Ruta graveolens: Phytochemistry, Pharmacology, and Biotechnology

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Sonia Malik, Denise Fernandes Coutinho Moraes, Flavia Maria Mendonça do Amaral, and Maria Nilce Sousa Ribeiro

Abstract

Ruta graveolens L., popularly known as rue, is a multipurpose herb belonging to family Rutaceae. It is a rich source of secondary metabolites mainly: coumarins, alkaloids, volatile oils, flavonoids, and phenolic acids. It has been used abundantly worldwide due to its diverse medicinal properties. Extract and essential oil obtained from this plant species have been shown to possess various pharmacological activities including contraceptive, anti-inflammatory, antimicrobial, antipyretic, antioxidant, analgesic, antihyperglycemic, free radical scavenging, hypotensive, antiviral, and antiplasmodial effects. In vitro assays performed with human cell lines have indicated the anticancer potential of furanoacridones and acridone alkaloids isolated from *R. graveolens*. In vitro approaches have been carried out for rapid clonal multiplication of *R. graveolens.* Application of hairy root culture has effectively observed to be beneficial for enhanced production of bioactive compounds from this plant species. A review of literature suggests that it is an interesting plant species to pharmaceutical industry due to its potential to produce several pharmacological effects.

Keywords

Bioactive compounds • Hairy root culture • In vitro culture • Medicinal plant • Ruta • Rutaceae • Pharmacology • Toxicology

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S. Jha (ed.), *Transgenesis and Secondary Metabolism*, Reference Series in Phytochemistry, DOI 10.1007/978-3-319-28669-3_4

Contents

1 Introduction

Plants produce several variety and number of secondary metabolites that play a major role in adaptation with the environment. These compounds also represent important sources of pharmaceutical drugs due to their pharmacological activities. The metabolism from plants is quite diverse with long and complicated chemical reactions which, in many cases, is impossible to be carried out in laboratories. Thus many active raw materials used in pharmaceutical industries are not synthesized by the human and are being obtained just from plant source.

Biotechnology is being used as a tool to obtain bioactive compounds from plants which are unlikely to be synthesized in laboratories. It is also possible to improve the production of secondary metabolites from plants by using various biotechnological means [1–3]. Several strategies have been used to obtain the enhanced and improved production of compounds of interest, which include plant cell culture as well as modification in the gene expression to improve the enzymatic reactions of the metabolism [4, 5].

Among the interesting species to the pharmaceutical area, *Ruta graveolens* has gained considerable importance because of its medicinal purpose, and it is also a source of several secondary metabolites that have demonstrated different biological activities.

2 Botanical Characteristics

R. graveolens L. (Fig. 1) belongs to family Rutaceae, which comprises approximately 160 genera and 2070 species, distributed in tropical and temperate regions of the world, mainly America, Africa, and Australia [6, 7]. According to Engler [8, 9], the Rutaceae Jussieu family belongs to Rutales order. Takhtajan [10, 11] and Thorne

Fig. 1 Photograph of *Ruta graveolens* L.

[12] also placed this family into Rutales although Cronquist [13] and APG III [6] classified it as Sapindales.

Engler [8, 9] divided Rutaceae family into seven subfamilies: Rhabdodendroideae, Aurantioideae, Flindersioideae, Spathelioideae, Dictyolomatoideae, Rutoideae, and Toddalioideae. Thorne [12] has combined Toddalioideae to Rutoideae and suppressed Rhabdodendroideae, thus making five in total. Takhtajan [10, 11] classified it into six subfamilies, excluding also Rhabdodendroideae and the APG III recognized four subfamilies (Cneoroideae, Amyridoideae, Rutoideae, Aurantioideae) [6]. Cronquist [13] did not mention subfamilies. In all these classifications, *Ruta* genus is placed into Rutoideae subfamily.

Some of the species from Rutaceae family, which are having economic importance, include *Ruta*, *Citrus*, and *Pilocarpus*. This family includes species with edible fruit such as *Citrus aurantium* L. (sour orange), *C. sinensis* (L.) Osbeck (sweet orange), *Citrus limon* (L.) Osbeck (lemon), and *Citrus reticulate* Blanco (tangerine) [14]. Some plant species have been used as ornamental plants due to their attractive flowers such as *Hortia, Correa, Boronia, Choisya*, and *Clausena* [15, 16]. Species from *Ruta*, *Zanthoxylum*, and *Casimiroa* are known for their medicinal purposes. Genus *Pilocarpus* has species native from Brazil and Paraguay that produce pilocarpine, an alkaloid used to manufacture medicine to treat glaucoma [17].

Ruta graveolens L. is popularly known as rue, herb of grace (English), ruda (Spanish), raute (German), and arruda (Portuguese), and it has *Ruta hortensis* Mill. as scientific synonym [18]. It is a herb or sub-shrub with branches grown in clumps up to 60 m high. The plant is covered by trichomes, and flowers are small and yellow in color. Plant has scent, alternate, petiolated, and compound leaves that can reach up to 15 cm length. Each leaf has 2–5 leaflets that are fleshy, sessile, and with color varying from light green to bluish-green [19–21].

There are reports concerning difficulty in identifying *R. graveolens*, mainly to differentiate it from *R. chalepensis*. In this regards, Kanan and Babu [22] performed

pharmacognostic studies in stem and leaves of *R. graveolens* and recognized microscopic characteristics that can be used to identify this plant species. The stem of *R. graveolens* has single layer epidermis followed by hypodermis. The cortex is divided into two layers: one of chlorenchyma and the other parenchyma. The chlorenchyma layer has a lot of air spaces between the cells, characterized by an aerenchyma and the parenchyma has normal intercellular spaces. The pericycle is made of fibers with wide and clearly visible lumen. The xylem and phloem vessels have usual elements. In the center of the stem, there is large pith formed by undifferentiated parenchyma. Starch grains and calcium oxalates are found in the stem. The lamina of the leaves has a single layer epidermis and dorsiventral mesophyll. Druses of calcium oxalate are abundant in leaves.

In our study about microscopic features of leaves in *R. graveolens*, we observed a single layer of epidermis in both sides (in cross section), mesophyll dorsiventral, numerous crystals of calcium oxalate as described by Kanan and Babu [22], and also frequent secretory cavities that store essential oil (Fig. 2). These characteristics can be used to identify *R. graveolens*, avoiding misidentification when it is used to produce interesting materials for human being.

3 Distribution and Medicinal Importance

R. graveolens is known for its medicinal properties since a quite long time ago. This plant species is native from Mediterranean region. Nowadays, it can be found in many different countries, including Brazil, Peru, Italy, India, South Africa, and others. There are many reports about the medicinal usage of this species by the ancient Greeks and Romans [23]. In several traditional medicine systems, *R. graveolens* is used as tonic, anthelmintic, emmenagogue, sudorific, antifertility, and also for respiratory disease, headache, heart problem, gastrointestinal disorders, neck pains, rheumatism, gout, intestinal cramps, convulsions, diabetes, fever, worms, kidney problem, earache, bladder and sinus [23–29]. Rue is also a plant used in magic rituals to treat supernatural folk illness as well as against evil eye or bad spirits influence [30, 31].

In Amazonic region, *R. graveolens* leaves have been used against headache, dizziness, brain weakness, flu with cough, fever, hoarse voice, stroke, toothache, numbness after bug sting, and intestinal pain. It is also used for personal protection as a magic herb against evil energies [32, 33]. Thring and Weitz [34] performed an ethnobotanical study in South Africa and described *R. graveolens* as the second most medicinal plant as reported by the questionnaire. Above description shows how important is *R. graveolens* as a medicinal plant worldwide.

Besides the medicinal purpose of this species, there are some reports on its toxic effects in liver and kidney and as abortive. According to Prabhu et al. [24], *R. graveolens* may damage important organs of the body, when taken in high dosage. The leaves of this species can cause chemical irritation in the skin. Some dermatitis has been reported mainly in children [30, 35].

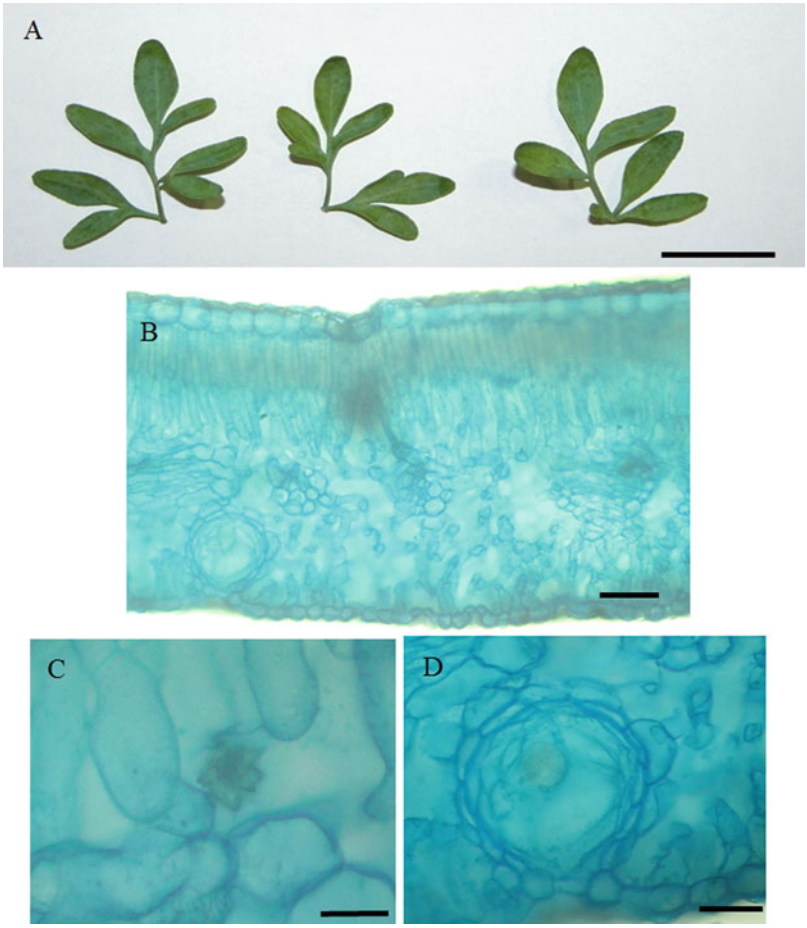
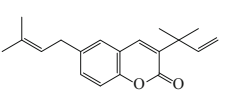


Fig. 2 *Ruta graveolens* L. (**a**). Leaves, bar 2 cm; (**b**). Cross section of leaves, bar 150 μm; (**c**). Detail of druse, bar 20 μm; (**d**). Detail of secretory cavity, scale 50 μm

4 Phytochemistry

4.1 Secondary Metabolites in *R. graveolens*

R. graveolens is a rich source of secondary metabolites mainly: coumarins, alkaloids, volatile oils, flavonoids, and phenolic acids [36–38]. The bioactive compounds of this species have been widely studied not only because of their interest in the chemistry of natural products but also because of the several biologically active compounds which provide a base for the use of *R. graveolens* in folk medicine and in the search of more biologically active compounds. Figure 3 depicts the



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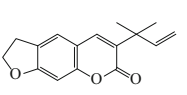

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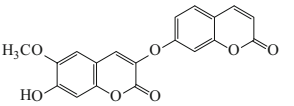

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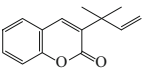

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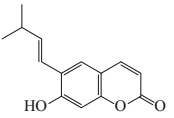

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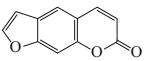

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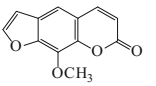

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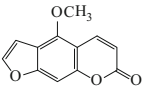

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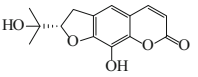

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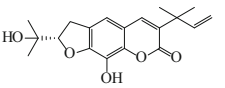

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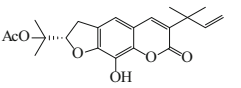

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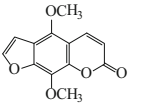

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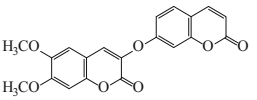

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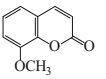

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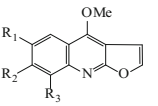
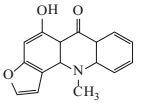


	R1	R2	R3
15	H	H	H
16	OMe	OMe	H
17	H	H	OH
18	H	OMe	OH
19	H	H	OMe



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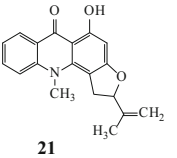

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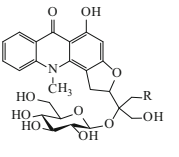
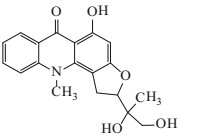


27 R= H
28 R= OH



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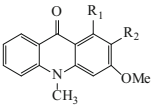
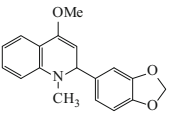


29 R1 = OH, R_2 = H
30 R1 = OH, R_2 = OMe



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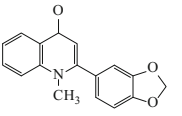

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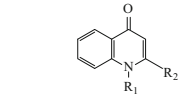
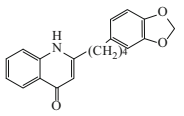
Fig. 3 (continued)



	R_1	R_2
33	Me	
34	Me	*n*-nonyl
35	Me	*n*-decyl
36	H	*n*-undecyl
37	H	*n*-heptyl
38	H	*n*-octyl
39	H	*n*-nonyl
40	H	*n*-decyl
41	H	*n*-undecyl



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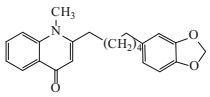

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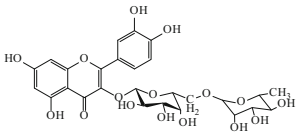

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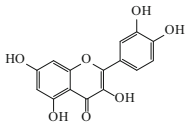

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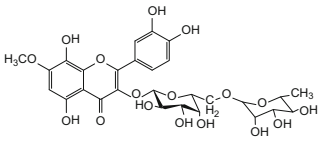

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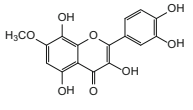

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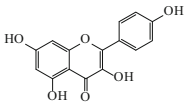

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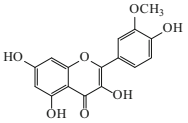

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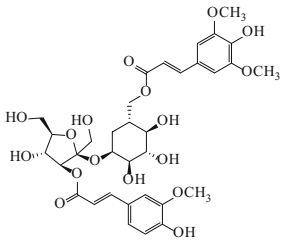

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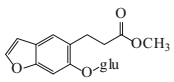

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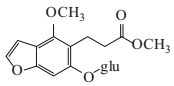

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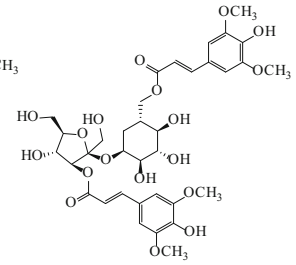

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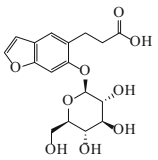

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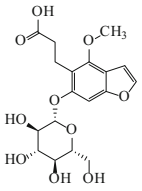

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Fig. 3 Secondary compounds present in *R. graveolens*

common secondary compounds present in *R. graveolens*; the details of these compounds are presented in the following sections.

4.1.1 Coumarins

The main types of coumarins identified in *R. graveolens* are: simple coumarin, furanocoumarins, dihydrofuranocoumarins, and isocoumarins among others [36, 39, 40].

The coumarins isolated from the roots of the *R. graveolens* for the first time were gravelliferone methyl ether (**1**) chalepensin (**2**), daphnoretin (**3**) [41], 3-(1′,1′-dimethylallyl)-herniarin (**4**), and gravelliferon (**5**) [42, 43]. The furanocoumarins (FCs) with linear structure are produced in different parts of the species; studies of field-grown plants showed that FCs' concentration was proportionally related to both the plant's phenological stages and the organs in which the substance is stored [44]. Fruits accumulate higher concentrations of FCs, followed by leaves, roots, and stems. On the leaf surface, concentrations and proportions of FCs as psoralens (psoralen **(6),** xanthotoxin **(7),** and bergapten **(8)** vary during aging [45, 46]. Xanthotoxin prevails in absolute amounts regardless of the species' age [47].

Other furanocoumarins as rutamarin **(9)**, rutarentin (**10**) [48], chalepin (**11**) [49], isopimpinellin (**12**), and a biscoumarin, O-methyl-daphnoetin (**13**) [50] has been found in many parts of the plant species in different amounts. Abyshev et al. [51] isolated from the epigeal part of *R. graveolens*, the furonocoumarins, bergapten (**8**), chalepin, and chalepensin (**2**). The novel and, besides, recently a new coumarin derivative, 8-methoxy chromen-2-one (**14**) was isolated by Debasis et al. [52].

4.1.2 Alkaloids

Rue is a valuable natural source of alkaloids. The alkaloids found in this species can be classified as furoquinolines, acridones, quinolines, and alkylquinolinones [37, 53, 54].

The furoquinoline alkaloids such as dictamine (**15**), kokusagenin (**16**), peleteine (**17**), skimmianine (**18**), and fagarine (**19**) occur, in different amounts, on the leaves, shoots, roots, and flowers [37, 55]. In the roots and leaves, one of the main alkaloids present was skimmianine (**18)** [56]. According to Mancuso et al. [50] kokusagenin **(16)** and skimmianine (**18**) are almost equally distributed in the plant's aerial parts.

Furacridone (**20**) and rutacridone (**21**) were the first representatives from acridone alkaloids, to be extracted from the roots of *R. graveolens* [57], from that a series of rutacridone derivatives have been obtained such as rutacridone epoxide (**22**), gravacridonol (**23**) [58–61], gravacridondiol **(24)**, gravacridondiol acetate (**25**) [62], gravacridontriol (**26**), glycosides gravacridondiol-*O*-18-β-D-glucoside (**27**), and gravacridontriol-*O*-18- β-D-glucoside **(28)**, which accumulate in different parts of the roots [53, 63].

Distribution and compartmentalization studies revealed the accumulation of acridone alkaloids in intact roots of *R. graveolens* [60]. Rutacridone (**21**) was found in the differentiation zone where the root hairs are located, suggesting its accumulation in the roots trichomes. More hydrophilic acridones such as gravacridondiol **(24)** and 1-hydroxy-3-methoxy-*N*-methylacridone (**29**) [53] and

other metabolites, in turn, experience a decrease in concentration in this specific root segment. Gravacridondiol glucoside (**27**) was shown to be the major compound in the root tips [53]. Arborinine (**30**) [55, 64] is another acridone alkaloid identified in *R. graveolens*.

Graveolinine (**31**) and (4S) 1,4-dihydro-4-methoxy-1,4-dimethyl-3-(3-methylbut-2-enyl) quinoline 2,7-diol **(32)** [65] are quinoline alkaloids isolated from the leaves of the species.

R. graveolens is also an important source of alkylquinolone alkaloids (AQs) of the series 4-quinolones. Graveoline **(33)**, an alkaloid of the 2-aryl-4-(1H)-quinolone series, has been identified by Grundon and Okeley [56], Oliva et al. [66], and Ghosh [67]. At a subsequent time, analogues of 2-n-alkyl **34**–**42** [37], such as 2-[4′-(3′,4′-methylenedioxyphenyl) butyl]-4-quinolone (**43**), [68] and 1-methyl-2-[6′-(3″,4″-methylenedioxyphenyl] hexyl]-4-quinolone (**44**) [69] were also identified.

According to Eun-Tae et al. [54], the AQs without methyl groups (HAQs) and 1-methyl-AQSs (MeAQs) with 2-nonyl substituents were the most abundant in leaves while in root, the one with 2-undecyl group were the dominant metabolites.

4.1.3 Volatile Oils

The volatile oils obtained from *R. graveolens* fruits, leaves, roots, flowers, or stems have a yellowish color, as well as an intense and penetrating odor. They are composed mainly of oxygenated compounds (ketones, alcohols, acetates), sesquiterpenes and monoterpenes hydrocarbons, aromatics hydrocarbons, and coumarins.

Within the series of methyl nonyl ketones, 2-undecanone and 2-nonanone are the predominant constituents in essential oils extracted from the aerial parts of *R. graveolens,* even if it is grown in different parts of the world such as Malaysia [70], Italy [71], Venezuela [72, 73], Egypt [74], Iran [75], Algeria [76], Ukraine [77], and Brazil [78].

Monoterpenes and sesquiterpenes have been identified in this plant species include, α-pinene, limonene, 1,8-cineol, α-thujene, camphene, terpinolene, camphor, *trans*-*p*-menth-2-en-1-ol, β-phellandrene, germacrene-B, 3-carene, D-cadinene, β-caryophyllen, β-humulene, elemol, geijerene, and geyrene [71, 76]. Pregeijerene and geijerene, sesquiterpenes compounds, are the major constituents of the essential oil from *R. graveolens* roots [79, 80]. Xanthotoxin was also found in the oil extracted from aerial parts and roots of the plant [71]. Aromatic hydrocarbons such as trans-anethole are also found in essential oil [75].

The composition of the essential oil is affected by climatic, seasonal, and geographic conditions; harvest period; chemotypes; and/or extraction procedure [81].

4.1.4 Flavonoid Glycosides and Flavonoids

R. graveolens is a rich source of flavonoid glycosides, for example, rutin (**45**) [82, 83].

A yellow pigment containing gossypetin 7-methyl ether 3-rutinoside (**46**) and gossypetin 7-methyl ether (**47**) has been isolated from the flowers of *R. graveolens* [84].

The leaves and flowers of this plant species have been reported to possess flavonoid, quercetin (**48**), kaempferol (**49**), and isorhamnetin (**50**) [83, 84].

4.1.5 Miscellaneous Compounds

Besides the constituents listed above, other metabolites have also been reported in *R. graveolens*, such as glycosides and phenolic compounds. Chien-Chih et al. [85] have reported six glycosides, 3′-sinapoyl-6-feruloylsucrose (**51**), methylcnidioside A (**52**), methylpicraquassioside A (**53**), 3′,6-disinapoylsucrose (**54**), cnidioside A (**55**), picraquassioside A (**56**), isolated from aerial parts of *R. graveolens* plants. Phenolic acids gentisic acid, caffeic acid, ferulic acid, and p-coumaric acid were isolated from the leaves of this plant species [86].

5 Pharmacological Studies

R. graveolens is found abundantly around the different parts of the world with wide and diversified use for medicinal purposes, which have stimulated the development of several studies evaluating the biological activity to support its therapeutic use in both allopathic and homeopathic approaches.

In proof of view of therapeutic efficacy of homeopathic preparations, Rosi Cruvinel et al. [87] found that administration of *R. graveolens* 6 CH interferes favorably in the feeding, water intake, and weight gain in experiments carried out with chickens.

In vivo test with patients (18–60 years old, candidates for surgery of anterior cruciate ligament) has shown that homeopathic complex containing *Arnica montana* 5 CH, *Bryonia alba* 5 CH, *Hypericum perforatum* 5 CH, and *R. graveolens* 3 DH showed similar results to placebo group in reducing the morphine consumption 24 h after the surgery to fix the knee ligament [88].

Study by Khan [89] indicated the potential for topical homeopathic preparations with *Symphytum officinale, Thuya occidentalis*, *R. graveolens, Rosemary officinalis, Bellis perennis, Hypericum perforatum, Calendula officinalis, and Tagetes* sp. to treat lesions of foot.

Arora et al. [90] evaluated the in vitro action of the mother tincture (MT), *R. graveolens* 30C, 200C, 1 M, and 10 M against cell lines deriving from tumors of human colorectal carcinoma (COLO-205), showing that homeopathic preparations had highly significant effects in the respective cancer cell lines, cytotoxicity and decrease in cell proliferation, thus indicating potential of these preparations as anticarcinogenic.

In search of anticancer effect of homeopathic preparations based on *R. graveolens* on COLO-205 cell line, another study showed that the mother tincture (MT) and *R. graveolens* 30C were able to cause a decrease in cell viability with reduced clonogenecity and migration capabilities, causing also with morphological and biochemical changes indicative of cell death by apoptosis, demonstrating the potential of these preparations in the treatment of colon carcinoma [91].

Patients with locally advanced solid tumors or metastases and previously treated with conventional anticancer drugs showed a transitory improvement in quality of life when given homeopathic preparation of *R. graveolens* 9C by oral administration; however, there was no influence on the tumor progression [92].

The ultradiluted potencies and mother tincture (MT) of *R. graveolens* showed anticancer activity when avoided the proliferation and cytotoxic effects on normal kidney epithelial cell model [93].

To study the antineoplastic properties of homeopathic medicines, homeopathic preparations of *R. graveolens* were administered orally in mice and evaluated its effect on immune system. It was found that higher potencies caused significant enhancement of hematological parameters including the total white blood cell count, bone marrow cellularity, and the number of α-esterase positive cells. Other parameters of immune response such as antibody titer circulating and the number of plaque forming cells, enhanced proliferation of B and T lymphoid cells were also observed, which suggest the immune modulatory activity of homeopathic preparations in high dilutions [94].

Besides the homeopathic preparations of *R. graveolens* to check out pharmacological properties, this plant species has been also analyzed to find out the new alternatives or therapeutic supplements for employment in allopathy using isolated compounds as well as using the whole components (phytocomplex) to produce herbal medicines. A number of studies have been carried out to show the activities such as contraceptive, anti-inflammatory, antimicrobial, and analgesic, and these will be discussed in the following sections.

In view of development of new contraceptive drugs, Guerra and Andrade [95] developed an in vivo study using pregnant albino primiparous female rats subjected to intramuscular or oral administration of *R. graveolens* extract and noted marked contraceptive effect with loss of implantation of eggs. Similarly, Gandhi et al. [96] demonstrated the efficacy of oral administration of powder of aerial parts from *R. graveolens* and its extracts (petroleum ether and methanolic) in the model female rats.

Evaluation study on the pregnancy of the ethanol extract of aerial parts of *R. graveolens* developed in model in vivo (CF1 mice) showed that the extract did not cause preimplantation embryonic loss or reabsorptions, but it led to fetal death [97].

Sailani and Moeini [98] evaluated the effect of ethanol extracts of *R. graveolens* and *Cannabis sativa* on spermatogenesis in the adult male Wistar rats and found that the extracts cause decrease in spermatogenesis. In the search for male contraceptive drugs, Harat et al. [99] developed an in vitro study with human sperm using aqueous extract of *R. graveolens* and proved the potential effect of extract. Oral administration (5 g/kg) of an aqueous extract of *R. graveolens* in male rats resulted in reduced sperm motility only after one hour of administration without any change in other sperm characteristics, indicating the potential of this plant extract in male contraception [100]. Intraperitoneal administration of aqueous extract of *R. graveolens* in rat resulted in reduced number of spermatogonia, thus suggesting that this species can be used in birth control [101].

Nasirinezhad et al. [102] investigated the effect of aqueous extract of *R. graveolens* on the reproductive system of immature female mice, BALB/C line. They reported that the extract of *R. graveolens* can interfere with reproductive system function in immature female mice by alterations in sex hormonal level and ovarian morphology and thus it might be useful as a source of antifertility substance.

In research of the anti-inflammatory effect of *R. graveolens* extract and rutin (flavonoid present in the species), Raghav et al. [103] showed that the extract has better anti-inflammatory activity than the rutin using different models such as murine macrophage cells (J-774) challenged with lipopolysaccharide (LPS), induction of inflammatory response by nitric oxide, and other mediators.

Li et al. [104] performed a study with extracts of this plant species to analyze their NGF-potentiating activities on the NGF-mediated neurite outgrowth from PC12D and proved that the methanol extract of the leaves of *R. graveolens* markedly increased the proportion of neurite-bearing cells.

The isolated compound from methanolic extract of *R. graveolens*, identified as 3-(1′-allyl-1′-dimethyl)-6-hydroxy-7-methoxy-coumarin has been measured in iNOS, COX-2 genes, and some cytokines pro-inflammatory. This compound revealed ability to inhibit protein and mRNA expression of iNOS and IL-1β in LPS challenged macrophages, showing also anti-oxidant activity [105].

The ability of methanol extract of *R. graveolens* has been demonstrated to inhibit inflammation and oxidative stress in adjuvant induced arthritis in rats [106]. Polyphenols and alkaloid fractions obtained from *R. graveolens* extract showed anti-inflammatory activity in a model of acute and chronic inflammation in rats [107].

The in vivo studies of methanol extract of *R. graveolens* in hypercholesteremic rats showed reducing oxidative damage, inflammation, and aortic pathology and indicated that the species has potential for therapeutic use in clinical conditions associated with atherosclerosis [108].

The evaluation of anti-inflammatory effect of skimmianine quinoline alkaloid isolated from *R. graveolens* extract proved decrease in mRNA levels of TNF- α and IL-6 as well as the levels of NO and PGE.sub 2, COX-2 and 5-LOXm activities, thus proving anti-inflammatory action of the substance by several mechanisms involved in the response cascade of events [109]. Methanol extract of leaves of *R. graveolens* showed antinociceptive, anti-inflammatory, and antipyretic activities in mice [110]. Kataki et al. [111] have reported the antioxidant and anti-inflammatory activities of methanol extract of leaves of *R. graveolens* in vitro and in vivo models and showed potent inhibitory effects on the arachidonic acid pathways. The antioxidant effect of *R. graveolens* extract has also been also evaluated in two models including free radical scavenging using DPPH and inhibition of lipid peroxidation by the ferric thiocyanate method [112].

Aqueous extract of *R. graveolens* leaves has been reported to possess antimicrobial effect against *Fusarium solani*, *Pyrenochaeta lycopersici, Trichoderma viride, Penicillium sp., Thielaviopsis basicola, and Verticillium dahliae, Bacillus cereus, Staphylococcus aureus, and Listeria monocytogenes* [66, 113]. In another report by Ivanova et al. [114], the methanol, petroleum ether, ethyl acetate, and water–methanol extracts of aerial parts of *R. graveolens* were found to possess

cytotoxic as well as antimicrobial activity against *Streptococcus pyogenes, Listeria monocytogenes,* and *Bacillus subtilis*.

Extract and essential oils obtained from *R. graveolens* were evaluated against *Pseudomonas aeruginosa* strains*, Staphylococcus aureus, Candida albicans,* and *Candida krusei* isolated from patients suffering with acute external otitis. All the tested strains were found to be resistant to the extract, while essential oil (4%) of *R. graveolens* inhibited four *Staphylococcus* and all *Candida* strains (inhibition halos between 10 and 13 mm diameter) [115].

Oils obtained from *R. graveolens* inhibited the growth of Gram positive (*Staphylococcus aureus* and *Enterococcus faecalis*) and Gram negative (*Escherichia coli* and *Klebsiella pneumoniae*) bacteria [116]. However, the aqueous leaf extract and essential oils isolated from this plant species was found to be ineffective against *Trichophyton mentagrophytes* and *Pseudomonas aeruginosa* [117, 118]. Hydro-alcoholic and aqueous extracts of *R. graveolens* have shown to be ineffective against *Enterococcus faecalis* [119, 120].

In search of new therapies to fight against *Helicobacter pylori, R. graveolens* extract was found to exhibit strong inhibitory activity against IL-8 secretion [121]. Its essential oils possess antifungal activity, more significantly against *Aspergillus fumigatus* and *Cladosporium herbarum* [122]. Essential oil obtained from fresh leaves by hydrodistillation in a Clevenger-type apparatus and characterized by GC-FID and GC-MS showed antibacterial activity against Gram-positive and Gram-negative bacteria, especially *Bacillus cereus* and *Staphylococcus aureus* [78]. Aerial parts essential oil showed activity against different strains of *Legionella pneumophila* [123].

Figueroa-Valverde et al. [124] reported that methanol extract of the leaves (0.5 g/kg) is able to induce hypoglycemic effect, which is attributed to the presence of flavonoids in this species. In vivo experiments in hyperglycemic rats demonstrated that oral administration of the methanol extract resulted in decreased concentration of blood glucose [125].

Van Huyssteen et al. [126] conducted a study on popular practice in Africa for the treatment of *Diabetes mellitus* and reported that hydroalcoholic extract of aerial parts of *R. graveolens* produced the highest increase in glucose utilization in C2C12 muscle cells.

Effect on lipid and glucose levels as well as hematological parameters were studied upon administration of *R. graveolens* extract in rats with diabetes induced by injecting streptozotocin. This study showed that *R. graveolens* extract caused a significant decrease in cholesterol and LDL-C but no alterations were in the levels of glucose, triglycerides, VLDL-C, and HDL [127].

In vivo assay using acetic-acid-induced writhing and hot-plate-induced thermal stimulation in mice has demonstrated the antinociceptive activity of *R. graveolens* extract [128].

Park et al. [129] performed in vivo studies using various experimental models of pain and found that antinociceptive effect of *R. graveolens* extract was mediated by opioidergic and α 2-adrenergic receptors but not by serotonergic receptors.

Comparative studies between *R. graveolens* and *Matricaria chamomilla* (70% ethanol as solvent and fractions of petroleum ether, ethyl acetate, and *n-butanol*) showed that both of these plant species can be effectively used as analgesics, which is attributed to the presence of flavonoids and alkaloids [130].

Gilbert et al. [131] carried out in vitro assays to find out the antiparasitic activity *of R. graveolens* and proved that ether extract of leaves was active against *Strongyloides stercoralis*, a nematode that causes strongyloidiasis.

Mendes et al. [132] studied the molluscicidal activity of hexane and ethanol extracts from leaves and stem of *R. graveolens* and found to be effective against the intermediate host of *Schistosoma mansoni.*

In vitro study performed with hydroalcoholic extract of aerial parts of *R. graveolens* showed the reductions in viability, potential for invasion, and multiplication rate of the parasites *Leishmania amazonensis and Trypanosoma cruzi* and indicated that these herbal extracts may be potential candidates for developing drugs to treat leishmaniasis and Chagas disease [133]. Queiroz et al. [134] reported that aqueous extract of aerial parts of *R. graveolens* possessed leishmanicide activity against promastigotes and amastigotes of *Leishmania amazonensis*.

In view of new drugs for the treatment of neurodegenerative diseases such as Parkinson's and Alzheimer's, in vivo model for assessing the inhibition of the oxidative deamination of tyramine by monoamine oxidase (MAO) isolated from rat liver have been studied and they found that ethyl acetate extracts and oil extracted from leaves of *R. graveolens* have a significant ability to inhibit this enzyme [135]. In another study [136], hexane extract of this plant species caused potent inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) demonstrating potential to be used to treat Alzheimer's disease. Russo et al. [137] also indicate that *R. graveolens* has potential for therapeutic use in this disease, which is attributed to the presence of coumarin in the hexane extract with anti-AChE activity.

In order to find new anticonvulsant drugs, Keihanian et al. [138] investigated the effect of *R. graveolens* extract in vivo model of seizures induced by pentilentetrazole (PTZ) in mice and demonstrated that its ability to reduce seizures. In vivo study developed by Amabeoku and Ahmad [139] showed anticonvulsant activity of the methanol extract of the leaves of *R. graveolens* probably involving potentiation of gamma aminobutyric acid (GABA). Study by Bohuslavizki [140] indicated the action of infused *R. graveolens* in blocking potassium channels, thus signaling the potential of this plant species in the treatment of Encephalomyelitis disseminata.

Aqueous and ethanol extract of *R. graveolens* leaves have been shown to exhibit binding affinity to GABA-benzodiazepine receptor in the flumazenil-binding assay, which was attributed to the presence of furanocoumarines [141]. Adsersen et al. [142] reported that the extract of *R. graveolens* exhibited moderate inhibition of the acetylcholinesterase.

In vitro assays performed with human cell lines HeLa, MCF7, and A43 have shown the anticancer potential of furanoacridones and acridone alkaloids (arborinine and evoxanthine) isolated from *R. graveolens* [64]. Fadlalla et al. [143] investigated the effect of the methanol extract of *R. graveolens* on colon, breast, and prostate

cancer cells and found that it inhibited the proliferation and survival of cancer cells via multiple targets. In vitro studies by Ghosh et al. [144] have demonstrated that graveoline, a compound isolated from *R. graveolens*, showed cytotoxicity against A375 skin melanoma cells, causing cell death by apoptosis and autophage. In vitro (skin melanoma cells) and in vivo (7,12-dimethylbenze (a) anthracene induced skin cancer in Swiss albino mice) assessment of ethanol extract of *R. graveolens* demonstrated the potential of this plant species to treat skin cancer without causing any acute or chronic toxicity [145]. The anticancer potential of aqueous extract of this species has been also evaluated in model of different glioblastoma cell lines (U87MG, C6, and U138 [146].

Al-Nimer and Ali [147] studied the effect of aqueous leaves extract of *R. graveolens* on nitric oxide (NO)–peroxynitrite (ONOO-) cycle biochemistry and found that the improved bioavailability of nitric oxide (NO), indicating that its use in coronary artery disease with nitrate tolerance.

Essential oils obtained by hydrodistillation of aerial parts of *R. graveolens* and isolated constituents showed repellent and larvicidal activity against *Aedes* L. and could be useful in mosquito control [148].

Methanolic extracts from aerial parts of *R. graveolens* have demonstrated significant ability to inhibit aldehyde oxidase, partially purified from liver homogenates of mature male guinea pigs by heat treatment and ammonium sulfate precipitation [149].

Extract from the aerial parts of *R. graveolens* obtained by different extractive procedures (extraction in Soxhlet percolation and ultrasound) were investigated for cytotoxicity (G-929 cells) and tyrosine inhibition capacity (spectrometry at 481 nm). It has been observed that extraction procedure interferes with the activity of the compounds. Extraction obtained by percolation method showed less cytotoxicity and higher percentage of inhibition of tyrosine [150]. Hypotensive effect of aqueous extract of *R. graveolens* under in vivo system in normotensive rats has been shown by Chiu et al. [151]. Studies by Khori et al. [152] indicated that the methanol extract of *R. graveolens* and its alkaloidal fraction has potential to treat supraventricular tachyarrhythmia.

Ueng et al. [153] evaluated the effect of aqueous extract of aerial parts of *R. graveolens* and isolated substances (rutin and furanocoumarins) on activities of enzymes; cytochrome P450 (P450/ CYP), uridine diphosphate (UDP)-glucuronosyltransferase, and reduced nicotinamide adenine dinucleotide (phosphate) (NAD (P) H): quinone oxidoreductase. They observed that oral administration of the extract in mice/rats caused an increase in the levels of CYP1A and CYP2B in a dose dependent manner and by inducing increased hepatic UDP-glucuronosyltransferase activity.

In vivo studies have indicated that hydroalcoholic extract of *R. graveolens* caused relaxation in the rings of the trachea of rat and the effect is mediated by noncompetitive antagonistic mechanism [154].

A recent review on genus *Ruta* by Hammiche and Azzouz [155] reported the diversity of biological activities of *R. graveolens*, especially the anticancer activity, action on pigmentation of the skin to stimulate melanin synthesis signaling for use in

vitiligo or psoriasis, effect on the central nervous system by inhibiting the activity of MAO-B, anti-inflammatory, antimicrobial, cytotoxic, hypotensive, antiviral, and antiplasmodial affects.

6 Toxicological Studies

To study the safety assessment parameters in the use of *R. graveolens*, some studies have been carried out to assess the toxicity. There are a few clinical case reports of poisoning in humans.

Simon et al. [156] present a patient developed severe dermatitis as a result of contact with *R. graveolens*, which is attributed to the toxic bioactive compounds furanocoumarin constituents: 5-methoxypsoralen (bergapten), 8-methoxypsoralen (xanthotoxine), and furanoquinoline dictamnine. Sharma et al. [157] reported the case of 26-year-old pregnant woman led to abortion after consuming *R. graveolens* tea in combination with chocolate and cinnamon. In another case report, use of decoction of *R. graveolens* by 78-year-old woman led to poisoning by bradycardia, renal failure with acute hyperkalemia, and coagulopathy [158]. Adams et al. [159] reported the case of acute phytophotodermatitis in 2-year-old child after contact with this plant species. A 12-year-old patient who developed lesion with linear distribution in the lower limbs has been diagnosed with phototoxic reaction, attributed to the use of alcoholic extract of *R. graveolens* [160].

Experimental study in goats has indicated that oral administration of *R. graveolens* leaves (1 and 5 g/kg) led to toxicity in animals with pathological changes in various organs. The changes included alterations in serum aspartate and copper, iron, zinc, manganese, calcium, and phosphorus with animal death record [161]. Lyophilized hydroalcoholic extract of aerial parts of *R. graveolens* resulted in fetal death when administered orally (1000 mg/kg/day) during the pregnancy [97].

In the research of potential antiparasitic property against *Vampirolepis nana* (gastrointestinal parasite), hydroalcoholic extract of the leaves and seeds of *R. graveolens* was administered by gavage to Swiss albino mice and it was proven mild to moderate hepatotoxic with weak antiparasitic effect [162].

Intraperitoneal administration of *R. graveolens* extract (30 and 100 mg) in Wistar rats for 3 days resulted in morphological changes in the liver and led to hepatotoxicity [163].

Aqueous extract (20%) of *R. graveolens* leaves when administered orally in rats caused a significant decrease in the number of normal embryos and increase in cases of persistent late embryonic development thus demonstrating toxic effect of the extract [164]. Shama et al. [165] reported that ethanolic and aqueous extracts of *R. graveolens* seeds resulted in change in body weight, biochemical, and hematology parameters and thus caused toxicity when administered orally to male Wistar rats at 200 mg/kg/day (for 4 weeks). The ethnobotanical study carried out in health care service of Provincia del Chaco at Argentina has reported the potential dangers and poisoning effects (which may lead to death) associated with the use of medicinal plants (highlighted *R. graveolens*) [166].

7 Biotechnological Studies in *R. graveolens*

7.1 In Vitro Regeneration and Mass Propagation

From the above literature, it has been evidenced that *R. graveolens* is a multipurpose herb. Biotechnology using in vitro technique provides a viable tool for mass multiplication and germplasm conservation of aromatic and medicinal plants of interest [167, 168]. Faisal et al. [169] developed a protocol for rapid clonal multiplication of *R. graveolens* through high frequency shoot induction from nodal explants and reported the successful outdoor establishment of regenerated plants. Various concentrations and combinations of plant growth regulators, (PGRs) viz., BA, Kn, IAA, and NAA have been tried. The highest shoot regeneration frequency (98.5%) was reported on MS medium containing BA (10 μM) and NAA (2.5 μM). The regenerated shoots were found to be rooted best on MS medium with 0.5 μM IBA [169]. There are other reports on tissue culture studies of *R. graveolens* to monitor alkaloids and coumarins content in callus, shoots, and regenerated plants [170–172].

7.2 Use of Elicitors

Commercial production of secondary compounds is generally hampered by their low yield. Elicitation is a strategy to enhance the secondary metabolite production and is considered an integral part of any large-scale process for secondary metabolite production. Elicitor can be biotic and abiotic depending on its origin. The mechanism of both the elicitors is different and quite complex. Chitin and chitosan are the elicitors inducing phytoalexin accumulation in plant tissue. Orlita et al. [173] studied the effect of chitin and chitosan on alkaloids and coumarins in in vitro shoot cultures of *R. graveolens* and found that elicitation induced a significant increase in quantity of all the metabolites. The application of abiotic elicitors saccharin and benzothiadiazole has been reported to increase the production of simple coumarins, linear furanocoumarins, dihydrofuranocoumarins, and furoquinolone alkaloids by several times in in vitro shoots of *R. graveolens*. [174]

Effect of polyamines, spermine and putrescine, have been studied on growth and furanocoumarins in *R. graveolens* cultures. Spermine was found to increase the multiple shoots formation and furanocoumarins production by 2.5 and 1.47-fold, respectively [175].

7.3 Scale Up Studies

Bioreactors of different types are generally used for the large scale production of shoots and bioactive compounds for commercialization. In *R. graveolens*, bioreactor system has been reported to be an effective technique for large scale production of shoots [176]. A one-step protocol with improved regeneration efficiency for

multiple shoots induction employing liquid culture system has been reported by Diwan and Malpathak [175]. They scaled up selected shoot line, RS2 from 250 mL to 5 L culture vessels, with 1.53-fold increase in biomass without affecting the productivity of cultures. Gontier et al. [177] developed bioreactor for economic and efficient production of furocoumarins from *R. graveolens* shoots. The traditional systems of in vitro propagation have been compared with simple aerated bioreactor for large scale shoot biomass in same species [176]. Shoots cultured in the simple aerated bioreactor systems showed 4.1-fold increase in 2 l bioreactor vessel and 5.2-fold increase in 5 l bioreactor as compared to 500 mL conical flask culture.

7.4 Genetic Transformation and Plant Hairy Root Culture

Transgenic or hairy roots obtained after transformation with *Agrobacterium rhizogenes* present the fast growing system as compared to nondifferentiated plant cells and therefore have become a promising source of bioactive compounds of interest in several plant species. Chemical synthesis of furocoumarins in *R. graveolens* is very expensive and does not always lead to biologically active compounds [178]. To study the distribution and compartmentalization of alkaloids in intact roots, Kuzovkina [179] established hairy root cultures. There are several factors determining the efficient transformation starting from explant selection to *Agrobacterium rhizogenes* strain and media used. Certain tissue or plant organs are more appropriate for transformation in its particular developmental stage. Usually young tissues are more responsive/ susceptible to transformation as compared to mature ones. The commonly used explants for transformation with *A. rhizogenes* are young seedlings or their parts, roots, and shoots [180]. Hypocotyls, callus, and shoots were inoculated with *A. rhizogenes* strains (LBA 9402 and A4) in *R. graveolens* [181]. Hypocotyls were found to be more responsive with *A. rhizogenes* strain LBA 9402 due to low level of coumarins and furanocoumarins as compared to other explants. Hairy roots obtained as a result of transformation indicated high level of coumarins, furanocoumarins, and alkaloids. The content of pinnarin, rutacultin, bergapten, isopimpinelin, and xanthotoxin in hairy cultures has been reported to be twofold higher than in shoot cultures. The two novel coumarins, osthole and osthenol have been also found, which are known to possess many biological activities including anti-inflammatory, anti-oxidative, and anti-tumorigenic [181]. Genetic transformation system has been developed for *R. graveolens* by co-cultivation of hypocotyls with *A. tumefaciens* strain C58C1Rif^{R} containing a plasmid harboring neomycin phosphotransferase and β-glucuronidase encoding genes [182]. The stable transgene integration was confirmed by growth on selection medium for *npt*II, by PCR and southern blot analysis [182].

8 Conclusions

R. graveolens is considered as an important plant species with various pharmacological activities. Although it is a plant used with medicinal purpose in many parts of the world, safety assessment parameters indicate that there are a few clinical case reports of poisoning in humans with the use of *R. graveolens.* It may damage the important organs of the body, when taken in high dosage. Therefore, it is important to consume the correct concentration and dose of this plant species within safer limits. The pharmacological and toxicological studies indicate the correct way to use this plant (or compounds derived from it) to produce safe medicines that can be used around the world. Regarding the improvement in production of secondary compounds from *Ruta graveolens*, there is still gap in the knowledge about the biosynthetic pathway of various bioactive compounds, and future studies could be focused to solve this problem in order to rise the production of bioactive compounds from *R. graveolens* by using strategies like immobilization, two phase culture system, as well as metabolic engineering approach to improve the production.

Acknowledgments Authors would like to acknowledge the Foundation for the Support of Research, Scientific, and Technological Development of the state of Maranhão – FAPEMA for financial support.

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