Mallappa Kumara Swamy
Mohd Sayeed Akhtar *Editors*

Natural Bio-active Compounds

Volume 2: Chemistry, Pharmacology and Health Care Practices

Springer

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ISBN 978-981-13-7204-9 ISBN 978-981-13-7205-6 (eBook)
https://doi.org/10.1007/978-981-13-7205-6

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The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

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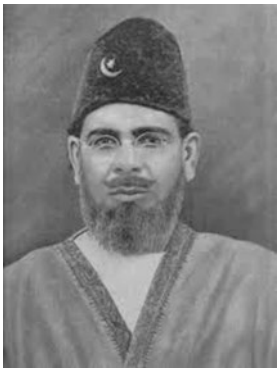

Maulana Mohammad Ali Jauhar (1878–1931)
A great scholar, historian, educationist and social reformer of the twentieth century

Foreword

Bio-active compounds derived from various natural sources, such as plants, fungi, lichens, etc., have become an integral part of the present-day medicine. They have been widely used as a source of medicine since ancient times in various forms of traditional medical practices. Even now, many health issues are being addressed using natural products or naturally derived bio-active compounds worldwide. Many traditional medicinal systems, including Ayurveda, Unani, Siddha, and Homeopathy depend on plant products or their phytocompounds. Some examples include alkaloids, glycosides, polyphenols, resins, saponins, tannins, terpenoids and oils. Notably, natural compounds improve human health and vitality without causing adverse side effects as compared to synthetic drugs. Also, they are relatively cheaper and easily available. Natural compounds exhibit high chemo-diversity with exceptional molecular scaffolds, and thus offer the possibility of synthetic alterations to increase their bioactivity. Therefore, natural resources are highly preferred in developing new drug molecules with therapeutic efficiency. Considering this scenario, researchers and pharmaceutical industries are paying more attention to natural product research for innovating novel key drug molecules. Further, several natural products are beneficial to plants as they encourage growth and development. Importantly, they allow plants to withstand environmental stress and pathogenic attack. Initially, natural bio-active compounds are extracted using various extraction techniques, and their bioactivity is identified using *in vitro* and *in vivo* testing. However, the drug molecule has to pass the clinical trials to be used as a functional drug. As with any promising field, pitfalls and drawbacks are inevitable; these include poor bioavailability and unknown pharmacodynamics/pharmacokinetics. Overall, natural resources provide unlimited opportunities to discover new drug leads. Thus, understanding the chemistry, pharmacology and healthcare practices of natural bio-active compounds could significantly support the modern drug discovery processes.

The present book *Natural Bio-active Compounds – Volume 2: Chemistry, Pharmacology and Health Care Practices*, includes 18 chapters contributed by academicians, scientists and researchers from different parts of the globe. Chapter 1 by Khan et al., summarizes the secondary metabolites of *Rosmarinus officianalis* and their protective action against neurological disorders, while Chap. 2, by Anwar et al., elucidates the plant metabolites as drug molecules with a focus on their

screening, based on drug likeliness features and properties. Chapter 3, contributed by Indian authors, discusses the current insights on the role of terpenoids as anticancer agents, while Chaps. 4 and 5 uncovers the botanical details, pharmacological and toxicological aspects of *Myristica fragrans* and *Coscinium fenestratum*. Chapter 6 is a joint collaboration of authors from UK, Spain, Brazil and France, highlighting the relevance of pharmacogenomics and computational design in drug discovery, including information on the benefits of using plant secondary metabolites for the production of anti-malarial compounds, while Chap. 7 by Malaysian authors provides a range of *in vitro* and *in vivo* studies and discusses the anticancer efficacy of various curcumin nano-formulations. Likewise, in Chap. 8, Hussain et al. discuss the techniques for extraction, isolation and standardization of bio-active compounds, and Chap. 9 by Indian investigators highlights the role of phytocompounds in treating *Glioblastoma multiforme*. Chapters 10 and 11 by Indian contributors, deal with the cosmetic potential of natural products for industrial applications, and use of natural compounds and their utilization in the treatment of diabetes and hypertension. Similarly, Chap. 12 by Sasidharan and Saudagar explains the plant metabolites as new leads to drug discovery, while Chap. 13 by Faujdar and Priyadarshini explains the role of natural compounds in treatment of cardiovascular diseases and kidney diseases including acute renal failure and chronic kidney diseases. Chapter 14, by Ravindran et al., discusses the dietary polyphenolics and their bioavailability along with their beneficial mechanism of action in treating various diseases. Subsequently, the chapter by Lodh and Swamy discusses the major medicinal plants of North-East India and their phytochemical aspects to cure various gynaecological disorders. Similarly, Chap. 16 of Makbul et al., highlights the role of bio-active compounds extracted from Unani plants in treating diverse pathological conditions of urolithiasis, while Chap. 17 by Kalam et al., updates the knowledge on the importance of rhizomatous plants with special reference to their therapeutic application in Unani system of medicine in the management of different diseases. Finally, the last chapter by Perihan et al., describes the extraction techniques of bio-active compounds derived from plants.

Overall, the chapters included in this book volume show that various natural bio-active compounds are effective in the treatment of various human health problems. These bio-active compounds exhibit copious pharmacological activities, including antioxidant, anti-inflammatory, antibacterial and anticancer properties. The information contained in this book will be very useful for researchers, undergraduate and postgraduate students studying the chemistry and pharmacognosy of natural bio-active compounds and biomedical sciences, and also for healthcare professionals. Altogether, this edited book provides detailed aspects of natural sources, their

bio-active compounds and their pharmacological significance. I applaud the editorial board members, Dr. Mallappa Kumara Swamy and Dr. Mohd Sayeed Akhtar, as well as the book chapter contributors for successfully bringing this book volume.

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Khalid Rehman Hakeem

Preface

Secondary metabolites are a unique group of compounds produced by plants to protect itself against various biotic and abiotic factors (diseases, pests, pathogens, herbivores, environmental stresses, etc.). These compounds, however, do not influence primary metabolic activities, such as growth and reproduction of plants. The major classes of secondary metabolites include phenolics, alkaloids, tannins, saponins, lignins, glycosides, and terpenoids. Some of these compounds have become an integral part of plant–microbe interactions towards adapting to environmental irregularities. They regulate symbiosis, induce seed germination, and show allelopathic effect, i.e., inhibit other competing plant species in their environment. Moreover, these compounds induce adverse physiological activities such as reduced digestive efficiency, reproductive failure, neurological problems, gangrene, goiter, even death and also possess high toxicity. The discovery of such unique compounds has inspired many scientific communities to explore their potential applications in various fields including agriculture and biomedicine. For instance, plant secondary metabolites are utilized to manufacture eco-friendly bio-pesticides and as drug sources in medicine. Due to numerous health-promoting properties, these compounds have been widely used as a source of medication since ancient times. The assessment of plant secondary metabolites for their wide-ranging therapeutic potential has led to the discovery of many drug leads in recent times. Therefore, this field of research has become a significant area for researchers interested to obtain understanding of the chemistry, analytical methodologies, biosynthetic mechanisms, and pharmacological activities of these plant secondary metabolites.

Use of natural bio-active compounds and their products are considered as most suitable and safe as alternative medicine. Thus, there is an unprecedented task to meet the increasing demand for plant secondary metabolites from flavour and fragrance, food to pharmaceutical industries. However, their supply has become a major constraint as their large-scale cultivation is very limited. Moreover, it is difficult to obtain a constant quantity of compounds from cultivated plants as their yield fluctuates due to several factors including genotypic variations, geography, edaphic conditions, harvesting and processing methods. In addition, medicinal plants have become endangered due to ruthless harvesting in nature. Alternatively, the plant tissue culture approaches can well be explored to produce secondary

metabolites without practising of conventional agriculture which requires more land space. *In vitro* cell and tissue cultures requires less space and are grown under controlled lab conditions, and hence offer advantages of producing the desired compounds continuously without affecting their biosynthesis and quality. Furthermore, these cultures can be scaled up to produce metabolites in very large bioreactors and also, using genetically engineered cells/tissues, novel products can be obtained. The proper knowledge and exploration of these *in vitro* approaches could provide an optional source to produce plant secondary metabolites from many medicinal plants in large scale.

Natural Bio-active Compounds: Volume 2 – Chemistry, Pharmacology and Health Care Practices is a very timely effort in this direction. This book volume with 18 contributions from Brazil, France, India, Malaysia, Spain and UK well discusses the Chemistry, Pharmacology and Health Care Practices of natural bio-active compounds need of time for human welfare aginst various human diseases in also well discussed. This book will be a valuable resource for researchers working towards identifying and characterizing new bio-active agents from diverse flora, enabling the discovery of novel therapeutic leads in the future against various diseases and also for the graduate and undergraduate students, teachers, industry persons and healthcare professionals involved in natural products and therapeutic research areas.

We are highly grateful to all our contributors for readily accepting our invitation for not only sharing their knowledge and research, but also meticulously integrating their expertise in diverse fields in composing the chapters and enduring editorial suggestions to finally produce this venture. We greatly appreciate their commitment. We are also thankful to Professor Khalid Rehman Hakeem for his suggestion and writing the foreword for this volume. We also thank the team of Springer International, especially Dr. Kapila Mamta and Raagaipriya Chandrasekaran, for their generous cooperation at every stage of publication.

Bengaluru, Karnataka, India — Mallappa Kumara Swamy
Shahjahanpur, Uttar Pradesh, India — Mohd Sayeed Akhtar

About the Book

This volume is a compiled resource of systematically refined information on the sources, chemistry and pharmacological and biological properties of natural bio-active compounds used in common healthcare practices, such as natural, modern, Ayurved and Unani systems of medicine. The latest evidences on the botanical details and pharmacological and toxicological aspects of bio-active compounds of plant sources are highlighted. In addition, the book discusses the pharmacogenomics and computational designs towards drug discovery, nano-formulations involving bio-active compounds and their efficacy, and safe uses against some of the human diseases. *Natural Bio-active Compounds: Volume 2-Chemistry, Pharmacology and Health Care Practices* is a very timely effort in this direction. This book will be a valuable resource for researchers working towards identifying and characterizing new bio-active agents from a diverse flora and enabling the discovery of novel therapeutic leads in future against various diseases. Moreover, it benefits the graduate and undergraduate students, medicinal chemistry, natural product research and also teachers, industry persons and healthcare professionals involved in natural products and therapeutic research areas.

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About the Editors

Dr. Mallappa Kumara Swamy (PhD) is a Professor and Head of the Department of Biotechnology at East West First Grade College of Science, Bengaluru, Karnataka, India. He completed his postdoctoral research at the Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia. Before that, he had worked as an Associate Professor and as Head of the Department of Biotechnology, Padmashree Institute of Management and Sciences, Bangalore University, Bengaluru, India. He received his PhD (Biotechnology) from Acharya Nagarjuna University, Guntur, India, in 2013. He has more than 15 years of teaching and research experience in the fields of plant biotechnology, secondary metabolite production, phytochemistry, and bio-active studies. Dr. Swamy has authored 80 research publications in peer-reviewed journals and 24 book chapters. He has edited four books published by Springer Nature Singapore Pte Ltd., Singapore. Recently, he has edited one book published by CRC Press LCC, USA, and one book published by Studium Press Pvt. Ltd., India. He also serves as an editorial board member and reviewer for several high-impact international journals. His current research is focused on cell and tissue culture technology for bio-active compound production, phytochemistry, phytocompounds isolation, production and their biological evaluation, and on nanotechnology for medical applications.

Dr. Mohd Sayeed Akhtar (PhD) is an Assistant Professor at Gandhi Faiz-e-Aam College, Shahjahanpur, UP, India. He received his PhD degree from Aligarh Muslim University (AMU), India, in 2008, prior to conducting postdoctoral research at the Botanical Institute, University of Basel (BIB), Switzerland (2008–2010), and at Chonbuk National University (CBNU), Republic of Korea, in 2011. He was an Assistant Professor at Jimma University, Ethiopia (2011–2014), and a fellow researcher at the Institute of Tropical Agriculture, Universiti Putra Malaysia (UPM), (2014–2015). Dr. Akhtar has more than 15 years of research and 10 years of teaching experience in soil microbiology, applied microbiology, environmental microbiology, molecular biology, plant pathology, and plant nanobiotechnology. He has authored or co-authored more than 100 articles in peer-reviewed journals, confer-

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1 Secondary Metabolites from Rosemary (*Rosmarinus officinalis* L.): Structure, Biochemistry and Therapeutic Implications Against Neurodegenerative Diseases

Sahir Sultan Alvi, Parvej Ahmad, Maleeha Ishrat, Danish Iqbal, and M. Salman Khan

Contents

Abstract

Rosemary (*Rosmarinus officinalis* L.), the representative of Lamiaceae family is known for its various medicinal uses that are accompanied by their hallmark secondary metabolites, i.e., carnosol, carnosic acid and rosmarinic acid (mostly the poly-

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M. K. Swamy, M. S. Akhtar (eds.), *Natural Bio-active Compounds*,
https://doi.org/10.1007/978-981-13-7205-6_1

phenolic diterpenes). In the age of medicines and methodologies, when we are floating through the advancements and achievements, we are being hijacked by various diseases leading to increased number of young deaths. Neurological disorders are one of them and characterized by any impairment in the nervous system, brain or spinal cord. The majority of young and aged people around the globe are manifested by neurological disorders, i.e., stroke, epilepsy, dementia, Alzheimer's disease (AD), Parkinson's disease (PD) and migraine. A large number of therapeutic approaches mend the symptoms in early stages of these disorders, but with the span of time, patients become progressively more disabled as they may suffer from drug-associated adverse effects. Emphasizing on the urgent need of alternative therapeutic regimens, natural products are encouraged worldwide in terms of safety and to minimize the aforesaid loss. In this order, the current chapter summarizes the protective role of *R. officinalis* L. and its bio-active metabolites against various neurological disorders via targeting amyloid-beta (A-β) aggregation, neuronal cell death, acetylcholinesterase (AChE), neuroinflammation, β-secretase (BACE-1) activity, mitochondrial redox status, etc. Based on the multifunctional nature due to effective bio-active secondary metabolites, *R. officinalis* can be a terrific alternative therapeutic source against many neurodegenerative diseases.

Keywords
Secondary metabolites · Essential oils · Neurological disorders · Molecular markers

1.1 Introduction

Alzheimer's disease (AD) has been considered as the major worldwide health anxieties as it shares about 60–80% of the pathologies of dementia (Wortmann 2012). According to a recent estimation, there are more than 45–50 million subjects suffering with epilepsy and dementia across the globe, and this count is increasing with a rise of 7.70 million newly diagnosed cases annually, whereas the contribution of migraine is also influential among neurodegenerative disorders (Wilmo and Martin 2010). Among distinct pathologies of AD, "amyloid hypothesis" has gained the most attention, which refers to the aggregation of amyloid-beta (A-β) as a major determinant of the continuous death of brain neuronal cells. The most challenging pathologies of AD are erratic emotion, impaired memory, sleep disorders and loneliness which have been linked to A-β-stimulated injury to cholinergic neurons, inflammation, reactive oxygen species (ROS) and excitotoxicity mechanisms (Whitehouse et al. 1982). The site of the origin of the cortical cholinergic neurons, forebrain region, is a crucial target for most of the AD pathologies, and neuronal loss in this part of the brain reflects the degree as well as severity of AD

symptomatology (Whitehouse et al. 1982). Till date, various classes of drugs have been tested to alleviate AD which are the acetylcholinesterase (AChE) inhibitors, e.g. rivastigmine, tacrine and donepezil, and antagonist of N-methyl-D-aspartate (NMDA) receptor such as memantine (Raschetti et al. 2007). The current strategies of therapeutic management have a potential to resist the degenerations by restoring the symptoms and manifestations in initial phases of disease, but with the span of time, patients become progressively more disabled as they may suffer from drug-related side effects.

In medicinal aspects, Lamiaceae is a very important family of the plant kingdom and also recognized as mint family. Their capability to produce essential oils allows most of the members of this family to survive high temperatures of the Mediterranean countries. The rosemary (*Rosmarinus officinalis* L.), representative of Lamiaceae family, has been reckoned as a perennial herb which was originated in the Mediterranean area and is now widely distributed across the globe due to its great ornamental, cosmetic, nutritional and medicinal values (Barbosa et al. 2015; Habtemariam 2016). *R. officinalis* L. have shown numerous pharmacological effects, i.e. antioxidant, anticancer, hepatoprotective, antidiabetic, antispasmodic, antiseptic and sedative properties (Rašković et al. 2014; González-Vallinas et al. 2014; Wang et al. 2012; Felicidade et al. 2014; Barbosa et al. 2015). *R. officinalis* L. has been established as a promising herbal remedy for the treatment of headaches, circulatory disorders and inflammation as well as physical and mental fatigue (Yu et al. 2012; Takaki et al. 2008).

Various parts of the *R. officinalis* L. can be used either freshly or dried and extracted to obtain essential oils, a colourless or pale yellow liquid, owed to the richness of its bio-active compounds (Barreto et al. 2014). Rosemary essential oils (REO), generally volatile and aromatic in nature, are metabolically synthesized as well as extracted from almost each and every part of the plant (Li et al. 2015; Teixeira et al. 2013). These products are highly valuable for the economy due to their applications in cosmetic, medical and food industries (Harkat-Madouri et al. 2015). Most of the pharmacological effects of *R. officinalis* L. extract or REO are attributed to their hallmark secondary metabolites such as carnosol, α-pinene, camphor, carnosic acid (CA), 1,8-cineole and rosmarinic acid (mostly polyphenolic diterpenes) (Barreto et al. 2014; Borrás et al. 2011; Jordán et al. 2012; Pérez-Fons et al. 2010; Li et al. 2014).

Recently, REO and their active metabolites are under the spotlight due to their salubrious effects. Therefore, we hypothesized that REO and its bio-actives may be used as alternative to classical therapeutic regimens for the treatment of neurological disorders. In this chapter, chemistry and pharmacology of *R. officinalis* L. and its secondary metabolites have been explored especially focusing our attention to their therapeutic efficacy against neurological disorders via targeting various markers, i.e. A-β aggregation, neuronal cell death, β-secretase (BACE-1), AChE, neuroinflammation, mitochondrial redox status, etc.

1.2 Secondary Metabolites from *Rosmarinus officinalis* L.: Structure, Biochemistry and Bioavailability

The REO is a complex remedy containing various independent compounds, volatiles, distinct classes of terpenes, aromatic compounds, proteins, fibres, vitamins as well as minerals, which have shown their pharmacological importance (Lovkova et al. 2001) (Fig. 1.1). As far as the *R. officinalis* is concerned, the major bio-actives are rosmarinic acid, camphor, caffeic acid, ursolic acid, betulinic acid, CA and carnosol (Begum et al. 2013; Ulbricht et al. 2010). Thus, the key phytochemicals of *R. officinalis* L. are phenolic metabolites, di- and triterpenes and essential oils (Aumeeruddy-Elalfi et al. 2015, 2016). Leaf-derived REO is generally colourless to very light yellow and insoluble in aqueous solutions and represents a characteristic aroma of camphor (Faixová and Faix 2008; Begum et al. 2013). The main constituents of the REO are 1,8-cineole, α-pinene, borneol, camphene, camphor, β-pinene and limonene, and their content may be varied depending on the age and growth stage of the vegetation as well as physiological and ecological settings (Begum et al. 2013; Satyal et al. 2017).

Polyphenols from *R. officinalis* L. show antioxidant properties and potentially aid in fruit colouring, and these are further categorized in subcategories, i.e. phenolic acids, flavonoids and non-flavonoids (Doughari 2012). The structure of various bio-active secondary metabolites from *R. officinalis* L. has been illustrated (Fig. 1.2). These metabolites participate in the defence mechanisms mounted towards the

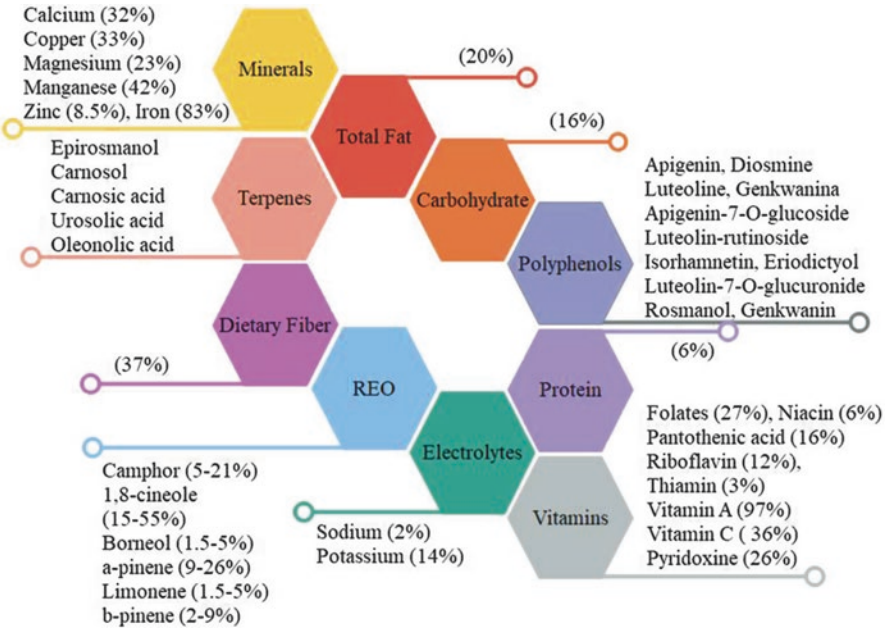

Fig. 1.1 Types of major phytochemicals present in *R. officinalis* L. and REO

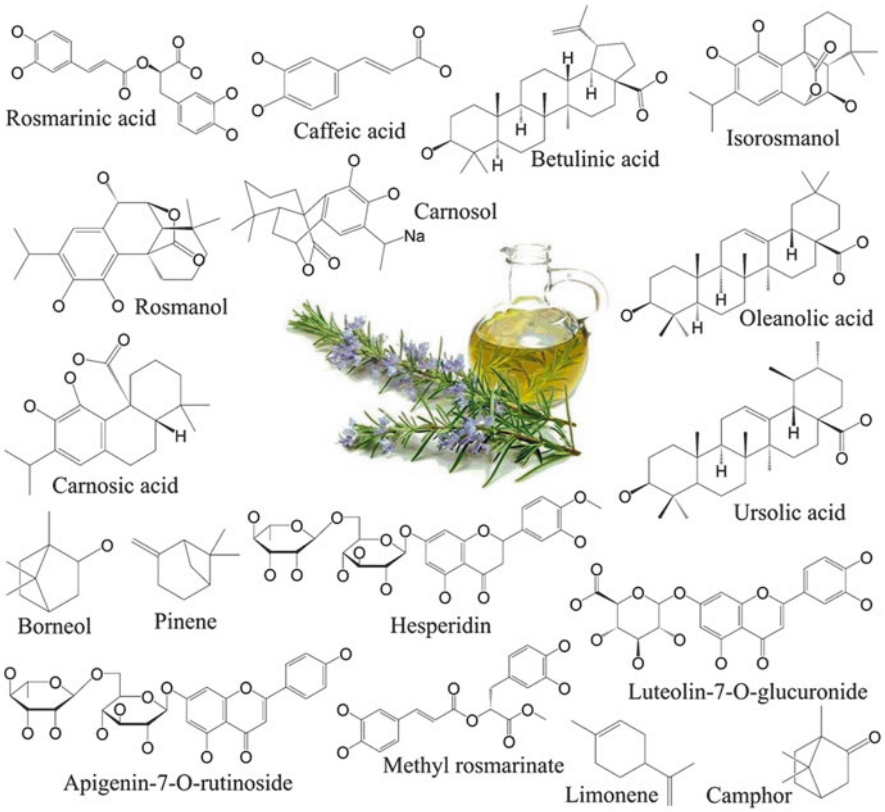

Fig. 1.2 Structural representation of various potent secondary metabolites from *R. officinalis* L. and REO

herbivores and pathogens; hence, these have been implied against the infectious agents of humans (Doughari 2012). In *R. officinalis* L., the most common polyphenols are apigenin, diosmin, luteolin, genkwanin and phenolic acids (>3%), especially rosmarinic acid, chlorogenic acid and caffeic acid (Samuelsson and Bohlin 2001; Al-Sereiti et al. 1999). Additional metabolites of rosemary are terpenes which include over 10,000 compounds differing in the number of carbon atoms and isoprene groups (C_5H_8) (Lovkova et al. 2001; Doughari et al. 2012). The most potent terpenes from *R. officinalis* L. are epirosmanol, carnosol, CA, ursolic acid and oleanolic acid (triterpenes) (Ulbricht et al. 2010). In addition, five novel officinoterpenosides (i.e. A1, A2, B, C and D) were also discovered from *R. officinalis* L. ethanolic extract (Zhang et al. 2014). CA showed the ability to penetrate the blood-brain barrier in mammals (Satoh et al. 2008a).

Aiming to the assessment of the therapeutic efficacy of CA in targeting neurological disorders, Doolaege et al. (2011) analysed the bioavailability of CA through absorption, distribution, metabolism and excretion (ADME) study in which CA was administered to the rats either intravenously (20.5 mg/kg.B.Wt./Rat) or orally

(64.3 mg/kg.B.Wt./Rat). They reported that after 6 h of the administration, the bioavailability of CA was up to 40.1%. This study also concluded that CA was detected in distinct tissues of the rats in its free form. Moreover, the excretion of CA through the faeces, after 24 h after oral administration, was only up to 15.6 ± 8.2% (Doolaege et al. 2011). In the next study regarding the bioavailability of CA, Vaquero et al. (2013) found that CA exists in the form of glucuronide conjugates (metabolic intermediates of CA) in various tissues. The other metabolites that were mainly detected in these organs were the 12-methyl ether and 5,6,7,10-tetrahydro-7-hydroxyrosmariquinone of CA (Vaquero et al. 2013). The bioavailability of *R. officinalis* L. diterpenes is very high as these have been detected within a short span of time (25 min) only, after the oral/gastric intubation. Most importantly, the CA and its derivatives were also identified in the brain tissues of the animal models, signifying their possible neuroprotective impact (Vaquero et al. 2013).

1.3 Mechanistic Insights into Pharmacological Effects of *R. officinalis* Extract, REO and Its Secondary Metabolites

1.3.1 Arresting Neurological Problems with *R. officinalis* Extracts and Its Secondary Metabolites

Comparing to more than previous two centuries in which the brain was considered only as a black box capable of transmitting input and output signals, without knowing the mechanistic insights of all the neurological entities, the third millennium became more advanced and focused much deeply into the actual causes and mechanisms of the neurological assets. These advancements are directed towards the development of alternative therapeutic approaches to overcome these brain-associated deadly pathologies. In addition to acting as a potent antioxidant, antimicrobial, anticancer and hepatoprotective agent, *R. officinalis* is far more serviceable as for neurological disorders. Some of its major neurological targets are appended below.

1.3.1.1 Protective Effect on Neuronal Cells

The applications of *R. officinalis* from the ancient age suggested that it may be implied in targeting ROS and apoptosis that triggered distinct neurological manifestations. In the same vein, Park et al. (2010) assessed the neuromodulatory properties of *R. officinalis* extract against H_2O_2-mediated apoptosis in human dopaminergic cells. Apart from the *R. officinalis* extracts, CA, one of the most potent bio-active compounds from REO, has been found to protect SN4741 cells from the toxicity of environmental neurotoxin dieldrin, an organochlorine pesticide, via enhancing brain-derived neurotrophic factor (BDNF) and limiting the apoptotic events (Park et al. 2008). Similarly, Kim et al. (2010a) also demonstrated that carnosol inhibits sodium nitroprusside-induced C6 glial cell apoptosis via induction of heme oxygenase-1 (HO-1) expression. Shimojo et al. (2010) also reported that extract from *R. officinalis* L. and particularly rosmarinic acid possess noticeable modulatory impact

on motor performance, body weight reduction, morphological features of motor neurons and clinical scoring in AD mice model. These findings strongly suggested that this herb could be one of the most prominent agents to control the symptoms of AD in addition to the other neurodegenerative diseases such as PD.

The modern predominant theory of neurodegenerative illness suggests that functioning of synaptic and extrasynaptic N-methyl-D-aspartate receptors (syn- and ex-NMDAR) influences the fate of the cell, whereas neuronal apoptosis is mainly regulated by the activation of ex-NMDAR (Zhou et al. 2013). Pretreatment with CA protected primary immature cortical neurons as well as primary mature cerebrocortical neuronal cells, expressing NMDAR, against N-methyl-D-aspartate (NMDA)-mediated excitotoxicity by facilitating Nrf2/ARE (Martin et al. 2004). A neurotoxin, 6-hydroxydopamine (6-OHDA), is believed to promote injury into dopaminergic neuron in PD models via robust ROS production, mitochondrial dysfunction and enhanced phosphorylated c-Jun N-terminal kinase (JNK) and p38, which have been also investigated in post-mortem PD brains (Kim et al. 2010b; Lee et al. 2010; Song et al. 2010; Tian et al. 2007; Hu et al. 2011). Administration of CA reduced the level of ROS to amend 6-OHDA-induced activation of JNK1/2 and p38 (Jiang et al. 2004; de Oliveira 2015). Therefore, CA was effective against different neurotoxic agent-induced neurological ailments via maintaining mitochondrial redox homeostasis.

Glutathione (GSH), a key endogenous antioxidant from various cell types including neurons and astrocytes, is responsible for the protection of these cells against ROS-mediated apoptosis and also performs detoxification (Wu et al. 2004; Martin and Teismann 2009; Jia et al. 2009). Studies have also confirmed that the fall in the GSH content promotes neuronal cell death via increased ROS, inhibition of mitochondrial complex I and impaired autophagy events (Verma and Nehru 2009; Vali et al. 2007). In this order, CA ameliorates 6-OHDA-mediated apoptosis in SH-SY5Y cells by promoting synthesis of GSH (Chen et al. 2012). CA is a better neuroprotective agent than carnosol and prevents oxidative glutamate toxicity in HT22 neuronal cells (Satoh et al. 2008b; Tamaki et al. 2010). Rosmarinic acid also attenuated motor neuron death in familial amyotrophic lateral sclerosis (ALS) mouse model (Shimojo et al. 2010), whereas CA possesses similar effects in a mouse model of AD (Azad et al. 2011). Administration of CA significantly reduced the infarct volume (by 52%) in the area of the cortex, caudate and putamen of all animals exposed to focal cerebral ischemia/reperfusion (Hou et al. 2012). CA also showed a neuroprotective effect in vitro in human-induced pluripotent stem cell (hiPSC)-derived neurons and against cyanide-induced brain damage in a mouse model in vivo (Zhang et al. 2015).

CA significantly protected overall integrity of cerebellar granule neurons (CGNs) against 5K-induced apoptosis at 10 and 20 μM via targeting PI3K pathway, whereas rosmarinic did not. Moreover, CA significantly protected CGNs from 5K-induced apoptosis in the presence of PD98059, a known MEK/ERK inhibitor. This suggests that carnosic acid prevents caspase activation through a mechanism independent of MEK/ERK signalling. Rosmarinic acid also protected HA14-1-mediated oxidative imbalance and mitochondrial apoptosis in aCGN model, where HA14-1 is a well-reckoned Bcl-2 inhibitor. It also markedly helped in regaining of the memory loss

caused by aggregation of A-β in models of both AD and ALS and prolonged the lifespan by delaying the manifestations (Alkam et al. 2007; Shimojo et al. 2010).

1.3.1.2 Protective Effect on Glial Cells

The beneficial effects of CA against neurodegeneration in both neuronal and glial cells are attributed to distinct mechanisms including its ability to cross blood-brain barrier. Kosaka and Yokoi (2003) showed that CA influences the production of neurotrophic factors in glial cells. Another study evidenced that CA up-regulates the expression of nerve growth factor (NGF) without affecting the levels of BDNF or neurotrophin-3 (NT-3) (Vigé et al. 1991). The CA-mediated up-regulation of NGF expression in activated glial cells was achieved by a Nrf2-dependent mechanism. These findings are advocating the beneficial neuroprotective impact of CA that was achieved by its antioxidant mechanisms, and it may be helpful in targeting neuronal plasticity (Maes et al. 2012; Mattson and Cheng 2006). CA also showed the potential to increase the HO-1 expression in mouse microglial cells (de Oliveira 2015).

Additionally, CA promotes the elevated bilirubin levels in the presence or absence of hemin, a substrate of HO-1 (Sperner-Unterweger et al. 2014). Moreover, different studies have confirmed that CA modulates the nitric oxide (NO) generation in LPS-challenged microglial MG6 cells in a concentration-dependent manner (Jiang et al. 2004; Yanagitai et al. 2012). Rosmarinic acid has also been demonstrated to decrease NO production in glial cells in order to protect dopaminergic neurons (Lo et al. 2002; Park et al. 2008). To sum up the above-mentioned pharmacological effects, it can be concluded that these metabolites from *R. officinalis* L. alleviate the nitrosative stress either by directly quenching the reactive nitrogen species (RNS), i.e. NO, or by reducing NO production from glial cells.

1.3.1.3 Effect on Neuronal Redox Homeostasis

Excessive generation of NO has been reported in cases of both PD and AD that may be attributed to neuroinflammation (Barres and Barde 2000). Therefore, *R. officinalis* and its secondary metabolites may be implied in targeting ROS generation and neuroinflammation. The in vitro antioxidant activity through DPPH assay showed that REO had a strong antioxidant activity (IC_{50} = 77.6 μg/ml) when compared to α-tocopherol (also known as vitamin E; IC_{50} = 25.3 μg/ml), reference standard drug. This potent radical quenching potential of REO was correlated with the phenolic content which was determined through Folin-Ciocalteu method (Viuda-Martos et al. 2010a; Rašković et al. 2014). REO has also been shown to reverse the CCl_4-induced oxidative damage to hepatocytes up to normal levels in rats via decreasing the level of malondialdehyde (MDA) and enhancing the activities of hepatic catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (Gred) (Rašković et al. 2014). Rosmarinic acid is also capable of scavenging the RNSs, peroxynitrite and various ROS (Choi et al. 2002; Qiao et al. 2005). Viuda-Marto et al. (2010b) suggested that REO can be reflected as a key source of various metabolites possessing strong antioxidant activity, which may be due to either the individual or synergistic action of distinct REO constituents.

Posadas et al. (2009) suggested that the administration of rosemary balanced the redox state in old rats by decreasing the lipoperoxidative events in brain tissue and stimulating the antioxidant enzyme activity particularly CAT as well as ameliorating the activity of nitric oxide synthase (NOS). Carnosol also up-regulated the expression of HO-1 through activation of the PI3K/Akt/Nrf2 signalling pathway in C6 cells (Sperner-Unterweger et al. 2014). CA and carnosol also participate in the signalling pathways regulating the control of the oxidative stress and immune functions in neuronal as well as glial cells (Jarrott and Williams 2015). CA enhances carcinogen detoxification and protects against oxidative stress (Manoharan et al. 2010). A report by Hou et al. (2012) showed that CA protected PC12 cells from hypoxia-induced cell injury at a concentration range of 0.1–1.0 μM. However, CA also reduced hypoxia-induced lactate dehydrogenase (LDH) release when used at a concentration of 1.0 μM and also increased cell viability. Similarly, PC12 cells under hypoxia accelerated the generation of ROS in culture supernatants, which was ameliorated by CA (1.0 μM) that neutralized up to 8% of hypoxia-induced ROS in PC12 cells. Treatment with CA also reduced MDA concentration by 25% and preserved SOD activity in hypoxia-induced PC12 cells (Hou et al. 2012).

Four diterpenoids, CA, carnosol, rosmanol and epirosmanol, significantly inhibited superoxide anion production in the xanthine/xanthine oxidase (XOD) system, representing their antioxidant potential (Haraguchi et al. 1995). All these metabolites markedly inhibited the NADH or NADPH oxidation-induced lipoperoxidative events at very low (micromolar) concentrations (Haraguchi et al. 1995). A recent report demonstrated that CA safeguards red cells against oxidative haemolysis (Miraj 2016). Furthermore, CA also binds to Kelch-like ECH-associated protein 1 (Keap1) in a dopaminergic cell line PC12h and activates the antioxidant response element (ARE) (de Oliveira 2015). In addition, rosmarinic acid and carnosol also showed similar antioxidant activity, but carnosol was found to be an extremely potent antioxidant, and this differential antioxidant activity suggested that factors other than the antioxidant capability may be responsible for the various pharmacological effects of these metabolites (Pérez-Fons et al. 2010).

1.3.1.4 Effect on Amyloid-β Processing and Aggregation

AD is the most frequent and fatal neurodegenerative ailment and is characterized by the structural and functional loss in neuronal cells (Baig et al. 2018). The progression of AD pathology is accelerated by the extracellular A-β plaque formation and aggregation of neurofibrillary tangles inside neurons; mainly hyperphosphorylated tau (τ) protein (VanItallie 2015). Such impairments subsequently lead to neuronal loss and cell death via apoptosis. Although the actual mechanism underlying AD progression remains a secret yet, the A-β cascade theory is the most influential one and reinforced by an array of studies (Drachman 2014; Herrup 2015). A-β is a key element of extracellular amyloid plaque, and the expression of A-β plays a major part in the progression of AD (Li et al. 2017). A-β peptide is the product of the b- and g-secretase-mediated sequential proteolytic cleavages of amyloid polypeptide (APP). These proteolytic cleavages generate two types of A-β isoforms (A-β 40 and A-β 42). A-β 40 is more ample than A-β 42 in various human fluids, whereas A-β 42

aggregates faster than A-β 40, and its regulation is believed to be more important for the survival of neuronal cells. Oligomers of diffusible A-β including protofibrils, prefibrillar aggregates and A-β-derived diffusible ligands (ADDLs) have been established as the key constituents of AD progression (Haass and Selkoe 2007; Shankar et al. 2008; Funke and Willbold 2012).

A-β homeostasis in the brain is mainly determined by distinct cellular events, i.e. its production, processing, degradation, localization out of the brain and the accumulation of insoluble aggregates. These events may be targeted to reduce A-β aggregation in the brain, whereas, among these, inhibition of Aβ expression is considered as the most promising marker in targeting AD (Menting and Claassen 2014). Numerous clinical trials based on amyloid reduction therapy (ART) have failed to improve AD pathologies (Grundman et al. 2013; Cheng et al. 2017), thus making an obvious call for the novel and alternative therapeutic regimen from natural sources as a substitute to presently available ART.

In this order, Yoshida et al. (2014) reported that CA blocks the production of Aβ42 and Aβ43 peptides via increased expression of the α-secretase TACE in U373MG cells. CA also reduced the production of Aβ40 and Aβ42 in both normoxia and hypoxia conditions but did not alter the expression pattern of α-secretase a disintegrin and metalloproteinase 1 (ADAM1), β-secretase BACE and γ-secretase PS1 (Yoshida et al. 2014). However, various reports have proven that CA upregulates the gene expression of the α-secretase TACE and also increased sAPPα protein expression, whereas the level of β-cleaved soluble fragment of APP was decreased in the above-mentioned cells. These findings suggest that CA promotes α-cleavage rather than β-cleavage leading to increased sAPPα production and decreased A-β production in U373MG cells (Mangoura et al. 1989; de Oliveira 2015). Such evidences suggest that rosemary and its secondary metabolites may down-regulate the production of A-β peptides and subsequently ameliorate the redox status in neuronal cells which may cause the neuronal cell death.

1.3.1.5 Effect on Cholinergic Functions via Acetylcholinesterase (AChE) and Butyryl-Cholinesterase (BChE) Activity

Persistent availability of acetylcholine (ACh), released into the neuronal synaptic cleft, has been correlated with the improved cholinergic function in AD via restricting acetylcholinesterase (AChE) activity (Kwon et al. 2010). AChE has been regarded as the most effective treatment strategy in targeting AD and other related ailments. Different extracts of *R. officinalis* L. had shown their ability to target the active pocket of both the AChE and butyryl-cholinesterase (BChE). Rosmarinic acid from *R. officinalis* L. CH_3OH extract and REO showed a potent ability to inhibit BChE activity in rats (Orhan et al. 2008). 1,8-Cineole and a-pinene were found as the two major monoterpenes in REO (Perry et al. 2000, 2003; Savelev et al. 2003). Moreover, the subchronic administration of complex *R. officinalis* L. extract significantly improved the long-term memory of rats via blocking the AChE active pocket to reduce the affinity of Ach and subsequent hydrolysis. The anti-AChE activity of *R. officinalis* L. extract opens the door for development of therapeutic agents against the risk of neurodegenerative diseases (Ozarowski et al. 2013).

1.3.1.6 Effects on the Functionality of the Synaptic Mitochondria

Recently, increasing attention is being focused on mitochondria, particularly on those located in synaptic compartments. These organelles are vital for brain health, since neurons have limited glycolytic capacity, high susceptibility to ROS-induced damage and elevated need for proper control of the cytosolic calcium levels (Moreira et al. 2010). Therefore, mitochondrial dysfunction may play a major role in AD aetiopathogenesis (Wang et al. 2009). Mitochondrion is a solo organelle responsible for the synthesis of ATP during respiratory chain activity in mammalian cells, and at the same time, it also produces ROS (Genova et al. 2005; Papa et al. 2012; Naoi et al. 2005). It also contains enzymes, i.e. monoamine oxidase (MAO) and nitric oxide synthase (NOS), in which the former produces the H_2O_2 and the latter is responsible for the production of NO in the influence of neuroinflammtion (Brown and Bal-Price 2003; Pun et al. 2010; Venditti et al. 2013; Wilkins and Swerdlow 2016). Considering the extremely high metabolic rates and O_2 consumption of neuronal cells, the ROS generation and mitochondrial dysfunction becomes a prevalent concern as it leads to the initiation and progression of neurological pathologies (Mena et al. 2015).

Mitochondria also contain both enzymatic and nonenzymatic antioxidant defences that play a very important role in the maintenance of the mitochondrial redox status (Venditti et al. 2013; Sies 2015). Oxidative stress, a major factor in neurodegenerative processes, is caused by an imbalance between the oxidants, i.e. ROS and RNS, and endogenous antioxidant defence enzymes. Constant supply of GSH is essential for the homeostasis of mitochondrial redox state (Lu 2013; Morris et al. 2014). The Nrf2 regulates the expression of various antioxidant genes via activation of the ARE of DNA (Itoh et al. 1999; Moi et al. 1994). Up-regulation of such antioxidant genes has been linked to potent neuroprotection by decreasing the production of 4-hyrdroxynonenal (4-HNE), a product of oxidative lipid modification. Miller et al. (2013) concluded that treatment with CA significantly increased the expression of HO-1 mRNA in 4-HNE-induced mitochondrial dysfunction in cortical tissue which ultimately resulted in the induction of the Nrf2-ARE pathway. Moreover, protein expression analysis confirmed that CA also reduced the mitochondria-associated 4-HNE levels. Carnosol, another bio-active from *R. officinalis* L., has also been reported to activate Nrf2 and antioxidant enzymes due to its high electrophilic activity (Satoh et al. 2013; Martin et al. 2004).

1.3.1.7 Antidepressant Activity of *R. officinalis* L. and Its Secondary Metabolites via Targeting HPA Axis and Ach Synthesis

Emotional/mood disorders, including stress and depression, result in atrophy and neuronal degeneration along with the reduction in the major brain structures that ultimately leads to the neuronal inflammation and brain microdamage (Chovatiya and Medzhitov 2014). Various studies have established that the level of brain catecholamine is directly associated with psychiatric diseases, whereas dopamine (DOP) level has been found to protect from depression (Hasler et al. 2008). In this order, REO was assessed for its therapeutic implications against mood disorders. Villareal et al. (2017) found that aroma of REO relieves stress by diminishing the

impact of serum corticosterone and raising the DOP level, clearly indicating the importance of REO in regulating the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic nerve system functions. Furthermore, REO also improves the in vitro brain neurotransmitter activity, ACh synthesis and translocation and induces neuronal differentiation (Villareal et al. 2017).

1.3.1.8 Modulation of Neuroinflammatory Cascades by *R. officinalis* and Its Secondary Metabolites

Recent epidemiological data have established a clear linkage between the inflammatory cascades and neurodegenerative pathways, and the pro-inflammatory markers aid in the progression of neurological ailments (Stephenson et al. 2018; Chen et al. 2016). Considering the drastic role of inflammatory cascades in neurodegenerative disorders, adjusting the release of these inflammatory cytokines in the circulation is considered a major aim of anti-inflammatory agents (da Rosa et al. 2013). Chronic pain and inflammation have worsen the wound healing process and subsequent production of redox mediators that further stimulate the inflammatory process (Backhouse et al. 2008). Therefore, the inflammation and the redox states are considered as the two major factors responsible for neurological disorders; however, some plants and their phytochemicals have shown the ability to reduce these factors (Peng et al. 2007).

Plants are the richest source of anti-inflammatory compounds, and these compounds exert their anti-inflammatory effects either by targeting these inflammatory mediators at transcriptional level or by regulating their activity in the circulation at post-translational level (Benincá et al. 2011; Alvi et al. 2015, 2016, 2017a, b). *R. officinalis* L. is reckoned for its anti-inflammatory properties including the treatment of bronchial asthma, inflammatory respiratory disease (Zanella et al. 2012). Different studies have shown the anti-inflammatory potential of the REO and its bio-active principal terpenes such as CA, carnosol, ursolic acid, betulinic acid, etc. (Benincá et al. 2011). Terpenes, such as 1,8-cineole and myrcene, have also been found to exert strong anti-inflammatory potential, in which 1,8-cineole showed the ability to protect against carrageenan-induced oedema and to reduce the capillary permeability (Santos and Rao 2000).

To some up the whole, it can be concluded that *R. officinalis* L., REO and its secondary metabolites possess strong neuroprotective effects via targeting various biochemical and molecular markers, i.e. A-β processing and aggregation, neuronal cell death, AChE and BChE activity, neuroinflammation, mitochondrial redox status, etc. The complete neuroprotective activity of *R. officinalis* L. has been represented in Fig. 1.3.

1.3.2 Other Potent Pharmacological Effects of *R. officinalis*, REO and Its Secondary Metabolites

R. officinalis L. and REO are known for their potent pharmacological effects. Some of these pharmacological effects have been diagrammatically represented in Fig. 1.4.

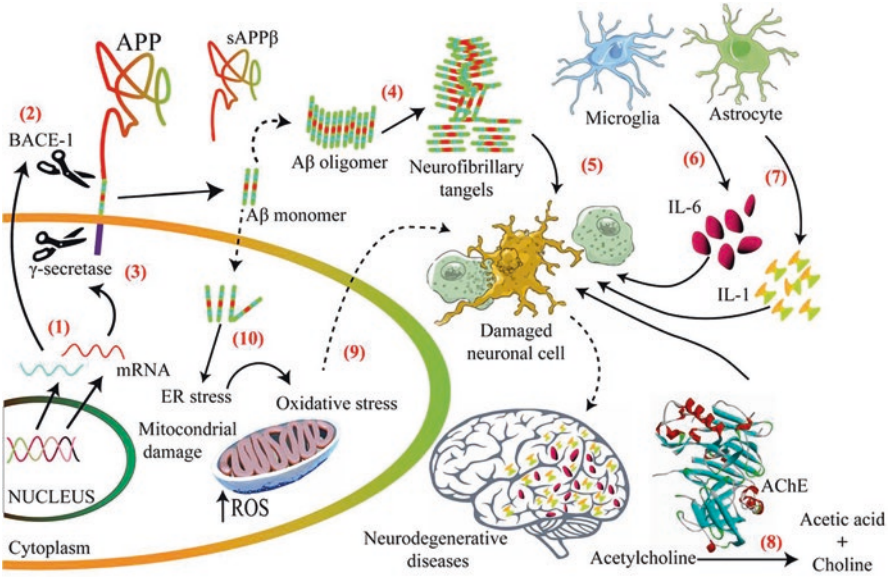

Fig. 1.3 Proposed regulatory mechanisms of *R. officinalis* L., REO and its secondary metabolites against neurodegenerative disorders

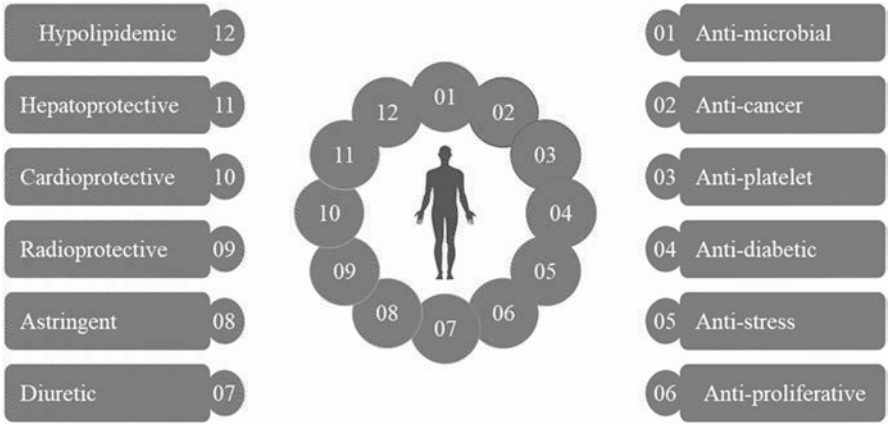

Fig. 1.4 Pharmacological effects of *R. officinalis* L., REO and its secondary metabolites other than neuroprotective effects

1.3.2.1 Protective Impact of *R. officinalis* and Its Secondary Metabolites in Targeting Cancer

Cancer remains a central cause of mortality worldwide nowadays; however, the drug-associated pronounced adverse effects associated with the currently available therapeutic approaches largely prevent their effectiveness, thus increasing the

demand for novel and safer anticancer therapeutic regimens (Xiang et al. 2014). *R. officinalis* L. is known to exert potent antioxidant activity and inhibits genotoxicity as well as protects from carcinogens or toxic agents (González-Vallinas et al. 2015). Moreover, the most fundamental characteristics of carcinoma cells are their excessive proliferation rates accompanied by retarded apoptosis (Hanahan and Weinberg 2011). Polyphenols, from *R. officinalis* L., have been found to regulate cell growth and differentiation thereby reducing rates of tumorigenesis (Kar et al. 2012). Some of these anticancer activities of *R. officinalis* L. against different cancer cells were accredited to its key bio-actives, such as CA, carnosol, ursolic acid and rosmarinic acid (Huang et al. 1994). Petiwala and Johnson (2015) reported that CA affected the cell viability of cancer cells via arresting them in G2/M phase in addition to stimulate the caspase-3-mediated apoptotic pathways. Another study by González-Vallinas et al. (2013) also reported the anticancer effects of carnosol as it decreases the cancer cell growth. These studies confirmed the potent anticancer effects of *R. officinalis* L. and its secondary metabolites.

1.3.2.2 Hepatoprotective Potential of *R. officinalis* and Its Secondary Metabolites

Gutiérrez et al. (2010) demonstrated that the methanolic extract of *R. officinalis* L. has shown profound effects against CCl_4-induced acute liver damage, whereas aqueous extract of *R. officinalis* L. prevented azathioprine-induced acute liver injury in rats. Rašković et al. (2014) also found that the methanol extract of *R. officinalis* L. could also prevent as well as may cause reversion of the CCl_4-induced liver cirrhosis in experimental models. Carnosol one of the main constituents from *R. officinalis* L. also alleviated acute liver damage via maintaining the structural integrity of the hepatocytes and scavenging CCl_4-triggered oxidants in order to limit the production of lipoperoxidative byproducts, i.e. MDA and HNE (Sotelo-Félix et al. 2002).

1.3.2.3 Antidiabetic Effects of *R. officinalis* and Its Secondary Metabolites

Diabetes mellitus is one of the most prevalent metabolic disorders across the globe. Despite the desired antidiabetic potential, insulin and other oral hypoglycaemic agents have been linked to various opposing effects (Rahimifard et al. 2014). *R. officinalis* L. and its extracts have been shown to alleviate the experimental diabetes in different in vivo studies (Tu et al. 2013). *R. officinalis* L. has been widely acknowledged as one of the valuable medicinal herbs with the highest hypoglycaemic and antioxidant activity. Bakirel et al. (2008) assessed the beneficial therapeutic effects of ethanolic extract of *R. officinalis* L. leaves on glucose homeostasis in alloxan-induced diabetic rabbits. In this study they observed that *R. officinalis* L. exerts significant hypoglycaemic potential that was accompanied via elevation in the level of serum insulin. *R. officinalis* L. also displayed antilipoperoxidative effects as well as stimulated the activity of enzymatic antioxidants in alloxan-induced diabetic rabbits. This strong antidiabetic effect of *R. officinalis* L. extract was attributed to the presence of potent antioxidant compounds (Bakirel et al. 2008).