REVIEW ARTICLE

Chemical Adaptation to the Environment: Secondary Metabolite Plasticity in Rutaceae with *Ruta graveolens* as a Model Species

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Abstract

The metabolites of this plant are classified into many groups, including flavonoids, essential oils, coumarins, furanocoumarins and acridone and furoquinoline alkaloids as well as various phenolic acids. The significant diversity in its chemistry has led to a long cultural history of use for many medicinal purposes, and as such has attracted renewed scientific research into the ecological and biological significance of R. graveolens. As an example, laboratory studies of extracts, essential oils and pure chemical constituents have demonstrated numerous biological activity, including antioxidants, anti-inflammatory, antibacterial, antiparasitic and the ability to modulate metabolic and cellular processes. Alkaloids derived from acridones and furanocridones are of particular interest because of their capacity to induce cytotoxic effects and promote apoptosis, both in vitro.; the author feels these compounds will continue to generate interest as additional studies are completed to provide a better understanding of their mode of action. Although these traits present an encouraging prospect, the high degree of variance observed in metabolite composition as a result of organ-specific accumulation factors, sampled environmental conditions and retrieval methodologies presents a large hurdle to replicate findings and utilize those outcomes through various means. Current trends within the field of plant biotechnology include a focus on establishing regulated in vitro culture systems, including the establishment of clonal uniformity through micropropagation, as well as providing an outlet for genetically stable and rapidly expanding biomasses with improved potential for specialized metabolite synthesis via the employment of hairy root cultures. This chapter provides an integrative review of the current understanding of the phytochemical profile, biological roles and biosynthetic pathways related to R. graveolens and utilizes this information to show that plant biotechnological approaches provide the means to produce standardized, reproducible, and scalable amounts of high-value metabolites from plants of the Rutaceae family.

Keywords: Rutaceae; *Ruta graveolens*; specialized metabolism; acridone alkaloids; hairy root cultures; elicitation.

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1. Introduction

The Rutaceae family, belonging to the order *Sapindales*, comprises approximately 2,000 species distributed across nearly 160 genera. Members of this family include woody shrubs, trees, and a limited number of herbaceous perennials that are widely distributed across the globe, with the highest species richness observed in warm temperate and tropical regions, particularly in Africa and Australia [1]. Most types of Rutaceae plants are found in semi-arid and Mediterranean climates, and many commercially valuable fruit trees, herbs, and flowers belong to Rutaceae. Aromatic foliage with oil glands on the leaf surfaces, clearly developed pores found in the stems of Rutaceae, and impressive cornucopia-shaped blossoms lead to their ability to be pollinated through insect visits.

The fruits of Rutaceae exhibit remarkable structural diversity, including capsules (*Ruta*), follicles (*Zanthoxylum*), drupes (*Amyris*), berries (*Triphasia* and *Citrus*), samaras, and schizocarps (*Helietta*). Among these, citrus fruits represent a specialized form of berry known as a hesperidium and account for some of the most commercially valuable fruit crops worldwide, including lemon (*Citrus limon*), sweet orange (*Citrus sinensis*), sour orange (*Citrus aurantium*), lime (*Citrus aurantifolia*), grapefruit (*Citrus paradisi*), mandarin (*Citrus reticulata*), and citron (*Citrus medica*) [1]. The Rutaceae family's fruit-providing plants contain several types of ornamentals: Poncirus, Skimmia, and Murraya paniculata, among others that are chemically unique, such as the Dictamnus albus, whose volatile oils readily ignite after mechanical damage to the leaf tissue.

The Rutaceae family of plants is famous for being a good source of many specialized (or secondary) metabolites. There is also a growing awareness of their potential for providing many benefits, both ecologically and pharmacologically. The metabolites from plants of the Rutaceae family will help defend the plant from herbivores and pathogens, will assist in the interaction of plants with pollinators and microbes, and will also help plants cope with environmental stress. Multiple studies have determined the different classes of metabolites that are produced by different species of

Rutaceae plants, as well as how plants produce these metabolites, such as the many different types of coumarin and furanocoumarin, alkaloids, flavonoids, phenolic acids, and terpenoid volatiles, and how often these types of metabolites are unique to the species when considering the tissue types [1, 2]. This chemical diversity reflects tight biosynthetic regulation, positioning Rutaceae as a chemotaxonomically informative and biologically rich plant family.

Biosynthetic pathways connected with specialized metabolic rifaux Rugaceae, such as phenylpropanoids, polyketides, and terpenes, are well-delineated based upon preservation of evolutionary pressures for metabolic conservation throughout history. The concentration of phenylpropanoids and other phenolic compounds, such as coumarins and flavonoids, within the plant cell supports photoprotection and antioxidant activity, while alkaloids of polyketide origin exhibit antimicrobial and cytotoxicity/inhibitory potential. The characteristic flavours/aromas of Rutaceae plants are determined by several terpene-derived compounds. This includes essential oils, as well as all major volatile constituents, which also contribute to characteristic flavours/aromas of Rutaceae plants [2]. Recent pathway-oriented investigations reveal that the spatial and temporal development of these biosynthetic pathways is organized in secretory and specialized storage tissues, highlighting the intricate variations in metabolic compartmentalization of these plant family members [3].

Despite the significant advances made in synthetic chemistry, many of the diverse Rutaceae metabolites are currently challenging or economically impractical to recreate in vitro, largely due to their structural complexity and stereochemical specificity. As a result, plant-derived production systems continue to be the dominant method of providing a significant number of pharmacologically relevant compounds [1, 4]. Consequently, the use of biotechnological techniques, including plant cell culture systems, organ-specific culture systems, and genetic manipulation of biosynthetic pathways, is increasingly valued for their ability to enhance metabolite yield, consistency, and sustainability, all while overcoming the limitations of field-based production and seasonally related variations of low availability of target compounds.

The availability of various Rutaceae species for use as research models to study the biosynthetic origin of specialized metabolites is well documented, but the publication of comprehensive reviews on the chemical composition of Ruta graveolens L. will demonstrate that this species is a particularly useful model for studying the biosynthetic pathways of specialized metabolites. Ruta graveolens possesses a long history of medicinal use and contains multiple families of specialized metabolites with similar biosynthetic pathways [5]. These metabolites include, but are not limited to, furanocoumarins, coumarins, acridone and furoquinoline alkaloids, flavonoids, and volatile oils. All of these specialized metabolites have been found to have distinct patterns of accumulation among various types of tissues and developmental stages, which allows for a more detailed study of the regulation of each pathway and the compartmentalization of each metabolite type [6].

In addition to its diverse chemical composition, Ruta graveolens also responds very well to various in vitro culturing systems such as callus culture, suspension culture, and hairy root culture. The use of hairy root cultures is a viable means of obtaining genetically stable and rapidly growing systems capable of the continuous production of alkaloids and other specialized metabolites, making them suitable for classes of research related to metabolic engineering and optimal pathway development [7]. The implementation of different elicitation methods, precursor feeding approaches, and an omics approach to metabolic engineering has further advanced the potential of Ruta graveolens as a model organism for the controlled biosynthesis and large-scale production of high-value natural products [8].

Ruta graveolens represents an extraordinary model plant for the involvement of the family Rutaceae regarding an understanding of how plants produce special chemicals or metabolites because it possesses all four criteria (i.e., botanic accessibility; chemical diversity; biosynthetic complexity; and ability to be used biologically through biotechnology). Furthermore, knowledge obtained from this plant can be utilized as a basis to develop tutorials that build on the knowledge obtained to create new products sustainably. Additionally, Ruta graveolens provides a good source for the discovery of natural products that could possess pharmaceutical properties [9]. The biosynthesis of specialized metabolites in Ruta graveolens is primarily driven by three major metabolic routes, including the phenylpropanoid, polyketide, and terpene pathways, which collectively contribute to the production of diverse bioactive compounds with ecological and pharmacological relevance (**Figure 1**).

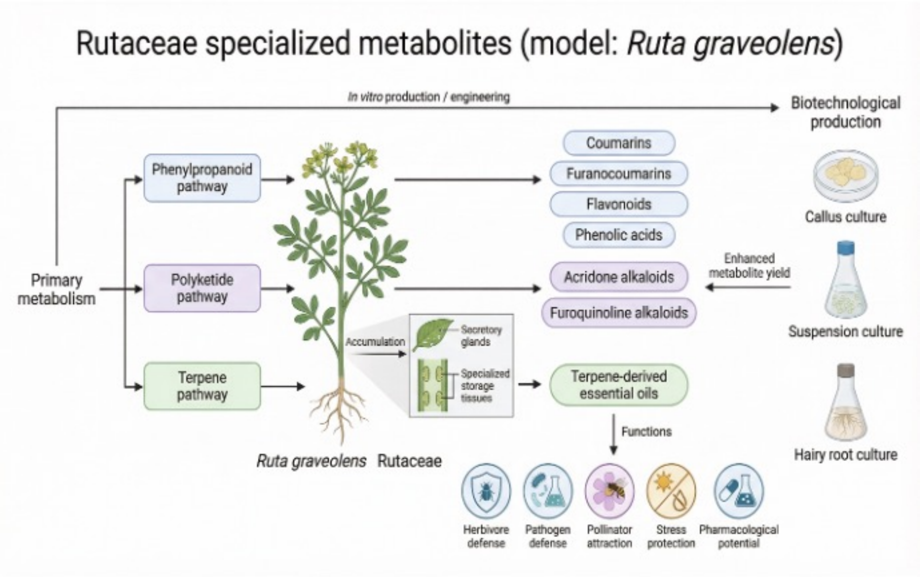

Figure 1. Schematic representation of specialized metabolite biosynthesis in Ruta graveolens. Primary metabolism feeds into phenylpropanoid, polyketide, and terpene pathways, generating compounds such as coumarins, alkaloids, flavonoids, and essential oils. These metabolites accumulate in specialized tissues and can be enhanced through biotechnological approaches (callus, suspension, and hairy root cultures), contributing to plant defense and pharmacological activity.

2. Natural Distribution, Morphology, and Medicinal Relevance of *Ruta graveolens* within Rutaceae

Rutaceae (order Sapindales) is a large family that includes 160+ genera and 2,000+ species found mainly in tropical/subtropical and warm temperate areas of the world. The family has numerous diversity centers in Africa, Australia, and the Mediterranean Basin. Most Rutaceae members are trees or woody shrubs; however, there are some exceptions, such as Ruta graveolens, which is herbaceous. Rutaceae is characterized by the presence of large secretory oil glands in the vegetative and reproductive parts of the plant, which are responsible for storing volatile and non-volatile secondary metabolites and giving the Rutaceae plants their distinct aroma [10].

Morphologically, Rutaceae species generally have hermaphroditic, hypogynous, and actinomorphic flowers, which often have a distinctive nectariferous disc beneath the ovary that facilitates entomophilous pollination. The corolla is typically composed of several free petals, and most species have obdiplostemonous stamens arranged in two whorls in the androecium, but there are notable intergeneric variations. Typically, the gynoecium has five or more carpels, which form a superior multilocular ovary, resulting in a wide variety of fruit types, which can include capsules, drupes, and berries, together with the specialized hesperidia found in species of the genus Citrus [11].

The varying diversity of vegetative organs amongst the traditionally known classification system of Rutaceae demonstrates how taxonomically informative vegetative organs can be. Leaf types in Rutaceae vary greatly, with the main variations including simple versus compound forms of leaflets (the latter is usually pinnate). In many of the genera within Rutaceae, including Citrus, winged petioles and thorn-like stems provide adaptations to specific environments and assist with the defence of the plants against herbivory. Some genera within the Rutaceae group contain anatomical features that are strongly related to storing and compound metabolites, thereby indicating a correlation between the morphology of plants (anatomy) and the phytochemical capacity of members of this family [12].

Ruta graveolens L. is a perennial herbaceous plant found throughout the Mediterranean region and is now cultivated worldwide; this plant has been cultivated for many centuries by the traditional medical systems of Europe, the Middle East, and parts of Asia. The characteristics that distinguish R. graveolens from the other herbs are bluish green pinnately divided leaves and a very pungent aroma from yellow terminal floral inflorescences, which represent traditionality for these groups of plants. The traditional medical systems of Europe, the Middle East, and Asia have utilized the plant for centuries to alleviate many forms of inflammation, gastrointestinal disturbances, parasitic infections, and some nervous disorders, thus demonstrating the long-term historical use of the plant's ethnopharmacological importance [13].

Ruta graveolens exhibits a high level of phytochemical complexity, underlining its importance as a medicinal plant. Studies performed over the last ten years have shown that *R. graveolens* contains a large variety of secondary metabolite groups such as furoquinoline, acridones, coumarins (especially furanocoumarins), flavonoids, phenolic acids, and volatile oils. The secondary metabolites in *R. graveolens* appear to be contained in different concentrations within the plant, in particular, the roots of *R. graveolens* tend to be more concentrated in alkaloids, while the other plant parts typically are the most concentrated in the volatile oils [14].

R. graveolens essential oils, along with some other Ruta species essential oils, contain a class of aliphatic ketones (e.g. 2-undecanone) and are also characterized by other ketones (e.g. 2-nonanone); however, many variations (both in type and amount) have been identified based on where the *R. graveolens* was grown, what climate it was grown in, and what harvesting methods were used to harvest the *R. graveolens*. These differences in phytochemistry are a result of the diverse nature of secondary metabolism in the Rutaceae family of plants, and thus, environmental influences have an important role in the chemical constituents of R. graveolens [15]. A combination of *R. graveolens*' morphology, secretion, and abundant phytochemical constituents makes it a premier example of a Rutaceae medicinal plant, and also a model for further studies on the biosynthesis, regulations, and biotechnological applications of specialized plant metabolites [16].

3. USES AND BIOACTIVE CONSTITUENTS OF *RUTA GRAVEOLENS* (FAMILY RUTACEAE)

3.1. Ethnomedicinal Uses and Systemic Pharmacological Effects

For thousands of years, *Ruta graveolens* has been used in traditional medicine throughout the Mediterranean and across Asia and the Middle East for both spiritual and medicinal purposes, and is still used by many today. As illustrated through multiple current reviews of ethnopharmacology, many of these traditional applications (e.g., treatment for circulatory disorders, gastrointestinal pain, nervous system disorders, inflammation, and pain) have been scientifically validated through pharmacological investigations. Furthermore, through scientific research, it has been established that there are vasoprotective, anti-spasmodic, sedative, anti-inflammatory, and analgesic effects of R. graveolens, which are achieved through antioxidant enhancement, smooth muscle relaxation, and modulation of inflammatory signaling pathways [17, 18].

In terms of cardiovascular benefits, Ruta graveolens provides increased resistance of capillaries to rupture, a decrease in the amount of fluid retained in the tissue (edema), and the ability to modulate endothelial nitric oxide signaling. These benefits validate its traditional use for treating varicosities and circulatory insufficiency. The gastrointestinal system benefits from the reduction of gastric spasms, heartburn, and indigestion through smooth muscle relaxation and control of oxidative stress. The neuroactive effects include anxiolytic, sedative, and sleep-promoting properties associated with GABAergic and monoaminergic modulation. Reproductive toxicity studies, however, demonstrate that R. graveolens exhibits uterotonic and abortifacient properties, reiterating absolute contraindication during pregnancy [19].

3.2. Anti-inflammatory, Antimicrobial, and Metabolic Activities

The multifactorial pharmacological analysis of Ruta graveolens has demonstrated that the plant has a very effective anti-inflammatory effect by blocking the principal inflammatory mediators—including tumor necrosis factor α (TNF-α), interleukin-6 (IL-6), cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS) by preventing the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and mitogen-activated protein kinase (MAPK) signalling pathways. The inhibitory properties of Ruta graveolens on these signalling pathways explain the plant's effectiveness in treating inflammatory diseases such as arthritis, rheumatism, and musculoskeletal pain, among others. In addition to anti-inflammatory action, the polyphenolic and alkaloidal fractions of Ruta graveolens are also known to protect tissues from oxidative damage by enhancing their antioxidant activity [20, 21].

Along with its anti-inflammatory activity, Ruta graveolens has broad-spectrum antimicrobial and antifungal properties. Several in vitro studies have demonstrated that both Gram-positive and Gram-negative bacterial membranes can be disrupted by a plant extract, causing mitochondrial damage and inhibiting bacterial enzymes. The extract also appears to have a metabolic-supportive role, through increasing the mobilization of fats, reducing fluid retention, and controlling the release of fatty acids by adipocytes; thus, it is likely that Ruta graveolens could effectively be an adjunct agent for managing metabolic syndrome, rather than a primary hypoglycaemic agent [22, 23]. An overview of the experimental evidence supporting the pharmacological activities of Ruta graveolens across in vitro, in vivo, and clinical studies is presented in Table 1.

Table 1. Experimental pharmacological effects of *Ruta graveolens* extracts and isolated constituents across different biological systems.

Study Type	Model / Cell Line	Extract / Compound	Dose / Concentration	Primary Outcomes	Limitations	Reference
In vitro	RAW 264.7 macrophages	Methanolic leaf extract	50–200 µg/mL	↓ TNF-α, IL-6, COX-2; Anti-inflammatory	Restricted experimental scope with reliance on a single in vitro cell model.	[24]
In vitro	Staphylococcus aureus, E. coli	Essential oil	25–100 µg/mL	Membrane disruption, bactericidal effect	Absence of validated in vivo or translational disease models.	[25]
In vivo	Sprague-Dawley rats (inflammation model)	Leaf ethanolic extract	200 mg/kg oral	↓ paw edema, analgesic effect	Evaluation limited to acute exposure without long-term toxicity profiling.	[26]
In vivo	Wistar rats (metabolic syndrome)	Aerial part extract	100 mg/kg/day	↑ lipid mobilization, ↓ fluid retention	Insufficient sample size and lack of human-based evidence.	[27]
In vitro	Caco-2 cells	Alkaloid fraction	10–50 µM	Inhibition of microbial	In vitro design precludes	[28]

				adhesion, antioxidant effect	assessment of systemic pharmacological effects.	
In vivo	Mice, induced pyrexia	Leaf aqueous extract	100 mg/kg IP	↓ rectal temperature; antipyretic	Single-dose, short-duration treatment limits dose–response interpretation.	[29]
Clinical	Healthy volunteers	Standardized leaf tincture	200 mL/day	↑ capillary resistance, improved microcirculation	Limited statistical power due to a small cohort and a brief study period.	[30]
In vitro	HepG2 liver cells	Phenolic fraction	25–100 µg/mL	↓ ROS, hepatoprotective markers	Lack of animal model validation to support cellular findings.	[31]
In vitro	Candida albicans	Essential oil	50 µg/mL	Antifungal activity; inhibition of hyphal growth	Narrow antifungal spectrum assessed using a single fungal strain.	[32]
In vivo	Mice (arthritis model)	Methanolic extract	150 mg/kg	↓ joint inflammation; ↓ TNF-α	Toxicological assessment is confined to short-term rodent studies.	[33]

3.3. Phytochemical Profile and Secondary Metabolite Diversity

R. graveolens has therapeutic versatility due to its high levels of phytochemicals and a wide range of secondary metabolites (coumarins, furanocoumarins, alkaloids, flavonoids, phenolic acids, and essential oils), which have been identified through many advanced methods of phytochemical and metabolomic analysis. The distribution of these secondary metabolites across the various parts of Ruta graveolens will differ based on plant age, growing conditions, and phenological stage, thereby necessitating the importance of standardization to be applied in the pharmacological use of this plant [34, 35]. The major classes of secondary metabolites identified in Ruta graveolens, along with their representative compounds, plant parts, and reported biological activities, are summarized in Table 2.

Table 2. Major bioactive compounds identified in *Ruta graveolens* and their associated pharmacological activities.

Metabolite Class	Representative Compounds	Plant Part	Reported Biological Activity	Key Analytical Method	Reference
Coumarins & Furanocoumarins	Xanthotoxin, Bergapten, Scopoletin	Fruit peel, Leaf	Anti-inflammatory, Antimicrobial, Enzyme modulation	HPLC, LC-MS	[36]
Flavonoids	Rutin, Quercetin, Kaempferol, Isorhamnetin	Leaves, Flowers	Antioxidant, Anti-inflammatory, Vasoprotective	LC-MS/MS, HPLC-DAD	[37]
Alkaloids (Furoquinolines & Acridones)	Skimmianine, Dictamnine, Arborinine	Root, Stem, Leaf	Cytotoxic, Antimicrobial, Anti-inflammatory	LC-MS, NMR	[38]
Volatile Oils (Terpenes)	2-Undecanone, 2-Nonanone, Limonene, γ-Terpinene	Leaf, Flower, Fruit	Antimicrobial, Allelopathic, Insecticidal	GC-MS	[39]
Phenolic Acids / Misc Phenolics	Gentisic Acid, Caffeic Acid, Ferulic Acid, p-Coumaric Acid	Leaves, Flowers	Antioxidant, Antimicrobial, and Inflammatory Modulation	LC-MS/MS, HPLC	[40]
Limonoids (Terpenoids)	Limonin, Nomilin	Fruit seeds, Peel	Antioxidant, Anti-inflammatory, Anticancer	LC-MS, GC-MS	[41]
Lignans / Fatty acids	Secoisolariciresinol, α-Linolenic acid	Seeds, Leaf	Antimicrobial, Antioxidant	GC-MS	[42]

3.4. Biotechnological Production of Secondary Metabolites

Biotechnological systems offer controlled means to sustainably grow valuable secondary metabolites in planta via controlled, mediated systems as opposed to extraction methods from wild or cultivated sources. Hairy root cultures from Agrobacterium rhizogenes are particularly advantageous because of their genetic and biochemical consistency, their ability to bring about rapid growth without reliance on growth hormones, and their ability to produce a range of specialized metabolites at levels comparable to or greater than those found in intact plants; thus, these systems are important for producing drug products [43].

Callus and cell suspension cultures serve as both in vitro examples of plant cells that establish undifferentiated masses of special plant cells capable of producing metabolites. Callus cultures are made by taking a sample of an entire plant and then placing the cut surface into a medium rich in sugar and salt without any other additives or hormones. Once the callus has established itself as a mass, it can be sub-sampled (obtained from within the original callus) to form a cell suspension for large-scale growth and cultivation in liquid media. This increases the capacity to grow plant cells and facilitates and complements the large-scale growth of plant cells using commercial processes. Thus, callus and cell suspension cultures allow for the continuous availability of the same metabolites during the entire year, whereas field-grown crops are limited by seasonal and climatic influences affecting the availability of the same metabolites [44, 45].

Transformed roots possess distinctive features that enable them to retain the organ-specific Metabolic expression of the source plant. Therefore, when used in the production of complex alkaloids and/or other metabolites, transformed roots will produce a compound with stable temporal and structural attributes. The application of transformed roots has been most commonly performed using plants from the Solanaceae; however, transformed roots can be beneficial to a greater number of useful species that produce bioactive secondary metabolites [7].

Optimization of metabolite production can also be achieved through the use of different strategies for eliciting plant metabolites. Biotic signals (such as yeast extracts or fungal polysaccharides) and abiotic signals (such as methyl jasmonate or salicylic acid) have been developed, which send a signal to the plant to initiate a plant defence reaction. This type of initiation stimulates the plant’s secondary metabolism. Elicitors should be optimized in terms of the type of elicitor used, the dose given, and the duration of time exposed to the stimulatory compound. Reports exist of the synergistic interaction between elicitors, feeding of precursors, and replenishment of medium [46].

Precursor feeding is another means of enhancing the production of metabolites. Precursor feeding means adding to the culture precursors that are necessary for the biosynthesis of a particular class of metabolite (such as flavonoids or Alkaloids). Optimizing the choice and concentration of the precursor is important, due to issues impacting both cellular uptake efficiency and/or cytotoxicity [47].

Genetic and metabolic engineering technologies are now being used to enhance production yields of plants by increasing production of limiting enzymes and regulatory transcription factors, and simultaneously decreasing production of competing pathways. In addition, the application of hairy root cultures or suspension cultures can create a way to generate specific metabolite profiles and increase biosynthetic throughput [48].

To be able to increase the productivity of manufacturing on a commercial scale, there must be a transition from using laboratory flasks to bioreactor systems. By using bioreactor technologies like agitated tanks and airlift bioreactors, larger numbers of plant cells and tissues will be able to grow at higher densities and to improve their ability to transfer nutrients and oxygen. Careful consideration must be given to the aeration rates, nutrient delivery method, and shear stress present in each type of bioreactor to ensure proper maintenance of plant cell viability and productivity of desired metabolites. While scalable systems have been developed for both cell suspension and organ culture systems, achieving high levels of scalability for the delicate hairy root system remains a formidable challenge due to the overall structure and sensitivity of these systems [45].

Nevertheless, the integration of biotechnological systems that combine in vitro culture systems, elicitation or induction of metabolite production, addition of precursors to the growth medium, and metabolic and genetic engineering provides a potential sustainable alternative to conventional plant harvest systems. Biotechnological systems may provide for the ability to consistently and at high volumes produce many of the most pharmacologically significant secondary metabolites produced by plants, such as flavonoids, coumarins, alkaloids, and terpenoids [45].

Table 2 summarizes the phytochemical diversity present in Ruta graveolens, which contains a plethora of structurally different secondary metabolites produced through natural accumulation processes influenced by both physiological and environmental conditions, resulting in variability in yield and consistency from environment to environment or plant to plant. Recent studies have indicated that in vitro plant culture systems (including callus, herbals, and hair root cultures) provide a way to circumvent these drawbacks by establishing an environment to control, scale up, and produce many different phytochemicals simultaneously [45]. Current biotechnological strategies aimed at improving the production of bioactive metabolites in Rutaceae species are summarized in Table 3.

Table 3. Core biotechnological strategies for metabolite enhancement in Rutaceae

Strategy	Target metabolites	Key benefit	Main limitation	Ref
Callus/cell culture	Phenolics, flavonoids	Controlled production	Low specialization	[49]
Hairy roots	Alkaloids, furoquinolines	High genetic stability	Technical complexity	[50]
Elicitation	Coumarins, flavonoids	Strong pathway induction	Stress variability	[51]
Metabolic engineering	Alkaloids, coumarins	Pathway-specific increase	Gene gaps	[52]
Synthetic biology	Volatile oils, alkaloids	High scalability	High cost	[53]

Coumarins and furanocoumarins represent the most common class of chemicals found in R. graveolens; they are primarily found in the fruit and above-ground structures of the plant. Photobiological, anti-inflammatory, enzyme-modulating, and antimicrobial activity are properties of these chemicals. The production and storage of these chemicals in the plant are spatially and temporally controlled, indicating that they have been adapted to act as a defense mechanism for the plant and that they have potential pharmacological benefits [18, 35]. Alkaloids make up one of the largest classes of metabolites produced by R. graveolens and have strong pharmacological relevance; furoquinolines, acridones, quinolines, and alkylquinolones are examples of alkaloids. These metabolites are cytotoxic, antimicrobial, enzyme-inhibiting, and anti-inflammatory. There is a pattern of tissue-specific accumulation of these metabolites, especially in the root and above-ground structures, and current research is starting to show that these metabolites may also have relevance for studying inflammation and cancer [54, 55]. The volatile oil extracted from R. graveolens is comprised primarily of oxygenated ketones, such as 2-undecanone and 2-nonanone, as well as monoterpenes and other compounds. These constituents of the volatile oil exhibit antimicrobial, allelopathic, and insecticidal activity. The chemical composition of the essential oils extracted from R. graveolens varies greatly depending on geographic origin, climatic conditions, time of harvest, and extraction method, all of which affect the biological efficacy of the essential oils [56, 57].

3.5. Flavonoids, Phenolic Compounds, and Synergistic Effects

Rutin, quercetin, kaempferol, isorhamnetin, caffeic, and ferulic acid are examples of flavonoids and phenolic acids, all of which play an important role in producing the medicinal properties of R. graveolens through their antioxidant, anti-inflammatory, and vasoprotective activities. The glycosylation of flavonoids enables them to be more bioavailable and enhances their ability to work together with alkaloids and coumarins, which together provide a multi-target approach to pharmacology. The synergy between all of these compounds is what allows for the extensive therapeutic properties of Ruta graveolens that have been documented in both experimental and traditional settings [34, 35]. Phytochemical research confirms the extensive flavonoid and flavonoid glycoside content within Ruta graveolens, which are significant contributors to the herb's anti-inflammatory, antioxidant, and vasoprotective properties. Rutin is the most abundant flavonoid glycoside in R. graveolens, while flowers produce unique yellow pigments such as gossypetin 7-methyl ether and its rutinoside derivative. Other flavonols include quercetin, kaempferol, and isorhamnetin, found mainly in leaves and flowers; they regulate redox balance, inhibit enzymes, and modulate inflammatory signalling pathways [34, 35]. Glycosylated and phenolic metabolites are an extensive group of metabolites produced by R. graveolens apart from classical flavonoids, and that enhance the pharmacological potential of R. graveolens. Recent studies have reported that many glycosides and phenylpropanoid derivatives based upon sucrose have been located in the aerial sections of R. graveolens, as well as several phenolic acids, including gentisic, caffeic, ferulic, and p-coumaric acids. The combination of these compounds has a synergistic antioxidant and antimicrobial action, therefore altering oxidative stress, inflammatory mediators, and limiting the growth of microorganisms. The presence of flavonoids, phenolic acids, and other glycosides indicates a multi-target mode of action, thereby enabling R. graveolens to exhibit a wide range of therapeutic effects [18, 58].

3.6.Toxicology and Safety Considerations

Ruta graveolens (rue) has considerable pharmaceutical possibilities. However, there are specific risks due to toxic furanocoumarins and alkaloids, especially when considering phototoxicity. Furanocoumarins, such as psoralen and xanthotoxin, are able to cause phototoxicity when they interact with ultraviolet A (UVA) light and through the process of phytophotodermatitis. Phytophotodermatitis is considered a non-immunological phototoxic response and has characteristics of erythema, blistering, and hyperpigmentation, which may develop on any portion of the skin that has been exposed to sunlight shortly after coming into contact with the sap or extracts from rue. The mechanism of phototoxicity involves the intercalation of DNA, as well as covalent binding (forming covalent adducts) after subsequent exposure to UVA light, resulting in damage to the DNA of the exposed cells [59].

The potential for phototoxicity following the handling of rue has been identified and documented through clinical observation. Handling rue has been observed to create phototoxic skin reactions in those individuals who previously have had an adverse reaction to handling the plant, such as those who have been out in the sun for a long time before making contact with rue, and who later experienced a reaction. Therefore, precautions must be taken for the safe handling of rue, such as wearing protective clothing and avoiding exposure to sunlight for at least 24-48 hours following contact [59].

Reproductive Toxicity - the Reproductive Toxicity of Ruta graveolens (Rue) is one of the main safety issues with this herb as the abortifacient/emmenagogue actions of the herb pose a risk of being a cause of reproductive toxicity. Ruta graveolens has a long history and there has been an extensive amount of research documenting the potential for high doses of Rue, as well as specific extracts from Rue, to have a stimulatory effect on necessary uterine contractions as well as producing embryotoxic effects and having a negative impact on reproductive outcomes in pregnancy models. Recent publications have begun to show that there are contraindications for the use of Rue during pregnancy; there is evidence suggesting that taking certain herbal preparations of Rue may have a detrimental effect on pregnancy outcomes [13].

Systemic Organ Toxicity. Laboratory animal studies using R. graveolens as a testing agent have revealed possible organ damage associated with systemic effects of this plant. The findings include signs of possible liver injury, and alterations to blood components in all cases where large amounts of Ruta or daily dosing over an extended time period were tested. The effects included increased levels of liver enzymes, and development of a type of anemia. These observations reinforce the conclusion that improper use or excessive dosage of Ruta extracts may lead to hepatic injury and also affect the immune system [60].

Drug–Herb Interactions. In addition to reproductive and organ toxicity, drug–herb interactions represent another level of risk associated with the use of R. graveolens. Specifically, furanocoumarins are known to interact with cytochrome P450 enzymes, which are responsible for metabolizing medications. The use of herbs that contain furanocoumarin compounds may alter the pharmacokinetic profiles of medications taken simultaneously. Although there is little information available regarding specific interactions between R. graveolens and medications, predictive models of herb–drug interactions for all herbs that cause furanocoumarin compounds suggest a likelihood of influencing the activity of CYP1A2, thereby necessitating caution in people taking medicines that undergo metabolism by that metabolic pathway [61].

Little is known about the way in which dose-response relationships are established, and currently available scientific evidence indicates that when large doses or very concentrated forms of the plant are ingested, there is a greater likelihood of experiencing negative side effects such as nausea, vomiting, liver damage, or other systemic toxic effects. Therefore, the consensus among published literature indicates that internally ingesting the plant should be done with caution; it also advises that topical application should be performed only with diluted extracts and avoiding sun exposure to minimize potential phototoxicity [13].

To further mitigate the risk of phototoxicity associated with prolonged UV radiation exposure from topical products containing furocoumarins, it will be necessary to ensure that extracts from medicinal plants contain only extensively characterized, well-concentrated, specific extracts and never make the assumption of equivalency between different preparation methods and/or parts of the plant as levels of phototoxic constituents differ widely based on the extraction solvent used, plant tissue source, and processing used to obtain the extract [59].

Ruta graveolens' harmful effects go beyond mere skin sensitivity to sunlight. Depending on how much you consume, how you take it (as a pill or liquid), etc., and other factors, the damage can go as deep as pregnancy difficulties for a woman, to problems later in life after she has delivered a baby. In almost all cases, the larger the dose that is taken, and/or the more concentrated the form of Ruta graveolens being used, the higher the chance that the user will experience negative results. To safely use Ruta graveolens and make sure it does not cause you harm, you should be aware of how to prepare your extracts from the plant to use them as drugs, and what guidelines, i.e., safe exposures, to follow. Finally, we do not yet fully know how the components of Ruta graveolens relate to human safety, thus creating a demand for controlled studies evaluating the dosages of compounds such as furanocoumarins and alkaloids and their potential safety margins [13].

4. Biosynthesis of Major Secondary Metabolites in Ruta graveolens and Rutaceae

The pathways through which plants convert basic substrates into complex, diverse, active secondary metabolites are connected via three main metabolic networks: phenylpropanoid, polyketide, and terpene. The proteins and enzymes involved in these pathways are highly specialized based on the types of compounds produced; the pathways often differ by a small number of key reaction steps. For instance, the formation of flavonoids, coumarins, and furanocoumarins involves polyketide synthases (PKS), while alkaloids (`furoquinoline' and `acridone') involve cytochrome P450s (CPY), O-methyl transferases, and phenyl transferases (from the phenylpropanoid pathways). All of these key enzymes significantly influence the assembly of carbon skeletons and determine how various modifications result in the final metabolite's chemical identity. Understanding how these biosynthetic pathways function is critical to understanding how plant species accumulate their products, how these compounds affect ecosystems, and how scientists can engineer metabolism via biotechnological methods (metabolic engineering) in both plants and in other species (heterologous hosts). Understanding the mechanisms that assemble and modify these structures where these compounds are produced is critical to understanding how secondary metabolites work in the biological context of organisms and how to increase yields through metabolic engineering and biotechnological means. Metabolic pathways produce secondary metabolites via interconnected enzymatic networks regulated by transcriptional and post-transcriptional control mechanisms [62, 63]. The diverse pharmacological activities of Ruta graveolens, including anticancer, neuroprotective, and cardiometabolic effects, are mediated through multiple molecular mechanisms such as apoptosis induction, oxidative stress modulation, and inhibition of tumor progression, as illustrated in **Figure 2**.

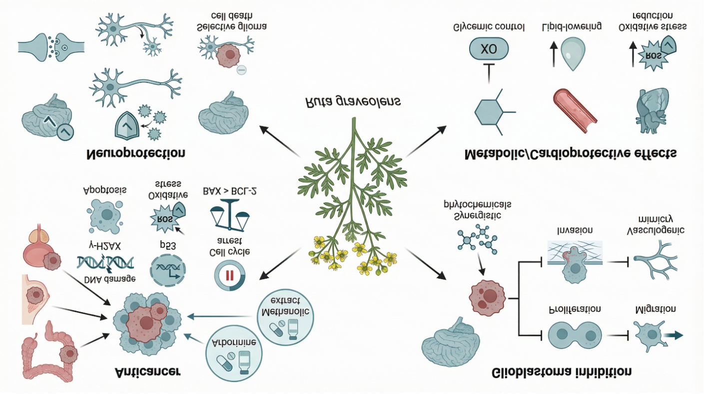

Figure 2. Schematic illustration of the biological activities of Ruta graveolens. Its phytochemicals (e.g., arborinine and methanolic extracts) exert anticancer effects via DNA damage, apoptosis, oxidative stress, and cell cycle arrest; inhibit glioblastoma progression; provide neuroprotection; and contribute to metabolic and cardioprotective effects through glycemic control, lipid regulation, and oxidative stress reduction.

4.1. Flavonoid Biosynthesis

Flavonoids represent the most diverse category of secondary metabolites in plants, occurring in many varieties worldwide. They are produced through phenylpropanoid biosynthesis: a two-step process. The first step involves the conversion of phenylalanine to cinnamic acid by *PHENYLALANINE AMMONIA LYASE* (PAL). This is followed by five enzymatic modifications of cinnamic acid (the original converted product) through *CINNAMATE* 4 *HYDROXYLASE* (C4H) and 4 *COUMARATE COA LIGASE* (4CL), resulting in 4 *COUMAROYL* COA, with C4H catalyzing the reaction of phenylalanine with 4 *COUMAROYL* COA to produce *CHALCONE*. The formation of chalcone requires one-third of the malonic acid added to 4 *COUMAROYL COA* through the action of *CHALCONE SYNTHESE* (CHS). Chalcone acts as a major precursor for flavonoids and undergoes further isomerization to produce *FLAVANONES* by the action of *CHALCONE ISOMERASE* (CHI), then further biotransformations occur to generate many individual flavonoid structures, such as *FLAVONOL, FLAVONE, FLAVANONE*, and *ANTHOCYANIN*. Various classes of enzymes are responsible for the final degradation of chalcone structure, including *FLAVONE SYNTHASE* (FNS), *FLAVONOL SYNTHASE* (FLS), and *O-METHYLTRANSFERASE* (OMT), resulting in the production of glycosides such as *RUTIN, QUERCETIN, and KAEMPFEROL*, which accumulate primarily in flowers and leaves, serving to protect these plant parts from UV damage and other oxidative stress. Transcriptional regulation of flavonoid biosynthetic gene expression is carried out by interaction of: *MYB bHLH WD-40*.which respond to developmental and environmental cues [64].

Flavonoids produced by the Rutaceae family, such as Rutin, Quercetin, and Kaempferol glycosides, originate from the preservation of several biochemical pathways. All parts of a plant are susceptible to UV radiation and oxidative stress, so the formation of flavonoids in plants depends on the availability of enzymes, the amount of precursors available for Flavonoid Biosynthesis, and the arrangement of the pathways' enzymes in the cell's cytoplasm [64].

4.2. Coumarin and Furanocoumarin Biosynthesis

Via the phenylpropanoid pathway, coumarins (furanocoumarins included) are produced. The biosynthetic precursor of coumarins is cinnamic acid, which is derived from the amino acid, phenylalanine, via the PAL enzyme. The main parts of coumarins' molecular structure originate from an intermediate that is formed from the phenylpropanoid pathway sequence of reactions involving hydroxylation and lactonization, catalyzed by the C2′H and F6′H enzymes [63].

During the biosynthesis of furanocoumarins (furanocoumarins, such as psoralen and xanthotoxin), two additional reactions (i.e., prenylation, cyclization, and phenyl transfer) take place through prenylated phenyl transferase(s) (PT) and Cytochrome P450 monooxygenase(s) (CYP). Through this process, PTs will provide a phenyl group to an existing furanocoumarin, while CYPs will use their monooxygenase activity to create a linear furanocoumarin (psoralen) and an angular furanocoumarin, respectively. The actions of these two types of enzymes are responsible for creating compounds that assist with both photoprotective and defensive functions for the plant [36]. The phenylpropanoid core is the source of coumarins and furanocoumarins because of a series of oxidation and ring closures by enzymes. The first step involves converting phenylalanine into p-coumaroyl CoA (with the help from PAL, C4H, and 4CL), followed by; addition of hydroxy groups (CYP98A-family of enzymes), followed by forming lactones such as umbelliferon (simple coumarins), followed by phenyltransferase (PT) steps where linear and angular furanocoumarins (psoralen, xanthotoxin, angelicin, etc.) are generated by phenyltransferases which create phenyl side chains, followed by oxidatively cyclizing intermediates (marmesin) via CYP71AJ-family of cytochrome P450 monooxygenases using oxidative cyclization, forming angular or linear structures depending on the type of P450. Functional genomics have identified CYP71AJ-genes with psoralensynthase activity in the coumarin pathway suggesting CYPs from the CYP71AJ family may help identify linear and angular patterns of furanocoumarins in Ruta species. The scaffold of the coumarin molecules are further modified by the action of O methyltransferases and UDP glycosyltransferases (UGTs) to make them soluble and storeable. Generally, these enzymes are located in the ER membrane (P450s) or cytosol (OMTs/PTs), whereas the final furanocoumarins usually accumulate in the epidermis or inside secretory tissues and may be exposed to UV rays [63].

Although complete pathways have been fully elucidated only in some plant families, the core steps are broadly conserved across species, suggesting similar enzymatic machinery likely exists in Rutaceae. The regulation of coumarin biosynthesis is influenced by both developmental factors and abiotic stresses, such as light and pathogen attack, indicating complex transcriptional control networks [65].

4.3. Alkaloid (Furoquinoline and Related) Biosynthesis

Plant Alkaloids are a large and varied group of biologically active nitrogen-containing compounds found in the Plant Family Rutaceae. These two classes of furoquinoline and acridone alkaloids originate from tryptophan (an amino acid) and anthranilate (an indole) through a series of biochemical processes involving polyketide synthases and multiple oxidation/methylation transformations [66].

Alkaloids from the plant genus Ruta contain many nitrogen atoms and exhibit many pharmacological activities as secondary metabolites of other organisms. The acridone alkaloid biosynthetic pathway employs an unusual type of polyketide synthase (PKS), acridone synthase (ACS), which combines N-methylanthraniloyl-CoA and malonyl-CoA to form "acridone cores" (i.e., 1,3-dihydroxy-N-methylacridone). The critical first step in this pathway is the N-methylation of anthranilate (the aromatic precursor) by its cognate methyltransferase (ANMT), directing the carbon skeleton toward alkaloid production. These biochemical reactions occur within the cytoplasm or possibly are influenced indirectly by relationships with ER-associated metabolomes (since the initial anthranilate-N-methylation requires an energy source, i.e., the CoA-compatible intermediate). Preliminary analyses of transcriptomes support the presence of additional PKS and tailoring enzymes (e.g., amine oxidases and methyltransferases) which modify hydroxyl functionality in furoquinolines and quinolines, resulting in new alkaloid structures. Cytochrome P450 monooxygenases perform regioselective oxidation of carbon and introduce the functional groups necessary for biological action. The coordinated regulation of PKS, methyltransferases, and cytochrome P450 genes provides the basis for the accumulation of different alkaloid classes in specific tissues [67].

The specific biosynthetic routes of alkaloids in the Ruta species may not be as fully studied and documented as those of other plant species (e.g., Catharanthus roseus and its indole alkaloids), but the general principles of alkaloid (or alkaloid-like) metabolism apply. Primary metabolites are converted into alkaloid skeletons through the action of various enzymes, e.g., amine oxidases, methyltransferases, cytochrome P450s, etc., that generate considerable structural diversity amongst the alkaloid compounds. Gene expression levels of the precursors for the biosynthesis of alkaloids and the transcriptional regulation of gene expression (e.g., WRKY and MYB transcription factors) in the related medicinal plants reveal the presence of multiple levels of regulation over the biosynthetic pathways for alkaloids [68].

The flux of metabolic intermediates toward the biosynthesis of alkaloids generally competes with the flux toward other metabolic pathways, and similar manipulation of both the supply of intermediates and the expression of the rate-limiting enzymes has been demonstrated to increase the accumulation of alkaloids within engineered biosynthetic systems, which suggests that such manipulations may apply to Ruta species to produce alkaloids of medical importance [68].

Cytochrome P450 monooxygenases play a key role in the diversification of alkaloid scaffolds within the Rutaceae family. These enzymes mediate regio- and stereo-specific oxidation reactions that dictate the divergence of alkaloid biosynthetic pathways toward either furoquinoline derivatives or acridone derivatives. The introduction of hydroxyl, epoxide, and methylenedioxy functionalities by cytochrome P450 enzymes, which are used in downstream modification of the alkaloid structure via methylation and ring closure, is essential for producing potent biological activity [69]. Functional studies of cytochrome P450 enzymes in related taxa that produce alkaloids suggest that cytochrome P450 activity represents a rate-limiting step during the biosynthetic pathway, is regulated both at the level of transcription and epigenesis and is responsive to both developmentally based cues and environmental signals. Therefore, differences in cytochrome P450 expression levels, or differences in the function of different forms of cytochrome P450 in the Rutaceae family, may give rise to variation in alkaloid profiles, underscoring cytochrome P450 enzymes as prime targets for metabolic engineering potential and optimization of alkaloid biosynthetic pathways [70].

4.4. Terpenoid Biosynthesis

It is known that all terpenoid compounds (monoterpenes, sesquiterpenes, diterpenes, etc.) have important roles both ecologically and medicinally. As previously stated, the two main biosynthetic methods for these compounds are the mevalonate pathway, which goes through cytosolic reactions to generate monoterpenes and triterpenes, and the 2-C-methylerythritol (MEP) pathway, localized in plastids, for monoterpenes and diterpenes [71, 72]

Acetyl CoA (the first precursor of terpenoids) is converted to isopentenyl diphosphate, or IPP, and dimethylallyl diphosphate (DMAPP), through a series of condensation and reduction reactions in the MVA pathway. In the MEP pathway, pyruvate and glyceraldehyde-3-phosphate form the same C5 building blocks. Prenyltransferases then condense these intermediates to yield geranyl diphosphate (GPP) and farnesyl diphosphate (FPP), which serve as precursors for a variety of terpenoid skeletons [71].

Terpenoids in *R. graveolens* encompass volatile oils and larger non-volatile constituents. Two distinct pathways generate the universal C5 building blocks: the cytosolic mevalonate (MVA) pathway and the plastidial 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway. Acetyl-CoA or glyceraldehyde-3-phosphate/pyruvate precursors are converted into isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). Sequential condensation by Prenyltransferases yields geranyl diphosphate (GPP) and farnesyl diphosphate (FPP) substrates for terpene synthases (TPSs) and further oxidative tailoring by P450s to produce monoterpenes, sesquiterpenes, and diterpenes. The compartmentalization of these enzyme systems occurs within plastids and the cytosol, respectively, whereby the combined effect creates an interconnection of the metabolic flux of these two pathways. There are regulatory nodes such as DXS and HMGR that regulate substrate supply to both pathways while there are also terpene synthases that determine the structural diversity of volatile oils [72].

4.5. Regulation of Biosynthetic Pathways

Multiple transcription factors (TFs) referred to as MYB, WRKY, bHLH, NAC and AP2/ERF form an intricate network of standing signals that coordinate the transcription of biochemical pathways by means of developmental signals (World Health Organization) as well as external environmental pressures (e.g., sunlight) that can create additional stress. Co-Expression Analysis from gene expression data has shown that TFs and enzymes associated with biochemical pathways often share co-expression patterns in response to stimuli (for example, MeJa), demonstrating multiple levels of control epigenetic, transcriptional after transcription, and feedback. Insights from these co-expression analyses can be used to control nodes of biochemical pathways and maximize desired products from them [68].

Multi-omics approaches integrating the use of metabolomics, transcriptomics, and proteomics have increased dramatically our capability to visualize putative biosynthetic gene clusters and infer potential functions for enzymes. The integration of new technologies such as those for analysis of the big data created from these three kinds of analysis along with machine learning algorithms has enabled us to rapidly discover new biosynthetic genes and regulatory networks, thus speeding up the process of identifying and elucidating complex secondary metabolites and their biosynthetic pathways [73].

5. Ruta graveolens L. (Common Rue): Modern Pharmacological Profile and Therapeutic Potential

5.1. Introduction and Traditional Context

Ruta graveolens L. (Rutaceae), also known as "Common Rue," is a perennial plant with deep roots in traditional medicine across cultures. Traditional uses of the plant include treating a variety of inflammatory disorders, addressing various microbial pathogens, treating digestive system issues, and alleviating some neurological disorders. These findings support earlier ethnopharmacological research into the use of R. graveolens and reflect continuing phytochemical and pharmacological studies on R. graveolens. These studies provide detailed information regarding the diverse chemical compositions and biological effects of the extracts and their active ingredients [13]

Ruta graveolens contains an extensive variety of bioactive compounds that include flavonoids (such as rutin), phenolic acid derivatives, tertiary and quaternary alkaloids, and essential oils, which together confer its ability to exhibit multiple biological activities. These constituents provide the basis for ongoing investigation to establish a modern scientific basis for support of its traditional medicinal applications [13]. The major phytochemical classes of Ruta graveolens, including flavonoids, coumarins, alkaloids, phenolic acids, and essential oils, are associated with diverse pharmacological activities such as antioxidants, antimicrobial, anticancer, neuroprotective, and cardiometabolic effects (**Figure 3**).

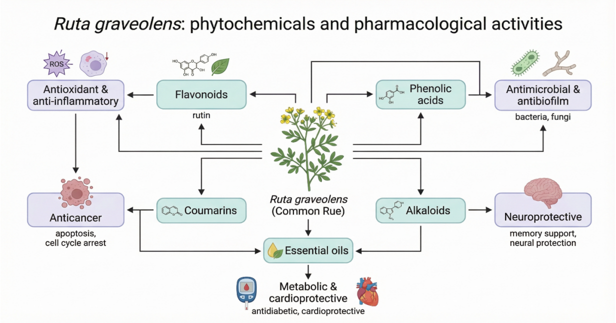

Figure 3. Overview of the main phytochemicals in Ruta graveolens and their associated pharmacological activities. Key compound classes include flavonoids, coumarins, alkaloids, phenolic acids, and essential oils contribute to antioxidants, antimicrobial, anticancer, neuroprotective, and cardiometabolic effects.

5.2. Phytochemical Constituents and Biological Basis

There is a large variety of chemicals in rue (Rue Graveolens), and to some extent, all of them are active biologically. Their main chemical groupings include the following: Flavonoids (rutin, naringenin), Phenolic acids (caffic, syringic acids), Coumarins and Alkaloids – many of which are shown to have significant biological activity when tested in vitro or vivo [74].

As an example, the flavonoid compounds and many phenolic compounds usually possess antioxidant activity, as well as an anti-inflammatory effect. Alkaloids and Coumarins are shown to possess anticancer and antimicrobial activity. Data generated by high-performance liquid chromatography analysis of rue extracts demonstrate similar bioactive compound profiles between extracts prepared in a series of different organic solvents [74].

5.3. Antioxidant and Anti-Inflammatory Activities

In several laboratory (in vitro) examinations, extracts from R. graveolens have shown that they have strong properties as antioxidants, based on their very high levels of flavonoids and other polyphenol compounds. Extracts from R. graveolens demonstrate a wide range of capability for scavenging radicals (both moderate and vigorous), which suggests that extracts may play an important role in reducing oxidative stress, which contributes to chronic inflammatory diseases and other forms of degenerative disease [75].

The inhibition of protein denaturation and stabilization of cell membranes are also indicative of the potential for the herb to provide therapeutic benefits for people with inflammatory diseases through an anti-inflammatory mechanism. At this time, however, there is still little in vivo evidence to support the findings mentioned above, but these preliminary studies provide evidence that compounds from rue may have an anti-inflammatory effect [75].

5.4. Antimicrobial and Antibiofilm Effects

5.4.1 Broad-Spectrum Antimicrobial Activity

Rich in phenolic compounds, R. graveolens has shown to inhibit the growth of Gram-positive and Gram-negative bacteria both in vitro and in vivo. Extracts that demonstrate zones of inhibiting activity against Staphylococcus aureus and Listeria monocytogenes had measurable and reproducible minimum inhibition concentrations that indicate potential for natural antimicrobial use [76].

R. graveolens essential oils also exhibit broad-spectrum antibacterial and antifungal properties by means of disruption of microbial cell membranes and interference with several important enzymatic pathways. These observations are also evidenced by molecular docking studies indicating potential inhibition of bacterial enzymes [76].

5.5. Anticancer Activity and Mechanistic Insights

5.5.1 In Vitro Cytotoxicity and Apoptosis Induction

R. graveolens extracts have been shown to have cytotoxic properties against various cancer cell lines as supported by mechanistic studies. Methylene extract of R. graveolens induces DNA damage, freezes cell cycle, and activates apoptosis via p53 and γ H2AX, respectively, in colon, breast and prostate cancer cell lines, demonstrating that it can affect proliferation and survival pathways of these cancer cells [77].

The anticancer alkaloid arborinine alone has been shown to have strong cytotoxicity towards MCF 7 breast cancer cells due to apoptosis via the alteration of BAX/BCL 2 ratio and elevation of oxidative stress markers. This indicates that different components of R. graveolens may help inhibit the growth of cancer cells [77].

5.5.2 Glioblastoma and Tumor Invasiveness

Water extracts from whole Rubus graveolens have inhibited the growth, migration, invasion, and ability to form vasculogenic mimicry in glioblastoma models. It has been noted that the specific inhibitory effect on tumor cells is not attributed solely to rutin, and thus, the collective contribution of numerous phytochemicals working together synergistically underlies this effect [78].

5.6. Neuroprotective and CNS Activities

All available preclinical studies illustrate that R. graveolens, along with its flavonoid constituent Rutin, has shown potential evidence of neuroprotection through enhancement of memory and learning in laboratory animal studies via oxidative stress management and neuroantioxidant levels modulation. This was also consistent with historical use of R. graveolens for cognitive/nervous disorders [79].

Additionally, extracts from R. graveolens were identified to facilitate neural plasticity, induce specific glioma cell death, and let neurons differentiate. They have possible future applications within degenerative brain disease use and CNS repair techniques [78].

5.7. Metabolic Effects: Antidiabetic and Cardioprotective Potential

Recent studies indicate that rue constituents are probably metabolically regulated. The antihyperglycemic and antioxidant activities of certain compounds (like rutin) may have implications for glucose maintenance within the body, as shown in experiment models. However, available data regarding humans remains scarce despite animal studies indicating improvement in lipids and oxidative stress markers and potential benefit for metabolism [80].

Some reports also describe the possible cardiovascular protective effects of xanthine oxidase inhibitors and potentially hyperlipidemic agents [75]

6. BIOLOGICAL ACTIVITIES OF FAMILY RUTACEAE

6.1. Pharmacokinetic considerations of Rutaceae-derived bioactive compounds

Pharmacokinetics (absorption, distribution, metabolism, and excretion) play key roles in determining the safety, efficacy, and overall bioavailability of the bioactive compounds described for this family. The majority of bioactive compounds found within plants of the Rutaceae family (flavonoids, alkaloids, coumarins, and volatile terpenoids) are administered by oral routes of administration (traditional and experimental), making the ability of the gastrointestinal tract to absorb these compounds critical for determining their systemic effects [81]. Rutaceae-derived bioactive compounds, including flavonoids, coumarins, alkaloids, and terpenoids, exhibit notable antibacterial and therapeutic activities, with their pharmacokinetic behavior encompassing absorption, metabolism, distribution, and elimination processes (**Figure 4**).

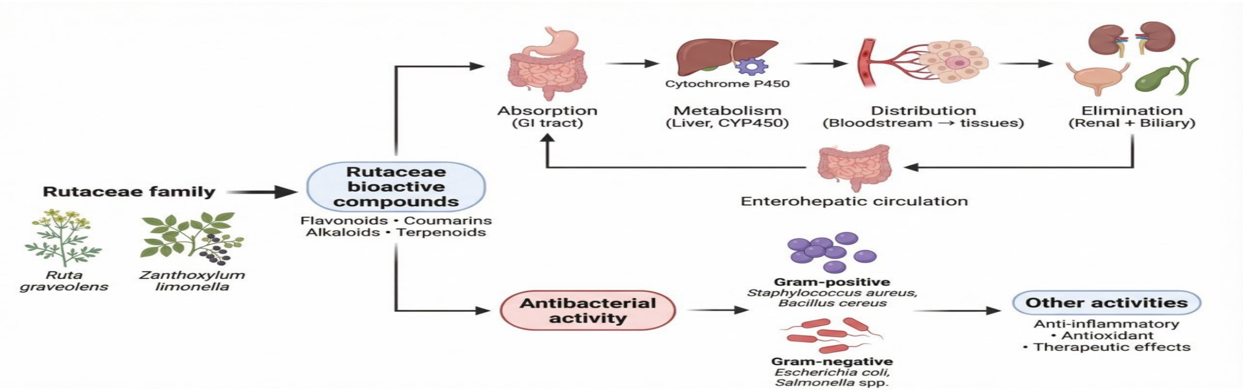

Figure 4. Overview of Rutaceae bioactive compounds and their pharmacokinetics and biological activities. Following absorption, these compounds undergo hepatic metabolism (CYP450), systemic distribution, and elimination, with possible enterohepatic circulation. They exhibit antibacterial activity against Gram-positive and Gram-negative bacteria, alongside additional anti-inflammatory, antioxidant, and therapeutic effects.

The oral bioavailability of phytochemical polyphenols can vary depending on their constituent types, as exemplified by the poor intestinal absorption of rutin (a glycoside of quercetin) due to its high polarity and dependence on colonic deglycosylation, compared to the higher absorption rate of quercetin aglycone after extensive phase II metabolism. Conversely, the lipophilic terpenoids and alkaloids are generally more permeable to membranes but can have limited systemic exposure due to rapid hepatic metabolism [82].

After absorption from the intestines, metabolites of plants of the Rutaceae family undergo significant metabolism in the liver via phases I and II of enzymatic activity (cytochrome P450-mediated oxidation, glucuronidation, and sulfation). The resulting metabolites can be pharmacologically active or inactive, which can modulate their respective potency and toxicity. In the particular case of Ruta graveolens, various alkaloids and furanocoumarins have been identified as having interaction potential with hepatic enzymes that can produce increased therapeutic potential, but also might create concerns related to potential herb–drug interactions and possible photo-toxicity [83].

The ability to distribute through systemic circulation to numerous target tissues allows for the exertion of antimicrobial, anti-inflammatory, anticancer, and antioxidant effects by the Rutaceae. Thus, these effects are influenced by both dosage and tissue type [83].

One primary route of elimination for some of the metabolites produced by members of the Rutaceae family is through renal (urine) and biliary excretion; others are involved in enterohepatic circulation such that after biliary excretion, they can re-enter the systemic circulation (into the intestines) and be reabsorbed back into the systemic circulation (i.e., recycled). The ability to recycle may result in prolonged biological half-lives and continual therapeutic effects of Rutaceae-derived metabolites, but it may also result in DOSAGE (i.e., cumulative) toxicity if the same dose is taken repeatedly. In summary, pharmacokinetic characteristics are an important link between *in vitro* bioactivity and *in vivo* efficacy and therefore should always be considered in assessing the biomedical value of any Rutaceae species [84].

6.2. Antibacterial activities

Zanthoxylum limonella is a member of the Rutaceae family and belongs to the kingdom Plantae. This herb has a remarkable potential against various infectious diseases. In vitro analysis of the study revealed that Z. limonella possesses antibacterial, antioxidant, anti-inflammatory, and antifungal properties [85].

Z. limonella fruits also possess antituberculosis properties with the minimum inhibitory concentration (MIC) value of approximately 200 µg/mL against certain gram-positive bacterias including Mycobacterium tuberculosis [11]. The antibacterial activity of the oil extracts separated from the fruits of Z. limonella was analyzed by Wannissorn and colleagues against five different gram-negative bacterias including *Escherichia coli*, *Campylobacter jejuni*, *Salmonella typhimurium*, *Clostridium perfringens,* and *S. enteritidis*. The results of this study concluded that all these bacterial pathogens showed antibacterial potential against Z. limonella fruit extract (Murraya species biological overview supporting broad Rutaceae bioactivities) [86].

A study confirmed the antifungal activity of the dichloromethane extracted from the stem of Z. limonella. Another study reported the antibacterial properties of oil extracted from Z. limonella against three gram-negative and three gram-positive bacterial species (*Escherichia coli*, *Salmonella Rissen*, *Pseudomonas fluorescens,* and *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus,* respectively [86].

Ruta graveolens L. plant also belongs to the Rutaceae family. This plant has several antibacterial and anti-inflammatory properties. To confirm its antibacterial potential, oil was extracted from this plant by the hydro-distillation method [87]. Further analysis of the essential oil was conducted by gas chromatography. Four gram-negative and gram-positive microbial strains were used in this study, including *Pseudomonas aeruginosa* (ATCC 10145), *Salmonella typhi* (ATCC 19430), *Escherichia coli* (ATCC 25922), *Enterobacter aerogenes* (ATCC 13048), *Micrococcus flavus* (ATCC 25923), *Bacillus cereus* (ATCC 11778), *Micrococcus luteus* (ATCC 9341), and *Staphylococcus aureus,* respectively. The disc diffusion method was used to check the antimicrobial activity. The result of this study concluded that the strongest antibacterial activity of essential oil was observed against two gram-positive bacteria: *Staphylococcus aureus* and *Bacillus cereus* [74].

8. Limitations

The pharmacological potential of Ruta graveolens, as well as other species of the Rutaceae family, has been well documented. However, in order to translate laboratory and animal test results into clinical medicine, there are still several limitations. A particular challenge is the variability of metabolite composition resulting from environmental, seasonal, and organ-dependent components of variability, which causes a major barrier to the ability to reproduce and standardize these products for pharmaceutical uses [88].

There are also many gaps in understanding the toxicity and optimizing these products for human use. While preclinical studies show that these products are effective in reducing inflammation, killing bacteria, and killing cancer cells, there is very limited information concerning their long-term safety, pharmacokinetics, and interactions with other pharmaceutical drugs [89].

One other barrier to the use of these products as human medicines is their low natural yield of bioactive metabolites, particularly alkaloids, furanocoumarins, and flavonoids, which limits the ability to develop large quantities of these products [90]. In addition, the concentration and efficacy of these metabolites are influenced by several factors, including the age of the plant, the environmental conditions in which it grows, and the extraction method [91-99].

Biotechnological methods can overcome these challenges through the use of callus, suspension, hairy root cultures, elicitation, and metabolic engineering, all of which enable the production of secondary metabolites in an environment where production is strictly regulated. These methods provide the potential for reproducible, scalable, and sustainable production of secondary metabolites without relying heavily on environmental variability [45]. By using these methods together, the biotechnological approaches allow for consistent production of secondary metabolites while minimizing their dependence on nature's variability [50].

9. CONCLUSION

Ruta graveolens L., a member of the Rutaceae family, has been employed in traditional medicine for generations as a source of numerous types of specialized and biologically active metabolites. The amount, type, and distribution of specialized metabolites produced by R. graveolens vary with developmental stages and locations within the plant. Primary examples of metabolite classes found in R. graveolens include coumarins and furanocoumarins; acridone alkaloids and furoquinolines; flavonoids; phenolic acids; and various types of volatile compounds. Collectively, these metabolite classes explain the wide-ranging biological effects attributed to R. graveolens in scientific literature. Numerous laboratory studies have also implicated R. graveolens in free radical scavenging effects, exhibiting antioxidant properties; supporting its anti-inflammatory properties; inhibitory effects on bacteria; showing activity against parasites; showing activity regulating nutrition; and possessing anticancer properties, establishing R. graveolens as a viable source for the extraction of bioactive compounds with substantial amounts of biological activity. However, while the extraction and purification of R. graveolens' specialized metabolites have been successful, environmental factors and plant age, organs and stages, and methods of harvest have resulted in considerable variability in the phytochemical profiles of R. graveolens metabolites.

Variability in phytochemical composition throughout the growth cycle of R. graveolens, combined with variability in environmental conditions, restricts the development and use of R. graveolens as a standardized and controllable source of bioactive metabolites. The development of biotechnology provides an opportunity for companies to find effective and affordable solutions to the issues surrounding phytochemical and pharmaceutical development. In culture systems, both callus and suspension growth allow for a more reliable and continuous production of plant material due to the increased possibilities for biomass production through new growth stimulation methods (improved support materials), feeding with new precursors, and applying omics-based metabolic engineering approaches to re-route metabolic pathways in producing targeted compounds. Furthermore, using these technologies, along with using bioreactors, quality control levels, and process development, will facilitate the transition of findings from laboratory investigations into commercially viable and reproducible production of high-quality biopharmaceuticals. One area that needs to be fully considered is biomedical product safety and toxicology, especially concerning the effects of rue (Ruta graveolens) on reproduction and the potential for furanocoumarin to be phototoxic. Rue (Ruta graveolens) serves as a prime model of a species in the Rutaceae plant family that can link specialized metabolite (primary and secondary metabolites) biosynthesis with commercially viable functional bioprocesses. Through continued integration between elucidating biosynthetic pathways, developing advanced bioprocess technologies, and performing translational studies for pharmaceutical applications, it is anticipated that the complete therapeutic and commercial usage of rue-derived metabolites will be achieved while maintaining sustainable safety and reproducible consistency in future applications.

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