and behavioral changes. Being the most prevalent form of dementia, it is estimated to affect up to 50 million people worldwide; this number is likely to increase threefold by the year 2050 [1]. The growing prevalence of AD has turned out to be one of the critical challenges for healthcare systems and societies worldwide, which has underscored the urgent need for effective diagnostic tools and treatments.

Biomarkers will, therefore, be very important in the early detection, diagnosis, and monitoring of AD. Biomarkers are quantifiable biological characteristics that indicate some feature of health or disease. They act as surrogates for disease processes even before the beginning of symptoms, and they can be objectively measured [2]. Biomarkers for neuroimaging and cerebrospinal fluid (CSF) analysis that detect amyloid-β (Aβ) and tau proteins have been widely used lately. These methodologies are often invasive, expensive, or not widely available; therefore, there is also a need to find novel, affordable biomarkers [3].

In the last years, natural products have represented a growing interest in being a valid source of neuroprotective compounds and biomarkers of neurodegenerative diseases. Among these, rosemary, *Rosmarinus officinalis* L., belongs to the Lamiaceae family and is an evergreen fragrant herbal plant that lately has been reported as a potential candidate in AD-related studies [4]. Rosemary contains some bioactive compounds such as rosmarinic acid (RA), carnosic acid (CA), and luteolin that, in pre-clinical studies, have been described to possess antioxidant, anti-inflammatory, and neuroprotective properties [5].

The Nilgiris district, part of the state of Tamil Nadu, India, is particularly famous for its geographical and climatic conditions, which enable it to grow superior qualities of aromatic and medicinal plants [6]. The altitude in this region varies within the range from 900 to 2,636 m above sea level, and merged with the average temperature that prevails along with high rainfall, it becomes an ideal place for rosemary cultivation [7]. The terroir of the Nilgiris differs from other places, which may influence the phytochemical profile of rosemary grown in the region in such a way to increase the therapeutic effect and biomarker potentiality for AD [8].

This review outlines the identification and quantification of biomarkers present in rosemary grown in the Nilgiris district with their therapeutic potentials related to AD. We will look at the unique phytochemical composition of Nilgiri rosemary, discuss biomarkers that may have potential for AD quantification, and further outline their various therapeutic applications in the treatment of AD. Further, we discuss various challenges and future directions of this promising field of research.

By focusing on rosemary of local origin from the Nilgiris, this review adds to the growing knowledge in the field of natural product-based AD interventions and also serves to underscore the relevance of regional biodiversity in drug discovery and development. The findings presented herein might form the basis for the development of novel, affordable, and efficient strategies for both diagnosis and treatment of AD, with the potential to benefit millions affected by this terrible disease.

The selections of articles for the present review were searched from specialized databases (Range of years: 2014–2024) such as Elsevier, Pubmed, and Cambridge using the keywords *R. officinalis* L., AD, Neuroprotection, Phytochemicals, Biomarkers, Cognitive enhancement. Other selections include articles from Springer Wiley, information from Internet sources, and online published articles from The Lancet Respiratory Medicine, Medscape, and StatPearls.

Herbarium specimen information

The details of the herbarium specimen of *Rosmarinus officinalis* L. (syn. *Salvia rosmarinus* Spenn.) are provided in the table below. The botanical name, common name, and family classification are specified, along with the collection site, identification authority, and the repository where the specimen is deposited. These details ensure proper documentation and authentication of the plant material used for research and reference purposes table 1.

ROSEMARY (*R. OFFICINALIS*) IN THE NILGIRIS DISTRICT

Geographical and climatic conditions of the Nilgiris

The Nilgiris is a district located in the western part of Tamil Nadu State, India. It descends in the middle of the Western Ghats mountainous continuous range. Due to its geographical entity and climatic conditions, it constitutes a region with high biodiversity and agricultural potential [9].

It covers an area of about 2,565 sq km with an altitude ranging from 900 to 2,636 m above MSL [10]. The Doddabetta is the highest elevation, at 2,636 m, which is considered the second-highest peak in the Western Ghats [11], presenting a different topography of microclimates within the district.

The Köppen classification of the Nilgiris is a subtropical highland climate with Cwb. On average, the annual temperature has fallen between 5°C and 24°C; however, there are sometimes areas of frost in high altitudes due to extreme winter temperatures during winter time. An average annual rainfall of around 1200 to 1500 mm falls the area, and the area sees precipitation throughout the year due to both the southwest and northeast monsoon seasons (June to September and October to December, respectively) [12].

The following climatic conditions – moderate temperatures, high rainfall, and well-drained soils – offer the best opportunities for planting several aromatic and medicinal plants, among which rosemary is included [6].

Cultivation practices and varieties of rosemary in the region

Rosemary cultivation has gained popularity in this district owing to the congenial growing conditions of the crop and an increasing demand from the aromatic and medicinal plants industry. Methods relating to cultivation practices have also been adapted to suit the local environment to bring about maximum yield and quality in this region [7].

Rosemary is normally propagated through cuttings in well-prepared soil with good light, alkaline conditions of pH ranging between 6.0 and 7.5 [13]. Spacing of plants in rows is normally done at 60–90 cm, with 100–120 cm between each row. Organic farming in this region is adopted by farmers, and compost and vermicompost are the major organic manures used by the local farmers [14].

Irrigation is usually not of major concern with the high rainfall in the area, but supplementary irrigation may be given during dry months. Plants are regularly pruned to maintain shape and to encourage bushy growth for higher leaf yield [15].

Although the most common type of rosemary cultivated in the Nilgiris is *R. officinalis* L., some farmers have introduced other cultivars such as "Tuscan Blue" and "Prostratus" with a view to diversifying their production for various market needs [8].

Unique phytochemical profile of Nilgiris rosemary

The terroir of the Nilgiris district varies from others; hence, it is this factor that may play a huge role in shaping the phytochemical profile of rosemary within this area. A few studies have pointed out that the high altitude, cold climate, and strength of ultraviolet (UV) radiation are the reasons for the peculiar chemical make-up of Nilgiris rosemary [16].

Through various comparative analyses, rosemary of Nilgiris has been indicated to contain a higher amount of certain bioactive compounds in comparison to rosemary grown in other regions. In the work of Ramakrishna *et al.* 2021, Nilgiris rosemary exhibited 15–20% higher content of RA and 10–15% higher content of CA than those collected from various lower altitude regions in India.

Some peculiarities have also been recorded in the essential oil composition of Nilgiris rosemary. Gas chromatography-mass spectrometry (GC-MS) analysis has revealed that, in comparison with rosemary oil obtained from other geographical sources, Nilgiris rosemary oil contains more 1,8-cineole (eucalyptol) and α-pinene [17]. Such differences in chemical composition might be associated with superior therapeutic properties, including those related to neurodegenerative diseases such as Alzheimer's [18].

In fact, the antioxidant activity of Nilgiris rosemary extracts has been reported to be higher, by DPPH and FRAP assays, than that from other areas [19]. This enhanced antioxidant activity could be because of the enhanced synthesis of phenolic metabolites for which the high-altitude environmental stressors are responsible [20].

The peculiar phytochemical profile of Nilgiris rosemary strongly supports the importance of its geographical origin when it comes to studying its possible therapeutic applications in the context of AD [21].

BIOACTIVE COMPOUNDS IN ROSEMARY WITH POTENTIAL ANTI-ALZHEIMER PROPERTIES

R. officinalis L. contains a myriad of bioactive compounds that may be useful in the amelioration of various features of AD pathology. The next sections will detail the most promising compounds and their possible mechanism of action involved in the prevention and treatment of AD.

RA

RA is a polyphenolic and major component of rosemary, which has been the subject of significant interest on account of its neuroprotective action and potential in AD treatment [22]. Its major mechanism is through its antioxidant effect, where RA achieves this by free radical scavenging and blocking oxidative stress, the latter being a major constituent of AD pathogenesis [23]. Sahu *et al.* described that RA treatment effectively reduced lipid peroxidation as well as increased antioxidant enzyme activity in an AD model mouse [24]. RA also possesses potent anti-inflammatory activity and is responsible for controlling neuroinflammation, a critical factor in AD development. RA exerts anti-inflammatory effects by inhibiting pro-inflammatory cytokines and regulating microglial activation [25]. Luo *et al.* demonstrated that RA inhibits NF-κB and MAPK pathway activation, both of which are major inflammatory response mediators in AD. RA was also depicted to disrupt Aβ aggregation and Aβ-induced neurotoxicity. In a Habtemariam study, RA not only suppressed Aβ aggregation but also promoted the disaggregation of preformed Aβ fibrils, thus shielding neuronal cells against Aβ-induced toxicity [26]. Together, these results indicate that RA holds great therapeutic promise in AD targeting oxidative stress, neuroinflammation, and Aβ pathology [54].

CA

CA, a phenolic diterpenoid from rosemary, displays antioxidative and anti-inflammatory activity with promising neuroprotective functions in AD [27]. One of its major modes of action includes the activation of the Nrf2 pathway, which is important in the regulation of antioxidant defense-related gene expression as well as cytoprotection. Satoh *et al.* showed that, in a murine model of AD, CA-induced activation of Nrf2 successfully protected neurons against oxidative stress and enhanced cognitive function [28]. CA has also been found to increase neurotrophic support by upregulating the expression of brain-derived neurotrophic factor (BDNF), an important molecule for neuronal survival and synaptic plasticity. A study by Meng *et al.* recently indicated that administration of CA increased levels of BDNF, which resulted in enhanced cognitive function in a transgenic AD mouse model [29]. In addition, CA has been found to be involved in modulating tau pathology,

an important signature of AD, as tau hyperphosphorylation is reduced. Wu *et al.* proved that the treatment of CA inhibited tau kinase activity but enhanced the activity of tau phosphatases, thus preventing tau pathology *in vitro* and *in vivo* [30]. The data indicate that CA has excellent neuroprotective activities through antioxidative, neurotrophic, and tau-modulating activities and is a potential medication for treating AD.

Luteolin

Luteolin is a flavonoid found in rosemary that has shown multi-faced protective actions in AD [31]. One of its principal modes of action is the inhibition of neuroinflammation, since luteolin is a strong anti-inflammatory agent with its effects being mediated through modulation of microglial activation and inhibition of pro-inflammatory cytokines. Zhu *et al.* noted that luteolin treatment decreased neuroinflammation profoundly and enhanced cognitive function in an AD rat model. In addition, luteolin has been found to inhibit cholinesterase, and this activity can be used for the treatment of AD. Jabir *et al.* (2018) established that luteolin was a more effective AChE inhibitor compared to some of the available anti-AD drugs, revealing its potential as a drug. Apart from that, luteolin has been found to control Aβ pathology by inhibiting Aβ generation and deposition. Gu JX, Cheng XJ, Luo X, Yang X, Pang YP, Zhang XF, *et al.* have described that luteolin treatment of an AD cellular model changed APP processing, resulting in decreased Aβ content with increased Aβ clearance [32]. Such observations indicate that luteolin has neuroprotective actions due to its anti-inflammatory, cholinesterase-inhibition, and Aβ-modulating activities, and such a compound is a potential therapeutic compound for AD.

1,8-cineole (eucalyptol)

1,8-cineole, or eucalyptol, is a predominant constituent of rosemary essential oil with potential for the treatment of AD [33]. It has strong anti-inflammatory and antioxidant activity, contributing to its neuroprotective activity. Khan *et al.* (2019) showed that the administration of 1,8-cineole in an AD rat model decreased neuroinflammation and oxidative stress, resulting in enhanced cognitive functions. Furthermore, in addition to its acetylcholinesterase (AChE) inhibition activity, 1,8-cineole was found to regulate the cholinergic system by enhancing acetylcholine release. In this regard, Porres-Martínez *et al.* found that 1,8-cineole administration in an AD mouse model improved memory and cognitive performance through the modulation of the cholinergic system. Its ability to easily permeate through the blood–brain barrier (BBB) is another beneficial feature of 1,8-cineole. Supporting this, Seol *et al.* demonstrated in a study that, following administration, 1,8-cineole reached high concentrations in the brain, revealing its potential as an AD treatment neuroprotective agent [34]. These findings suggest that 1,8-cineole has the potential to be a key element of AD therapy due to its anti-inflammatory, antioxidant, cholinergic-modulating, and brain-penetrant activities.

In general, rosemary appears to be a rich source of such bioactive phytoconstituents that may act as promising anti-Alzheimer's compounds based on the antioxidant and anti-inflammatory activities and modulation of Aβ and tau pathology by improving cholinergic function. Hence, the additive impacts of these kinds of compounds in rosemary extracts do offer an attractive strategy for AD prevention and treatment.

Phytochemical composition of *R. officinalis* L.

The phytochemical composition of *Rosmarinus officinalis* L. is detailed in the table below. It includes concentrations of total phenolic content, total flavonoid content, and condensed tannin content expressed in milligrams per gram of dry weight (mg/g DW). Major bioactive compounds such as carnosic acid and rosmarinic acid are quantified in mg/g DW. Additional phenolic compounds including catechol, p-hydroxybenzoic acid, caffeine, vanillic acid, rutin, ellagic acid, and salicylic acid are measured in mg per 100 grams. The table also lists diterpenes like carnosol and ursolic acid in micrograms per gram (µg/g), along with essential oil components such as 1,8-cineole, camphor, α-pinene, β-pinene, camphene, and β-caryophyllene, expressed as percentages of volume/weight (% v/w). These compounds collectively contribute to the antioxidant, aromatic, and therapeutic properties of rosemary Table 2.

Notes

- DW: Dry Weight
- GAE: Gallic Acid Equivalents
- QE: Quercetin Equivalents
- CE: Catechin Equivalents
- µg/g: Micrograms per gram
- mg/g: Milligrams per gram
- mg/100 g: Milligrams per 100 grams
- % (v/w): Percentage volume/weight

Validated analytical methods for key phytochemicals in rosemary

The analysis of key phytochemicals in *Rosmarinus officinalis* utilizes validated, reliable analytical methods tailored to the chemical nature of each compound. Rosmarinic acid quantification is most accurately performed by High-Performance Liquid Chromatography (HPLC) with UV detection at approximately 330 nm, providing precise measurement in plant extracts. Carnosic acid, a diterpene, is commonly analyzed using HPLC coupled with Diode-Array Detection (DAD) or tandem Mass Spectrometry (MS/MS), with detection typically around 284 nm, offering high sensitivity and specificity. Luteolin, a flavonoid, is analyzed by HPLC-UV or HPLC-MS/MS, often after hydrolysis, with UV detection between 350 and 360 nm. For volatile oil constituents such as 1,8-cineole (eucalyptol), Gas Chromatography-Mass Spectrometry (GC-MS) is the method of choice, employing retention time and mass fragmentation patterns for reliable identification and quantification.

In summary, HPLC methods (either UV or DAD) serve as the benchmark for polar, non-volatile compounds like rosmarinic acid, carnosic acid, and luteolin, whereas GC-MS is essential for analyzing volatile essential oil components such as 1,8-cineole Table 3.

Overview by methodology

- High-performance liquid chromatography (HPLC) (UV or diode array detector) is the benchmark for polar non-volatile chemicals such as RA, CA, and luteolin.
- GC-MS is needed for volatile compounds, particularly 1,8-cineole, in essential oil fractions.

BIOMARKERS IN ROSEMARY FOR AD QUANTIFICATION

Definition and importance of biomarkers in AD

Biomarkers are quantifiable signals of biological processes, disease, or pharmacologic effects of intervention [35]. For AD, biomarkers play a very crucial role in early diagnosis, disease monitoring, and assessment of the efficacy of treatment [2]. Their application is not only important in research but also in clinical settings, as they offer significant information regarding AD development and response to therapy. One of their main uses is in the early diagnosis since biomarkers can identify AD changes before clinical symptoms, which allows for earlier intervention. They also enable the monitoring of disease evolution in time through measurable parameters. Biomarkers assist in the development of drugs by assessing candidate drug efficacy during clinical trials [36]. Moreover, biomarker profiling supports personalized medicine by providing the potential for tailoring treatment regimens based on individual disease pathology differences [37]. Such multifunctionality underscores the value of biomarkers in advancing AD diagnosis, therapy, and therapeutic research.

Potential rosemary-derived biomarkers for AD

Rosemary contains a number of compounds that have biomarker potential for quantification of AD and can be broadly categorized into three groups: antioxidant markers, anti-inflammatory markers, and cholinesterase inhibition markers. Oxidative stress plays a dominant role in the pathogenesis of AD. Thus, antioxidant markers become very important. Of these, RA has been noted as a promising biomarker, with plasma and urinary excretion of RA and its metabolites

acting as indicators of the antioxidant state in patients with AD. Likewise, CA and its metabolite carnosol have been identified as a biomarker related to oxidative stress in neurotransmitter diseases [38]. Furthermore, the whole phenolic content analyzed after supplementation with rosemary may reflect on the body's overall antioxidant capability.

Table 1: Herbarium specimen information

Parameter	Details	References
Botanical name	*Rosmarinus officinalis* L. (syn. *Salvia rosmarinus* Spenn.)	[56]
Common name	Rosemary	[56]
Family	Lamiaceae	[56]
Collection site	Field Station, Allalasandra, Bangalore, Karnataka, India	[56]
Identification authority	Mr. Siddulu, Lecturer of Botany, Nagarjuna Government Degree College, Nalgonda, Telangana	[56]
Deposited at	Department of Pharmacognosy, Nalanda College of Pharmacy, Nalgonda, Telangana	[56]

Table 2: Phytochemical composition of *Rosmarinus officinalis* L.

Phytochemical	Concentration	Unit	References
Total phenolic content	44.56±0.29	mg GAE/g DW	[57]
Total flavonoid content	3.20±0.02	mg QE/g DW	[57]
Condensed tannin content	1.61±0.01	mg CE/g DW	[57]
Carnosic acid	101.04±6.25	mg/g DW	[57]
Rosmarinic acid	10.09±0.59	mg/g DW	[57]
Catechol	1474.25±5.83	mg/100 g	[58]
p-Hydroxybenzoic acid	309.65±1.66	mg/100 g	[58]
Caffeine	235.66±2.36	mg/100 g	[58]
Vanillic acid	64.38±1.10	mg/100 g	[58]
Rutin	54.25±0.58	mg/100 g	[58]
Ellagic acid	42.49±0.36	mg/100 g	[58]
Salicylic acid	271.54±1.02	mg/100 g	[58]
Carnosol	22,000.67±77.39	µg/g	[59]
Ursolic acid	5144.27±28.68	µg/g	[59]
1,8-Cineole	43.77	% (v/w)	[59]
Camphor	12.53	% (v/w)	[59]
α-Pinene	11.51	% (v/w)	[59]
β-Pinene	8.16	% (v/w)	[59]
Camphene	4.55	% (v/w)	[59]
β-Caryophyllene	3.93	% (v/w)	[59]

DW: Dry weight, GAE: Gallic acid equivalents, QE: Quercetin equivalents, CE: Catechin equivalents, µg/g: Micrograms per gram, mg/g: Milligrams per gram, mg/100 g: Milligrams per 100 grams, % (v/w): Percentage volume/weight

Another feature of AD is chronic inflammation, which underscores the importance of anti-inflammatory markers [39]. Plasma luteolin and its plasma metabolites could be markers of neuroinflammation, whereas urinary excretion of 1,8-cineole metabolites has been suggested as an anti-inflammatory activity marker in neurodegenerative disorders. In addition, elevated levels of proinflammatory cytokines such as interleukin (IL)-1β, IL-6, and tumor necrosis factor-alpha in CSF or blood can be indicative of rosemary compound-induced anti-inflammatory activity.

Since cholinesterase inhibition is a major target in AD treatment, biomarkers associated with this pathway are of particular interest. The plasma or CSF activities of AChE may indicate the potency of rosemary compounds as inhibitors of the enzyme, whereas body fluid butyrylcholinesterase activity is suggested as a surrogate biomarker for cholinesterase inhibition. These findings suggest that compounds from rosemary may serve as useful biomarkers for the evaluation of AD development and therapeutic treatment.

Biomarker detection and quantification techniques

Various biological samples of rosemary have been studied with different analytical approaches for the detection and quantitation of potential biomarkers. Among the most popular methods are chromatographic techniques. HPLC is still the major method for the separation and quantitation of rosemary constituents and metabolites in biological fluids. For example, Mulinacci *et al.* (2011) were able to quantify RA and its metabolites in human plasma and urine after dietary rosemary extract intake. Furthermore, gas chromatography (GC) is also favored for the analysis of volatile compounds such as 1,8-cineole. Horváthová *et al.* (2017) established the blood levels of 1,8-cineole following the inhalation of rosemary oil by GC.

Mass spectrometry-based methods provide highly sensitive and specific analyses of rosemary compounds. Liquid chromatography-mass spectrometry has been employed to establish the bioavailability and metabolism of rosemary polyphenols in human subjects [40]. GC-MS is particularly suited for determining volatile compounds, as demonstrated by Moss *et al.* (2003), who detected traces of 1,8-cineole in blood samples after oral administration of rosemary oil.

Spectroscopic techniques have also been utilized in rosemary biomarker studies. UV-visible spectroscopy is usually employed to determine total phenolic content and antioxidant capacity, as Sánchez-Camargo *et al.* utilized this in the determination of antioxidant activity in rosemary extracts [41]. Fluorescence spectroscopy is useful in the determination of enzyme activity, especially in cholinesterase inhibition assays. Wszelaki *et al.* (2010) employed fluorescence spectroscopy to assess the cholinesterase inhibitory activity of rosemary compounds.

Overall, several rosemary-derived compounds have the potential to serve as biomarkers for AD in their quantitative form. The quantification and identification of these biomarkers should be based on their antioxidant status, anti-inflammatory effects, and cholinesterase

Table 3: Validated analytical methods for key phytochemicals in rosemary

Compound	Preferred analytical method	Description and validation	References
Rosmarinic acid	HPLC	• The most reliable method for quantification in plant extracts • Uses UV detection at~330 nm	[60]
Carnosic acid	HPLC-DAD or HPLC-MS/MS	• Sensitive and specific for diterpenes • Often detected at 284 nm	[60]
Luteolin	HPLC-UV or HPLC-MS/MS	• Flavonoid analysis is typically done with UV detection at 350–360 nm • Often in hydrolyzed extract	[60]
1,8-Cineole (eucalyptol)	GC-MS	• Ideal for volatile oil components • Retention time and MS fragmentation are used for identification and quantification	[60]

HPLC: High-performance liquid chromatography, GC-MS: Gas chromatography-mass spectrometry, UV: Ultraviolet

inhibition, employing various analytical techniques. The validation of these biomarkers and their clinical utility should be closely linked to their relevance in AD diagnosis and treatment monitoring.

THERAPEUTIC POTENTIAL OF ROSEMARY BIOMARKERS IN AD TREATMENT

Rosemary contains bioactive compounds that may be biomarkers capable of offering useful therapeutic benefits towards the treatment of AD. The section below discusses how such compounds may form part of the treatment provided for AD.

Neuroprotective role

Compounds of rosemary have shown remarkable neuroprotective effects, an essential attribute in preventing AD-induced neuronal damage. RA has shown strong neuroprotective activity in different AD models. In a work reported by Sahu *et al.* (2021), RA treatment inhibited oxidative stress and thus enhanced cognitive function in an aluminum-induced model of AD in rats [42]. The neuroprotective activity of RA is due to its potent antioxidant action and the modulation of cellular redox state. Likewise, CA has been reported to exhibit neuroprotection through the activation of the Nrf2-Keap1 pathway, which is critical to cellular antioxidant defense. Satoh *et al.* (2019) indicated that CA treatment minimized neuronal lesions induced by oxidative stress and significantly enhanced cognitive function in an AD mouse model. Another rosemary compound, luteolin, has exhibited neuroprotective properties through various mechanisms. Precisely, luteolin was noted to mitigate oxidative damage and suppress apoptosis in an *in vitro* model of AD caused by Aβ toxicity [43]. These results point out the promise of rosemary compounds as therapeutic treatments for neuroprotection in AD [55].

Cognitive enhancement

Rosemary-derived biomarkers have been suggested to enhance cognitive abilities and are therefore of high interest for future AD treatment. A compound in this regard, 1,8-cineole, has been directly involved in improved cognitive performance in human beings. Moss *et al.* conducted a randomized controlled trial (RCT) where rosemary essential oil containing high levels of 1,8-cineole was utilized, resulting in an improvement in cognitive performance in healthy adults [44]. While there is a lack of evidence for its activity in AD patients, these observations indicate potential benefits to cognition worthy of further investigation in AD. Another primary compound, RA, has exhibited cognition-enhancing activities in a variety of AD animal models. Chronic RA treatment has been shown in a study by Hasanein and Mahtaj to enhance learning and memory performance in a streptozotocin-induced rat model of AD [45]. This cognitive improvement was linked to decreased oxidative stress and elevated acetylcholine levels in the brain. These results indicate that rosemary-derived biomarkers, especially 1,8-cineole and RA, may provide promising therapeutic options for cognitive enhancement in AD.

Inhibition of Aβ aggregation

Some rosemary compounds have also been reported to inhibit amyloid-beta (Aβ) aggregation, which is the hallmark of AD pathogenesis. CA has exhibited strong anti-amyloidogenic activity. CA treatment was reported by Meng *et al.* to decrease Aβ formation and aggregation in both *in vitro* and *in vivo* AD models [46]. The suggested mechanism of action was through modulation of amyloid precursor protein (APP) processing and promotion of clearance of Aβ. Likewise, RA is demonstrated to inhibit Aβ aggregation and reduce Aβ-induced neurotoxicity. Luo C, Zou L, Sun H, Peng J, Gao C, Bao L, *et al*. presented evidence from an *in vitro* model of AD showing that RA inhibited neuroinflammation and oxidative stress caused by Aβ [47]. These results indicate that CA and RA may be effective agents for AD prevention as well as treatment through action against Aβ aggregation and the related neurotoxicity.

Tau hyperphosphorylation reduction

The tau phosphorylation mechanism is an important component of AD pathology, and some rosemary-derived compounds have been shown to modulate this process. CA has been reported to inhibit tau hyperphosphorylation by inhibiting the activity of tau kinases while at the same time increasing the activity of tau phosphatases, thereby resulting in decreased tau pathology in an AD cell model [30]. Similarly, luteolin was found to be an effective modulator of tau pathology. Rezai-Zadeh *et al.* stated that luteolin treatment substantially lowered tau hyperphosphorylation in *in vitro* and *in vivo* AD models [48]. The mode of action was linked to its inhibition of glycogen synthase kinase-3 beta (GSK-3β), a central kinase for tau phosphorylation. In summary, bioactives isolated from rosemary are promising agents as potential biomarker molecules to be used as therapeutic agents against AD. Such compounds are protective to neurons, improve cognitive deficits, prevent the aggregation of Aβ, and regulate tau hyperphosphorylation. Collectively, they form a powerful basis for developing future clinical studies on the pharmacological efficacy of rosemary in human patients afflicted with AD.

CHALLENGES AND FUTURE DIRECTIONS

While rosemary-derived biomarkers currently possess promising potential for AD quantification and treatment, several challenges have yet to be dealt with, and future research directions should be considered for this principle to see successful implementation.

Standardization of rosemary extracts from Nilgiris

One of the immediate challenges in utilizing rosemary biomarkers is the complete lack of standardization in extract preparation, particularly considering the distinct phytochemical profile of rosemary from the Nilgiris district. One important issue is the lack of uniformity in bioactive compound composition, which depends greatly on cultivation conditions, harvest period, and accessible extraction methods. Napoli *et al.* have established excellent qualitative variations in the phytochemical content of rosemary cultivated and harvested from wild sources, citing standardization [8]. Standardization can be achieved through quality control standard formulation. It is crucial to have a standardized procedure for cultivation, harvesting, and extraction in the quest for uniformity and effectiveness. Li Y, Xie Y, He Y, Hou W, Liao M, Liu C also suggested a comprehensive quality control method for plant extracts, which can be readily applied in Nilgiris rosemary to ensure reproducibility and therapeutic activity [49]. Standardized protocols for cultivation, harvesting, and extract preparation can be established for future studies aimed at conserving signature bioactive moieties, increasing the potential of rosemary as an AD biomarker source.

Bioavailability and BBB penetration

The effectiveness of rosemary biomarkers for the quantification and therapy of AD relies on their delivery to the brain in sufficient amounts. One of the main challenges is the low bioavailability of most of the polyphenolic compounds present in rosemary, e.g., RA. Baba *et al.* (2005) concluded that only traces of RA are delivered to systemic circulation following oral gavage, restricting its therapeutic value. A key factor is the capacity of rosemary bioactives to permeate the BBB. Although some compounds, such as 1,8-cineole, demonstrate excellent BBB permeability [50], others such as RA, demonstrate poor permeability, limiting their neuroprotective actions. Research in the future must aim at designing new formulation strategies for increasing the BBB penetration and bioavailability of biomarkers from rosemary. Sophisticated drug delivery systems, such as nanoparticles and prodrug approaches, have been proposed as potential solutions to improve brain-targeted delivery of polyphenols, as noted by Gao *et al.* [51]. These technologies can potentially significantly improve the therapeutic efficacy of rosemary compounds in the management of AD.

Clinical trials and dosage optimisation

While pre-clinical research has shown encouraging outcomes, clinical evidence for the efficacy of rosemary biomarkers against AD is still limited. Most of the rosemary compounds' research has been conducted *in vitro* or in animal models, with sparse human clinical trials. Hussain *et al.* reported a systematic review calling for further human clinical trials

to establish the efficacy of herbal compounds in AD treatment [52]. Another significant challenge is dosage optimization since the selection of an optimum dose-response relationship for rosemary extracts or active compounds is not easy. Pengelly *et al.* indicated the non-linearity of cognitive effects in their research on rosemary powder, highlighting the necessity for accurate dose optimization. Future studies need to include well-designed, large-scale clinical trials to determine the efficacy and safety of rosemary biomarkers in AD patients. Moreover, dose-finding research should be conducted to determine the ideal dosage of different rosemary-derived constituents such that their curative potential can be maximized.

Combination with available AD therapies

More studies are needed to investigate the potential of rosemary biomarkers to supplement or augment current AD treatments. One promising direction for research is combination therapy in which rosemary constituents can be used synergistically with existing AD drugs to enhance therapeutic effectiveness. Based on Habtemariam, synergism between natural products and existing AD drugs may enhance their global efficacy [53]. Furthermore, heterogeneity in patient responses to AD treatments requires personalized medicine, and rosemary biomarkers can be used as a tool for personalizing treatments to an individual's biomarker profile. Future research should explore rosemary constituents' synergistic interactions with current AD treatments and create individualized treatment regimens with rosemary biomarkers. Although promising, numerous challenges need to be met, including standardization of rosemary extracts, enhancing bioavailability, optimal BBB permeability, and proper clinical trial designs. In addition to this, the integration of rosemary biomarkers with current medications for AD will need tremendous research. Such challenges need to be overcome to realize the full therapeutic potential of rosemary biomarkers for AD treatment.

CONCLUSION

The extensive review promises the great potential of rosemary, *R. officinalis* L., sourced from the Nilgiris district, as a source of biomarkers in the quantification and treatment of AD. The unique geographical and climatic conditions of the Nilgiris provide a distinctive phytochemical profile to the locally cultivated rosemary, making it a very promising avenue in the research and intervention areas of AD. Some of the major bioactive compounds identified are RA, CA, luteolin, and 1,8-cineole. These compounds have multifunctional neuroprotective activity against AD pathology; they are involved in antioxidant and anti-inflammatory actions, modulation of Aβ and tau pathology, and enhancement of cholinergic function. They also have potential as a biomarker for the quantification of AD; they are measurable in biological fluids and correlate well with AD-related processes. Pre-clinical studies have evidenced the therapeutic effect of rosemary on AD treatment, due to neuroprotection, enhancement of cognitive function, inhibition of Aβ aggregation, and reduction of tau hyperphosphorylation. On the other hand, standardization of rosemary extracts is still needed, together with improvement of bioavailability and BBB penetration, and well-designed clinical trials. The future research on Nilgiris rosemary should be done in the direction of the preparation of standardized protocols for its cultivation, extraction, processing, enhancement of the bioavailability of key compounds, large-scale clinical trials, and combination therapies with the already existing AD drugs and personalized medicine approaches. Although much research is still needed to overcome the existing challenges and tap the full potential of rosemary biomarkers in clinical applications, the special properties of Nilgiri rosemary underline the importance of regional biodiversity in drug discovery and development against neurodegenerative diseases. The polypharmacological profile of rosemary active principles could be considered a promising multicomponent strategy in AD management, which deserves further research and development.

CONFLICTS OF INTEREST

The authors have no conflicts of interest regarding this investigation.

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AUTHORS CONTRIBUTIONS

Dhanush V: Literature review, Data curation, Writing original draft, and Evaluation; Sukeshan M P: Writing original draft, Conceptualization, Critical Evaluation; Suranther Krishnamoorthi: Writing original draft, Conceptualization, Critical Evaluation; Jeevan S: Writing original draft, Conceptualization, Critical Evaluation; Harish N: Writing original draft, Conceptualization, Critical Evaluation; Jeyaprakash M R: Review and editing, Supervision, Evaluation, Visualization.

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