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Ruta montana (L.) L.: An insight into its medicinal value, phytochemistry, biological properties, and toxicity

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ABSTRACT

Ruta montana (RM) is a medicinal and aromatic plant (MAP) used in folk medicine, especially in North Africa, to treat digestive, infectious, respiratory, neurological, gynecological, and diabetic diseases. The current work aims to review the scientifically validated ethno-medicinal usage, bioactivities and phytochemistry of RM, in order to provide data support for further investigations. Data were procured from PubMed, Scopus, Google Scholar, Web of Science, ScienceDirect, and PubChem. The present study revealed that RM could be used to manage many diseases involved in public health problems, such as diabetes, hypertension, neurological disorders, infections, reproductive system disorders, and cancer. It might also replace chemical insecticides and fungicides since it exhibits antifungal, insecticidal, and larvicidal properties. RM extracts also contain mainly coumarins and alkaloids. The volatile oil of RM is characterized by an abundance of ketone compounds and 2-undecanone as major constituents. In the case of a high-dose administration, RM infusion can cause poisoning through the oral path. Thus, in-depth *in vivo* pharmacological studies and clinical trials are needed to transmute the traditional applications of RM into scientific-based information.

Implication for health policy/practice/research/medical education:
This review provides a detailed insight into *Ruta montana* (L.) L. extracts, which could be considered a potential source of biomolecules, representing new, safe, and effective agents for managing diabetes, cancer, hypertension, infectious, digestive, and respiratory disorders.

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Introduction

To date, 80% of the world's population relies on phytotherapy, which applies medicinal plants to meet healthcare needs (1). This explains the increased demand for medicinal and aromatic plants (MAPs) in both developed and developing countries since they are known to have little or no side effects when used correctly (2,3). That's why the World Health Organization (WHO) considers phytotherapy in its health programs and suggests basic ways to confirm drugs from plant origin (4).

In many countries, traditional medicine remains an important form of disease treatment and prophylaxis, especially in North African countries (5). They own a rich phytogenetic bank of MAPs due to the climatic and ecological heterogeneity, implying a close relationship between the local population and medicinal herbs. The practice of traditional medicine is mainly noticed in rural and urban areas where plants are the only available source of medicine or due to the high cost of modern drugs (4,6,7).

Although MAPs are necessary for traditional medicine, some of them are little exploited by scientific research (8). *Ruta montana* (RM) is a MAP with "*Ruta graveolens* var. *montana* L." synonymous botanical name. This plant is among the three most widely distributed and most extensively studied species of the genus Ruta and the Rutaceae family, namely *Ruta chalepensis* L., *Ruta graveolens* L., and *Ruta montana* L. They were already known as "herb of grace" in ancient Greece (9,10).

RM is a spontaneous species native to the Mediterranean region and the Middle East, and known by its huge use in traditional medicine. It is used in traditional medicine as

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hypoglycemic, antirheumatic, anthelmintic, antiepileptic, antispasmodic, diuretic, and antipyretic remedy (11-14).

The chemical composition of RM from different regions have been reported in previous studies. The species presents an important source of various phytochemicals, including alkaloids, coumarins, flavonoids, tannins, and volatile compounds. Essential oils (EOs) extracted from RM are characterized by a strong nauseous odor and predominated by 2-ketones, such as 2-undecanone and 2-decanone. Scientific data demonstrated a significant variation in the chemical composition of RM extracts due to many factors, such as the harvesting season, botanical part, and geographical origin (15).

Moreover, many *in vitro* and *in vivo* works have shown that RM, especially its EOs, exhibit various biological activities such as antibacterial (11,16-20), antifungal (11,16,20-22), antioxidant (11,16,23-25), antidiabetic (26), anticancer (24), anti-fertility (27), antihypertensive (28), insecticidal, and larvicidal properties (29,30).

Despite the research works already executed on RM, there are still more unevaluated biological and pharmacological activities. This review aims to document and analyze information on the species in order to identify gaps for further work. Thereupon, our review attempt to answer the following questions: How is this plant traditionally used? What are the biological and pharmacological activities carried out? Where is it distributed geographically? What are the studied extracts of this plant? What are the bioactive compounds that characterize the phytochemical composition of this plant? Does this plant have toxic effects? What are the gaps observed about pharmacology, toxicity, and phytochemistry of this plant?

To the best of our knowledge, no literature review was published to analyze the reports of RM. Therefore, it is significant to proceed with an inventory of research on this plant to help researchers design future studies about this plant species.

Literature review method

The literature search was carried out in a period of eight months (from June 1st, 2021 to January 25th, 2022). The bibliographic search was performed based on scientific databases, including Scopus (Ruta AND montana*), Web of Science (Ruta AND montana*), Google Scholar (allintitle: "*Ruta montana*" OR "*Ruta graveolens* var. *montana*"), Science Direct ("*Ruta montana*" OR "Rue de montagne" OR "Mountain Rue"), PubMed (Ruta AND montana*). We got 97 articles from Scopus, 94 articles from Web of Science, 80 articles from Google Scholar, 346 articles from Science Direct, and 31 articles from PubMed. In total, we got 648 articles through database searching and 141 additional records were found through other sources, including book chapters, book reviews, conference abstracts, short communications, and Encyclopedia. Exclusion criteria were duplicated studies, works with incomplete data, multiple publication or overlapping subjects, and studies with no original research results. Data provided in case reports, editorial/letters, patents, conference papers, and symposiums were excluded. The eligible studies to establish this review were: articles about ethnomedicinal and ethnobotanical information (18 studies), phytochemistry (12 studies), biological and pharmacological properties (30 studies), toxicological profile (8 studies), and botany of RM species (3 studies) (Figure 1). Articles published from 1990 to January 25th 2022 were included. Finally, 71 pertinent articles were included for the preparation of the present review. The botanical information was collected from websites such as http://www.worldfloraonline.org and http://www.worldplants.org, and http://www.gbif.org. The chemical structures presented in the manuscript were prepared through ChemDraw Ultra 12.0 Software. IUPAC names of the reported chemical compounds were checked using PubChem databases. The Mendeley software was used to manage references.

Common names

Ruta montana (RM) species comprises different common names. It is known as 'Fijel' in Arabic, and 'Awermi' in Berber. It is called 'Rue des montagnes' in Frensh and

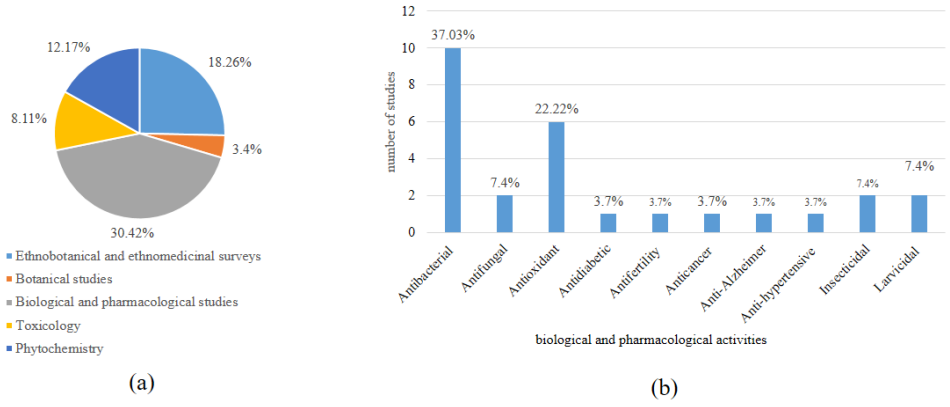

Figure 1. Number of studies by subject area (a); distribution according to the different categories of biological and pharmacological activities (b).

'Mountain Rue' in English. It was concluded that the vernacular name of this plant varies according to each country or region (Table 1). Indeed, RM can take various vernacular nominations including, Fidjel, Fijel, El Fijel, Aourmi or Awermi (Morocco, Tunisia, and Algeria), Sedef (Turkey), Arruda (Portugal), and Ruda de jardín (Spain).

Botany

Botanical characteristics

Ruta montana is an evergreen shrub with 20–60 cm tall and triangular and skinny leaves (Figure 2b); its flowers are small and yellow with two whorls of stamens, and are bisexual (Figure 2c). Its fruits are capsular with four rounded lobes (Figure 2d). The plant is characterized by a strong, foul-smelling, nauseating odor, due to an essential oil contained in huge bags containing secretarial glands (31,40). The botanical traits of RM are presented in Table 2.

Geographical distribution

Ruta montana is a plant of strongly fragrant evergreen subshrubs mainly found in temperate, tropical, and arid regions (Figure 2a). It is a spontaneous plant, responded broadly in the Mediterranean region (37). This species has a wide geographical distribution, particularly in Portugal, Greece, Turkey, Algeria, Tunisia, and Morocco (11). It is cultivated in many parts of the world for its medicinal properties and was the first plant introduced into the American continent (31,38).

Medicinal value

Several ethnobotanical and ethnopharmacological investigations have shown that the traditional use of *Ruta montana* depends on the plant's part used (Table 3). In Morocco, some preparation forms are used for mental, respiratory, and genitourinary systems disorders (oil, fumigation, infusion, and decoction forms). The RM poultice is recommended against poisonings by snake and scorpion venoms, and as a powder to treat facial paralysis (34,38). Also, fumigation, alone or combined with other species, is used to repel mosquitoes and snakes and to remove the evil eye in ritual preparations (38). A recent ethnobotanical study in the central Middle Atlas, Morocco, noticed that infusion and fumigation of RM flowers are used to treat bronchial congestion and Asthma (9).

In Algeria, the RM plant is used to relieve digestive disorders (14), toothache, joint pain, and to facilitate difficult childbirth. Furthermore, the *Ruta montana* essential oil (RMEO) was highly valued in the fragrance industry (38,39). In the Persian traditional medicine, the RM leaves powder has been used to treat epilepsy (40). The powder is also used in the kitchen as a spice; the bitterness of leaves stimulates the appetite, and their aroma gives a pleasant taste to fish dishes. Also in Italy, RM leaves flavor vinegar and alcohol called "Grappa" (38).

Table 1. Common names of *Ruta montana* in different countries

Country region	Common name	References
Morocco	Fijel, Aourmi	(31)
Algeria	Fidjel	(11)
Tunisia	El Fijel	(32)
France	Rue de montagne	(33)
Turkey	Sedef, sedef ağacı	(34)
Portugal	Arruda, Erva da inveja	(35)
Spain	Ruda, Ruda de jardín	(36)

Phytochemistry

Crude extracts

Based on the available reports, the plant extracts contain different bioactive compounds, which can be mainly grouped into three main chemical classes, including ketons, coumarins, and alkaloids (Table 4). The qualitative phytochemical tests of aerial part extracts fractionated by Soxlhet showed a richness in gallic and catechin tannins, while tests for saponins, free anthraquinones, and cyanogenic heterosides were negative. The test with Mayer's reagent revealed the presence of alkaloids such as leucoanthocyanins, whereas flavones, catechols, sterols, triterpenes, oses, holosides, and C-heterosides were identified in small quantities (10). An earlier study showed that the hydromethanolic extract of RM aerial part obtained by Soxhlet was rich in total phenols (33.684 ± 1.684 mg GAE/g extract) and flavonoids (0.843 ± 0.042 mg QE/g extract) (31).

The quantitative phytochemical analysis of RM extracts revealed that the diethyl-ether extract of a Turkish RM aerial part contained a high yield of coumarins through silica gel column chromatography. This group included bergapten, rutamarin, xanthotoxin, chalepensin,

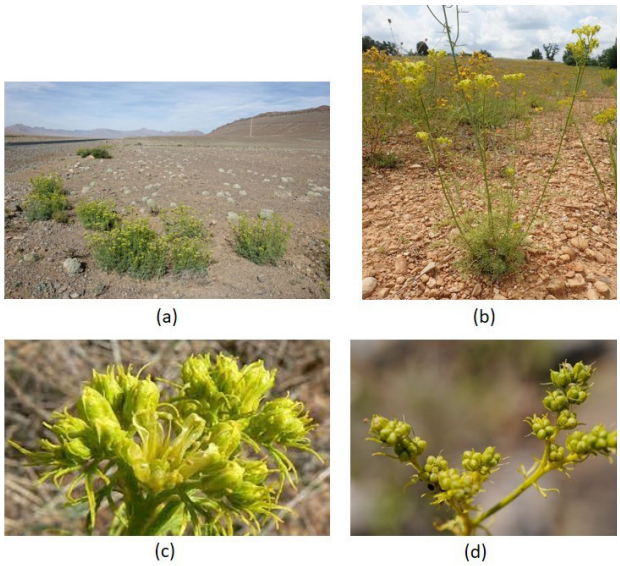

Figure 2. *Ruta montana* L. habitat (a); whole plant (b); flowers (c); and fruits (d).

Table 2. Botanical characteristics of *Ruta montana*

Type of plant	Height	General characteristics of the plant	Flowering period	References
Perennial shrub	20-60 cm	Spindly stems Leaves are light green– obovate Segments Flowers are small, yellow, borne in dichasial cymes. Fruits are rounded, small and lobulated.	May-August	(31, 38)

Table 3. Traditional uses of *Ruta montana* plant in different countries

Region and Country	Plant part used	Preparation mode	Traditional use	References
Bordj Bou Arreridj, Northeast Algeria	Aerial part	Decoction	Rheumatism	(39)
High Atlas, Morocco	Leaves	Decoction	Treatment of gingivitis	(41)
Meknes, Morocco	Leaves and roots	Decoction and Poultice	Dermatologic, respiratory and oral affections	(10)
Granada, Spain	Whole plant	Decoction	Birth	(36)
Ksar Lakbir, Morocco	Aerial part	Powder mixed with olive oil	Ear infection	(42)
		Infusion	Diuretic, anti-helmintic, pain killer (rheumatic pains)	
		Poultice (applied externally)	Headache	
Montalegre and Chaves, Portugal	Flowers and branch	Infusion	Sweating stimulation, laxative, internal and external parasitosis, nervousness and depression, menstruation and abortion induction	(35)
Errachidia, South-eastern Morocco	Aerial part, Leaves, Stem	Decoction, Infusion, Powder	Diabetes	(13, 43)
Djelfa, Algerian Central Steppe Region	Aerial part, Leaves	Decoction, Infusion, Powder	Anguish, Anorexia, Insomnia, Abdominal pain, Aerocoly, Colic, Nausea	(14)
Central Middle Atlas, Morocco	Flowers	Infusion, fumigation	Asthma, Bronchial disorders	(9)

(±)-oxypeucedanin with a lignan, sesamin, minor amounts of additional coumarins, daphnoretin methyl ether, and bergaptol. This analysis test also mentioned the presence of a new alkaloid named montanine, and two known alkaloids: 1,2-dimethyl-4-quinolinone and dictamnine (44). Likewise, six alkaloids were isolated from the chloroform extract of the whole Moroccan plant through the thin-layer chromatography (TLC) technique. Four alkaloids were new: 2-(nonan-8-one)-(1H)-4-quinolone, 2-(nonan8-one)-4-methoxyquinoline, 2-(nonan-8-one)-*N*-methyl-quinolone, and 2-(decan-9-one)-*N*-methyl-4-quinolone (45), and two known compounds; 1-methyl-4-methoxy-2-quinolone and evolitrine. The aerial parts of Algerian RM contains a dicoumarinyl ether named rutamontine, and two furocoumarins: heraclenol and isopimpinellin (46).

There are few studies examining the phytochemistry of RM. Its phenolic profile remains not well investigated. Most of the compounds have been isolated from the aerial parts, with coumarins and alkaloids being the main compounds. The other plant parts such as roots, stembarks, and fruits deserve to be explored (47,48). In fact, the phytochemical composition of RM plant varies depending on different factors, especially the development stage, harvest season, extraction solvent, plant's part used, plant's origin, storage conditions, and analytical method used (38). Table 4 summarizes the chemical compounds identified in some RM extracts collected from different zones. Their chemical structures are presented in Figure 3.

Volatile compounds

Numerous studies evaluated the chemical composition of RMEOs, described as a yellowish liquid of a strong smell and often obtained from the air-dried or the fresh aerial parts of the plant (29,50). RMEO collected at the flowering stage from Taza region of Morocco contained 2-undecanone (63.97%), camphor (3.82%), and cyclopropane carboxylic acid (3.66%) as the main components (16). Gas chromatography-mass spectrometry (GC-MS) analysis revealed that 2-undecanone (82.62%), 2-undecanol (2.87%), and 2-undecanol acetate (2.13%) were the most dominant components of RMEO from the middle Atlas mountains of Morocco (31). Some comparative studies on RM plant EOs collected from different regions of Algeria showed slight variation in the major compounds. In a study, the main constituents of the first sample were 2-undecanone (60.1%), 2-nonanone (08.6%), monoethylhexyl phthalate (6.4%), and decanone (6.2%), whereas those from the second sample were 2-undecanone (90.4%), 2-nonanone (4.0%), and decanone (1.4%) (51). This shows that the plant's origin influences the chemical composition of EOs.

Table 4. Reported phytochemical constituents of *Ruta montana* crude extracts

No.	Compound	Plant part used	Extract	Collection zone	Method	Ref.
			Lignan			
1	Sesamin	Aerial part	Diethyl ether extract	Marmara (Turkey)	Silica gel column chromatography	(44)
			Coumarins			
2	Rutamontine	Aerial part	Not described	Mila (Algeria)	Not described	(46)
3	Heraclenol	Aerial part	Not described	Mila (Algeria)	Not described	(46)
4	Isopimpinelline	Aerial part	Not described	Mila (Algeria)	Not described	(46)
5	Bergaptol	Aerial part	Diethyl ether extract	Marmara (Turkey)	Silica gel column chromatography	(44)
6	Daphnoretin methyl ether	Aerial part	Diethyl ether extract	Marmara (Turkey)	Silica gel column chromatography	(44)
7	Bergapten	Aerial part	Diethyl ether extract	Marmara (Turkey)	Silica gel column chromatography	(44)
8	Rutamarin	Aerial part	Diethyl ether extract	Marmara (Turkey)	Silica gel column chromatography	(44)
9	Xanthatoxin	Aerial part	Diethyl ether extract	Marmara (Turkey)	Silica gel column chromatography	(44)
10	Chalepensin	Aerial part, Root	Diethyl ether extract	Marmara (Turkey)	Silica gel column chromatography	(44,49)
11	(±)-Oxypeucedanin	Aerial part	Diethyl ether extract	Marmara (Turkey)	Silica gel column chromatography	(44)
			Alkaloids			
12	2-(Nonan-8-one)-(1H)-4-Quinolone	Whole plant	Chloroform extract	Rommani (Morocco)	Thin-layer chromatography	(45)
13	2-(Nonan-8-one)-4-methoxyquinoline	Whole plant	Chloroform extract	Rommani (Morocco)	Thin-layer chromatography	(45)
14	2-(Nonan-8-one)-*N*-methyl-quinolone	Whole plant	Chloroform extract	Rommani (Morocco)	Thin-layer chromatography	(45)
15	2-(Decan-9-one)-*N*-methyl-4-quinolone	Whole plant	Chloroform extract	Rommani (Morocco)	Thin-layer chromatography	(45)
16	1-Methyl-4-methoxy-2-quinolone	Whole plant	Chloroform extract	Rommani (Morocco)	Thin-layer chromatography	(45)
17	1,2-Dimethyl-4-quinolinone	Aerial part	Diethyl ether extract	Marmara (Turkey)	Silica gel column chromatography	(44)
18	Montanine	Aerial part	Diethyl ether extract	Marmara (Turkey)	Silica gel column chromatography	(44)
19	Evolitrine	Whole plant	Chloroform extract	Rommani (Morocco)	*Thin-layer chromatography*	(45)
20	Dictamnine	Aerial part	Diethyl ether extract	Marmara (Turkey)	Silica Gel Column Chromatography	(44)

The results of a study showed *approximately the same composition in essential oils but with* significant quantitative differences in the main compounds, including 2-undecanone (27.2–81.7%) and 2-nonanone (1.9–39.5%), while 2-nonanyl acetate ranged from traces to 24.8% (11). Nevertheless, RMEO of fresh aerial parts collected from eastern Algeria mainly characterized by undecan-2-one (37.74%), resorcinol (27.66%), and 2-acetoxytetradecane (9.19%) as major components (18), whereas they were not identified in other samples from Algeria, showing that the vegetative stage and plants condition (dry or fresh) also influence the chemical composition. Also, in a study the major components in the EO were 1-butene (38.33%), methylcyclopropane (15.47%), 2-butene (22.56%), and caryophyllene oxide (8.18%) (20). In another study the EO from Tunisian RM aerial parts dominated by the same major compounds identified in some samples from Morocco and Algeria precisely, 2-undecanone (86.77%), followed by 2-decanone (4.91%), and 2-nonanone (23.62%). Other studies mentioned that in addition to ketones there are also alcohols, aldehydes, and sesquiterpene hydrocarbons with the presence of aliphatic monoterpenes traces (17,32,52-54). Recently, Barbouchi et al found that chalepensin, elemol, and p*entadecane-2,4-dione* were the dominant compounds occurring in the volatile oil extracted from the roots of RM collected from Moulay Idriss Zerhoum, Morocco (49).

It is well seen that the amount and nature of these volatile compounds vary according to the harvest seasons and environmental factors, including geographical origin, phenological stage, and genotype. This variation also depends on the experimental conditions, including the drying process, storage, oil extraction method, and the plant's part used (55-58). According to the available studies, we notice that the RM plant is more studied in Algeria, it is therefore recommended to examine the RM plants of other regions and countries, and it is quite remarkable that besides leaves, flowers, and stems, other plant parts need further studies to reveal new bioactive molecules. Table 5 gives an overview of the main chemical compounds found in RMEOs from different regions extracted by hydrodistillation and Figure 4 shows their chemical structures.

Biological activities

Antibacterial activity

In order to search for new and potent antibacterial substances from natural sources, the antibacterial

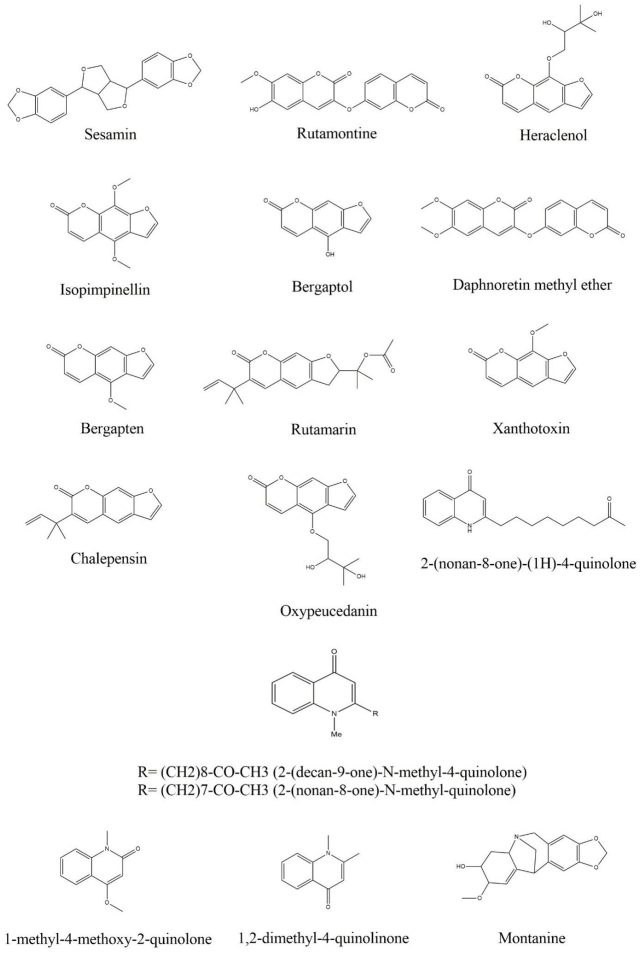

Figure 3. Chemical compounds isolated from *Ruta montana* extracts.

activities of RM extracts and EOs were tested against a panel of phytopathogenic and foodborne pathogens bacterial strains, including gram-positive (gram+) and gram-negative (gram-) bacteria (59). Table 6 summarizes previous works on antibacterial activities of RM including the parts used, the tested extracts, the methods used, the tested strains, and the main results obtained.

Indeed, Daoudi et al assessed the antibacterial activity of the aerial part of RMEO and crude extracts (decocted and infused) from Meknes city of Morocco, against *Staphylococcus aureus*, *Proteus mirabilis*, *Klebsiella pneumonia,* and *Escherichia coli*. Results showed that the highest activity was observed by the EO against *S. aureus* with an inhibition zone (IZ) of 32.66 ± 1.15 mm, which greatly exceeds the antibiotic activity; Gentamicin (IZ=19 mm). However, no effect was obtained on *P. mirabilis, E.coli*, and *K. pneumonia*, and the crude extracts showed no activity (10). This was due to the multi-resistance of bacterial strains clinically isolated. Moreover, the RMEO from Taza region of Morocco showed good antibacterial activity of RMEO against *Bacillus subtilis* and *P. mirabilis* strains with an IZs of 21.33 ± 1.52 mm and 16.66 ± 1.15 mm, respectively, followed by *S. aureus* (12 ± 1 mm) and *L. innocua* (10.33 ± 1.52 mm). The minimal bactericidal concentration (MBC) and minimal inhibitory concentration (MIC) values indicate that the EO exhibits a bacteriostatic effect against the previous bacterial strains. *E. coli* was the most resistant (IZ= 9.33 ± 1.52 mm) (16).

In another report, *S. aureus*, *P. aeruginosa*, *Mycobacterium kansasii,* and *Mycobacterium vaccae* were used for the antimicrobial study of RMEO from Algeria. Results showed that the inhibitory effect was dose-dependent against all the tested strains and the EO was effective even in weak concentrations. The average IZ was 0.6-5.8 mm (17). Another work demonstrated that EOs extracted from RM of Eastern Algeria exerted a high inhibitory activity against *Streptococcus enterococcus*, *P. aeruginosa, K. pneumonia,* and *E. coli* with IZ diameters of 27.5, 20, 19, and 19 mm, respectively (18). Also, RMEO from Tunisia had a low effect on *E. coli*, *K. pneumoniae*, and *P. aeruginosa*, with the IZs varying between 6 and 7 mm in comparison with gentamicin (17-19 mm) (19). An important antibacterial activity was shown against *S. aureus* (IZ = 21 mm). Findings in another study showed considerable activity against Gram+ bacteria, *S. aureus*, *B. subtilis,* and *Enterococcus faecium* (10.2 ± 0.7 mm ≤IZ≤ 15.1 ± 0.7 mm). However, the effects of standard antibiotics (piperacillin and kanamycin) were more notable than EOs

Table 5. Major volatile constituents of *Ruta montana* essential oils

No.	Chemical constituents	Plant's part	Method	References
1	2-Undecanone	Leaves and stems	GC-MS	(11, 16, 18, 29, 32, 51- 54).
2	Camphor	Aerial parts	GC-MS	(16)
3	Cyclopropanecarboxylic acid	Aerial parts	GC-MS	(16)
4	2-Nonanone	Aerial parts	GC-MS	(11, 32, 51-54).
5	2-Nonanyl acetate	Aerial parts	GC-MS	(11)
6	Resorcinol	Aerial parts	GC-MS	(18)
7	2-Acetoxytetradecane	Fresh flowering, aerial parts	GC-MS	(18, 52)
8	Monoethylhexyl phthalate	Aerial parts	GC-MS	(51, 52).
9	2-Decanone	Aerial parts	GC-MS	(29, 51, 52).
10	2-Tridecanol	Aerial parts	GC-MS	(52)
11	2-Dodecanone	Leaves and Stems	GC-MS	(29)
12	1-Butene	Leaves	GC-MS	(20)
13	Methylcyclopropane	Leaves	GC-MS	(20)
14	2-Butene	Leaves	GC-MS	(20)
15	Caryophyllene	Leaves	GC-MS	(20, 53).
16	2-Methyloctyl acetate	Leaves	GC-MS	(53)
17	1-Nonene	Leaves and Stems	GC-MS	(32)
18	Z-8-(3,5-Dimethyl-4-Hydroxyphenyl)-2-octene	Stems	GC-MS	(32)
19	Isomaturnin	Stems	GC-MS	(32)
20	Nonanol-2-acetate	Aerial parts	GC-MS	(54)
21	Psoralen	Aerial parts	GC-MS	(54)
22	*Pentadecane-2,4-dione*	Root	GC-MS	(49)
23	Elemol	Root	GC-MS	(49)

GC-MC: gas chromatography-mass spectrometry.

(21 mm ≤IZ≤ 26 mm). Other EOs had no inhibitory effect on *P. aeruginosa* while they had a moderate inhibitory effect on *E. coli* and *K. pneumonia* (9.2 ± 0.5 mm ≤IZ≤ 14.3 ± 0.7 mm) (11).

Interestingly, Hammami et al investigated the *in vivo* antibacterial activity of EO from air-dried leaves of a Tunisian RM plant on highly infected soil by *Agrobacterium tumefaciens*, inducing the crown gall. Results showed that infected soil treated with different concentrations of RMEO did not show symptoms of crown gall, and the enumeration of the *A. tumefaciens* population in the treated soil was significantly reduced (<10 CFU/g of soil) (20). More recently, Zeraib et al compared the antibacterial effect of RMEO alone and in combination with five conventional antibiotics (gentamycin, amoxicillin, cefazolin, tetracycline, and amoxicillin/clavulanic acid (Claventin)) against three pathogenic bacteria (*Staphylococcus aureus* ATCC25923, *Pseudomonas aeruginosa* ATCC27853, and *Escherichia coli* ATCC25922) using the agar disc method. Findings showed that the combination of RMEO with antibiotics, particularly with amoxicillin and cefazolin, induced significant synergistic effects against all bacterial strains tested. The inhibition zones of amoxicillin and cefazolin were 19.7-34 mm and 21.5-41 mm, respectively (15).

Antifungal activity

The use of synthetic fungicides is increasingly restricted due to their harmful effects on human health and the environment. This justifies the search for new bioactive molecules to fight against pathogenic fungi (21). Hammami et al assessed the *in vivo* and *in vitro* antifungal activity of Algerian RMEO and extracts obtained from leaves (hexane extract, chloroform extract, ethyl acetate extract (EAE), and methanol extract (ME)) against some phytopathogenic fungi, *Botrytis cinerea*, *Fusarium oxysporum*, *Verticillium dahliae, Aspergillus oryzae*, and *Fusarium solani* using the broth microdilution assay. The results revealed that 1000 µg of RMEO had potent *in vitro* activity with radial growth inhibition percentages of 40 to 80% confirmed with the MIC values ranging from 100 to 1100 µg/mL. On the other hand, RM extracts inhibited fungi growth less than EO (5 to 58.0% of inhibition for 1.5 µg) with MIC ranges from 250 to 3000 µg/mL. Likewise, 1.000 µg/mL of the oil showed 100% antifungal effect against leaf spot/scorch of tomato caused by *B. cinerea* compared to Benomyl as a positive control (20).

Mohammedi et al. tested *in vitro* antifungal activity of seven RMEOs from different locations in Algeria against *Saccharomyces cerevisiae* and *Candida albicans* using disc diffusion (30 µL per disc) and microdilution assays. Data showed that RMEOs had generally weak to moderate antifungal activity against the tested microorganisms (12.0 ± 0.4 <IZ< 17.9 ± 1.1 mm), and the fungi tested were more susceptible to the reference compound (Nystatin) than to EOs (18<IZ<19 mm). In addition, *C. albicans*

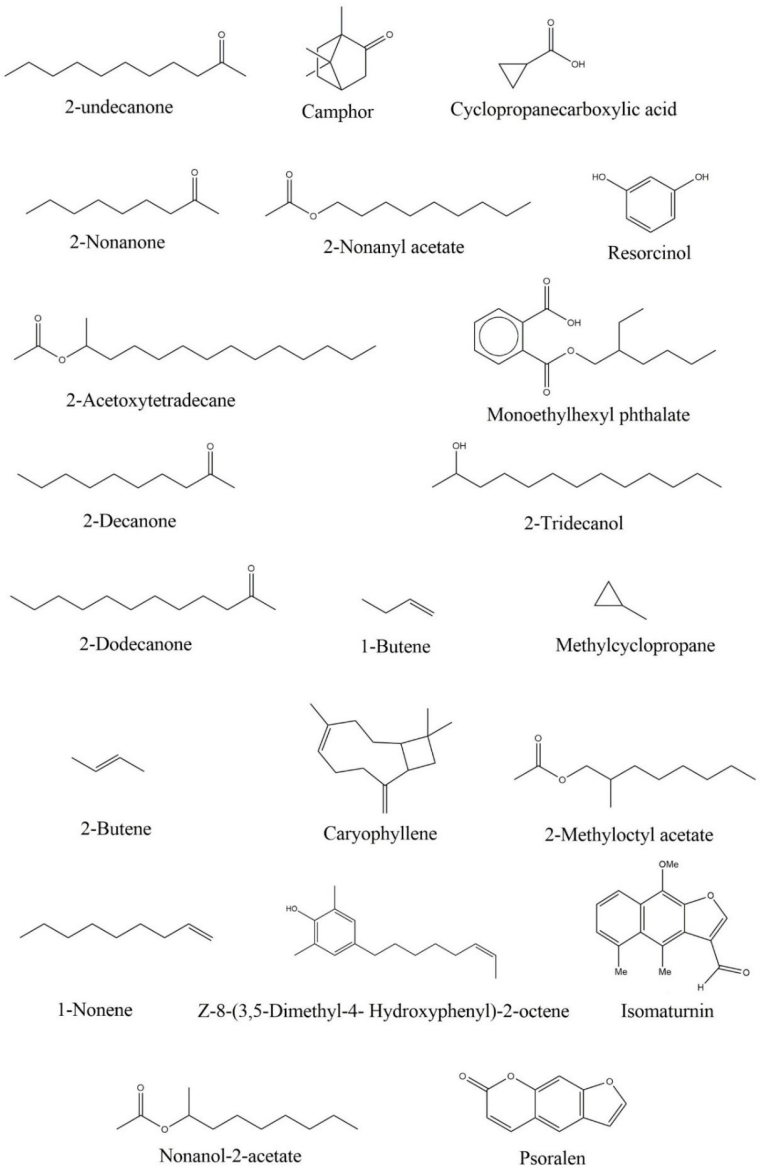

Figure 4. Chemical structures of major volatile compounds identified in *Ruta montana* essential oils.

showed high sensitivity to RMEOs at low doses (MIC ≤ 5 µL/mL) (11). Also, Benali et al evaluated the anticandidal activity of EO from Moroccan RM by using the filter paper disc diffusion and the microdilution methods against *C. albicans* ATCC 10231. The obtained results revealed an important antifungal activity with an IZ of 21.66 ± 0.57 mm, higher than Amphotericin 18.66 ± 1.15 mm, which was confirmed with a weak MIC value (6.25 µg /mL) (16).

Moreover, Gibka et al studied the antifungal effect of undecan-2-one, undecan-2-ol, and their derivatives against yeast *Candida mycoderma* and mold *Aspergillus niger* by the impedimetric method using a Bactometer M64 System (bioMerieux). Data showed that Undecan-2-one was most effective against yeast *C. mycoderma* and mold *A. niger*. Ketone and alcohol also exhibited great activity towards *A. niger*; the racemic mixture and S(+) enantiomer showed a potent static and cidal activity towards *A. niger* (22).

Antioxidant activity

Many studies have evaluated the antioxidant properties of RM to discover new natural antioxidant agents (Table 6). Merghem and Dahamna assessed the *in vitro* antioxidant activity of different extracts from RM aerial parts; ME, chloroform extract (ChE), EAE, and aqueous extract (AqE) using 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH) reduction, β-carotene bleaching, and ferrous iron chelation assays. Results showed that EAE had a strong scavenging capacity of DPPH with a half maximal inhibitory concentration (IC_{50}) of 0.044 ± 0.001 mg/mL compared with Quercetin (IC_{50}=3.491 ± 0.001 mg/mL), used as a standard antioxidant, followed by aqueous extract (AqE) (0.083 ± 0.003 mg/mL) compared to Rutin (4.179 ± 0.000 mg/mL), while ME (0.067 ± 0.002 mg/mL)

Table 6. Synopsis about biological activities of *Ruta montana* plant

Activity	Study type	Experimental model	Extract	Dose range	Controls	Key result	References
Anti-bacterial activity	*In vitro*	Disc diffusion method	Infused, decocted, essential oil from aerial part	25 µL	Gentamicin, oxacillin, imipenem, amoxicillin	Essential oil exhibited an important inhibitory activity against *S. aureus* (Z= 32.66 ± 1.15 mm) Extracts were inactive.	(10)
	In vitro	Agar disc diffusion and microdilution methods	Essential oil from aerial part	12.5 µL 0.097 to 25 mg/mL	Gentamicin, vancomicin, and amphotericin	Essential oil showed a strong effect against *B. subtilis* (IZ=21.33 ± 1.52 mm), and good minimal inhibitory and bactericidal concentrations (MIC= 0.39; MBC= 6.25 mg/mL)	(16)
	In vitro	Vincent method (Aromatogram)	Essential oil from aerial part	5 to 12.5 µL	Not identified	Essential oil had a moderate effect against *P. aeruginosa* (IZ= 5.8 ± 0.75 mm)	(17)
	In vitro	Disc diffusion and microdilution methods	Essential oil from aerial part	Not identified	Not identified	The best inhibitory zone was against *S. enterococcus* (IZ=27.5 mm), and the minimal inhibitory concentration was 20 µg/mL against *S. enterococcus, E. coli*, *P. aeruginosa*, and *K. pneumonia*	(18)
	In vitro	Agar disc diffusion method	Essential oil from aerial part	10 µL	Gentamycin	Essential oil showed an interesting inhibitory zone (21 mm) against *P. aeruginosa* ATCC 7624 and *S. aureus* ATCC76110	(19)
	In vitro	Agar disc diffusion method	Essential oil from aerial part	30 µL	Piperacillin and Kanamycin	Essential oil exhibited good inhibitory effect against *S. aureus* (IZ= 16.2 ± 0.6 mm and MIC= 2.5 µL/mL)	(11)
Antifungal effects	*In vitro* *In vivo;* on tomato plants infected by *B. cinera*	Disc diffusion and microdilution	Essential oil from leaves, CrE, EAE, and ME	1000 µg-4 µL (essential oil) 8-1500 µL (extracts)	Not identified	Essential oil showed significant percentage growth inhibition against *F. oxysporum* (69 %), *B. cinerea* (80 %), *A. oryzea*, (40 %), *V. dahliae*, (60 %), *R. solani* (58 %), and *F. solani* (76 %)	(20)
Antioxidant effects	*In vitro*	DPPH reduction, β-carotene bleaching, ferrous iron chelation	CrE, EAE, AqE	50 µL 350 µL 250 µL	Gallic acid, rutin, quercetin	EAE showed the best scavenging activity with a half maximal inhibitory concentration (IC_{50}) of 0.067 ± 0.002 mg/mL. AqE had the best lipid peroxidation inhibition (90.34± 0.46%). AqE had the best ferric chelating power with an IC_{50} of 0.005 ± 0.004 mg/mL	(23)
	In vitro	DPPH	ME of aerial part	0.3-0.001 mg/mL	Quercetin	ME inhibited the free radical DPPH with an IC_{50} of 0.12 mg/mL	(24)
	In vitro	DPPH reducing power assay	Essential oil from aerial part	2.5-100 µg/mL	Ascorbic acid	Moderate radical scavenging capacity IC_{50}=244.62 ± 0.34 µg/mL) Important ferric reducing ability (IC_{50}=1.39 ± 0.07 mg AAE/g of EO)	(16)

Table 6. Continued

Activity	Study type	Experimental model	Extract	Dose range	Controls	Key result	References
	In vitro	DPPH assay	Essential oil from aerial part	10-100 mg/L	BHT	EO showed good scavenging activity (IC_{50}=49.6 ± 2.7 mg/mL)	(11)
	In vitro	DPPH assay, Ferric reducing test	Decocted and ethanolic extract of Leaves and Stems	50 µL 1 mL	Gallic acid, BHT	Leaves decoction showed the highest free radical scavenging activity (IC_{50}=1.47 ± 0.1 µg/mL) Leaves decoction showed the highest Fe (III) reduction potential (IC_{50}=25.95 ± 1.22 mmol ascorbic acid/g extract)	(25)
Antidiabetic effects	*In vivo* (oral route)	Induction of diabetes by streptozotocin	Aqueous extract of aerial part	5 mg/kg	Distilled water	The extract exhibited hypoglycemic effect in normal rats and an anti-hyperglycemic activity in diabetic rats	(26)
Antifertility effects	*In vivo* (oral route)	Chronic oral administration to rats and histopathological examination	Aqueous extract of aerial part	100-600 mg/kg	Saline solution	The extract showed significant decrease in testis, epididymis, and seminal vesicles weights, number and the motility of spermatozoids	(27)
Anticancer activity	*In vivo*	MTT assay on resistant human ovarian cancer cell line (A2780)	Methanolic extract	10 and 40 mg/mL	Not identified	The extract induced a decrease of cell viability of ovarian cancer resistant cell line human	(24)
AchE inhibition	*In vitro*	Ellman's method	Decocted and EE of leaves and stems	100 µL	Not identified	EE had the highest inhibitory activity of AchE (IC_{50}=52 ± 0.4 µg/mL)	(25)
Anti-hypertensive activity	*In vivo*	Measurement of blood pressure parameters; systolic blood pressure (SBP), Mean blood pressure (MBP) and diastolic blood pressure (DBP) in L-NAME-induced hypertensive rats	Aqueous extract of aerial part	200 mg/kg	Distilled water	Significant decrease in SBP, MBP, DBP and heart rate. Dose-dependent relaxation in the aorta-precontracted with Epinephrine or KCl	(28)

EO, essential oil; AP, aerial part; IZ, inhibitory zone; MIC, minimal inhibitory concentration; Chlr, chloroform; EtA, ethyl acetate; Me, Methanol; MBC, minimal bactericidal concentration; ChlrE, chloroform extract; EAE, ethyl acetate extract; MeE, methanolic extract; DPPH, 2,2-diphenyl-1-picrylhydrazyl; AqE, aqueous extract; NI, Not identified; IC_{50}, inhibitory concentration; BHT, Butylated hydroxytoluene; MTT, (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide); AchE, Acetylcholinesterase; SBP, systolic blood pressure; MBP, mean blood pressure; DBP, diastolic blood pressure; KCL, Potassium chloride; EE, ethanolic extract.

and ChE (0.146 ± 0.015 mg/mL) had moderate antiradical capacities compared to controls, butylated hydroxytoluene (BHT) and gallic acid (0.056 ± 0.001 and 0.032 ± 0.001 mg/mL, respectively). All extracts exhibited strong iron chelating effects in comparison to Ethylene diamine tetraacetic acid (EDTA) (IC_{50}=5.32 ± 0.03 mg/mL). The chelating effect of AqE was the most important (IC_{50}= 0.005 ± 0.004 mg/mL) followed by ME (0.021 ± 0.005 mg/mL), ChE (0.146 ± 0.015 mg/mL) and EAE (0.771 ± 0.021 mg/mL). AqE and ChE showed the best inhibitory capacity of the coupled oxidation of linoleic acid/β-carotene (90.34 ± 0.46% and 89.92 ± 0.29%, respectively), which was near to that of BHT (100 ± 0.52%), following by EAE and ME (73.56 ± 0.63% and 63.27 ± 2.27%, respectively) (23).

Antioxidant activity of *Ruta montana* methanolic extract (RMME) is dose-dependent. The concentration of 0.3 mg/mL showed the best inhibition of DPPH (91.64%), while quercetin inhibited 96.10% of DPPH at the same concentration. These results were confirmed by the IC_{50} values of the extract and quercetin (0.12 mg/mL and 0.0013 mg/mL, respectively) (24). In addition, the results of a study demonstrated that RMEO had a moderate capacity to reduce the DPPH (IC_{50} = 244.62 ± 0.34 µg/mL). However, this activity was less potent than standards, Trolox and ascorbic acid (IC_{50} = 1.4 ± 0.04 µg/mL and IC_{50} = 1.82 ± 0.025 µg/mL, respectively). RMEO showed a strong reducing power IC_{50} = 1.39 ± 0.07 mg AAE/g of EO (16).

All RMEOs collected from seven different regions of Algeria exhibited significant free radical reducing capacity. Especially two EOs (EO6 and EO7) which proved to have strong inhibition activity of DPPH with the IC_{50s} of 49.6 ± 2.7 and 50.2 ± 3.3 mg/L, respectively. Other EOs exhibited DPPH scavenging capacity as follows: EO4 (51.4 ± 2.1 mg/L) > EO5 (55.0 ± 3.1 mg/L) > EO3 (57.2 ± 3.4 mg/L) > EO1 (63.4 ± 1.4 mg/L), while EO2 presented the weakest antioxidant activity IC_{50} =68.1 ± 2.4 mg/L in comparison with positive control BHT (IC_{50} = 60.6 ± 1.3 mg/L) (11).

In another work, the ethanol extract and decoction of RM leaves showed the highest free radical scavenging activities with IC_{50} values of 1.47 ± 0.1 and 1.54 ± 0.15 µg/mL, respectively, followed by ethanol extract of stem (2.66 ± 0.13 µg/mL) and decoction of stem (3.64 ± 0.09 µg/mL). These values were near to gallic acid, a known standard, (IC_{50}=0.95 ± 0.04 µg/mL) and better than BHT (IC_{50}=15.7 µg/mL). The results of Fe (III) reduction showed that RM leaf decoction (25.95 ± 1.22 mmol ascorbic acid/g extract) and ethanol extract (25.51 ± 1.15 mmol ascorbic acid/g extract) exhibited the most potent activities, followed by decoction and ethanol extract of RM stems (18.48 ± 1.04 and 16.48 ± 0.28 mmol ascorbic acid/g extract) respectively (25). More interestingly, the antioxidant capacity of RM decoctions was assessed after 4 hours of *in vitro* gastric digestion; the results showed that the inhibition capacity of the decoction had only a small decline, not statistically significant at the 95% level, indicating that this activity was also maintained during the digestion process (25).

Antidiabetic activity

It has been shown that RM has a positive impact on the management of diabetes (60,61). The antidiabetic effects of *Ruta montana* aqueous extract (RMAE) of the aerial parts were investigated in diabetic rats. Data showed an improvement in glucose tolerance and prevention of the increase in blood glucose levels, 30 and 90 min after administration of 3 g/kg of glucose solution and after 15 days of daily oral administration of 5 mg/kg of the RMAE to streptozotocin-induced diabetic rats (26).

Anti-fertility activity

Some plants may also have some properties as good as antifertility agents or oral contraceptives controlling fertility (62), such as the RM plant, which is used as an abortive agent in Algerian folk medicine (63). Merghem et al evaluated the effect of the RMAE on fertility in adult male and female rats. The results did not show any significant change in ovaries' weight; however, a significant decrease was shown in testes, epididymis, and seminal vesicle weights, and a reduction in the number and motility of spermatozoids in rats treated with 300 and 600 mg/kg of RMAE (27).

Anti-cancer activity

The percentage of cell viability of human ovarian cancer resistant cell line (A2780 DX3) decreased in the presence of RMME with doxorubicin (DXR), an anticancer drug used in chemotherapy, especially at the concentrations of 10 and 40 mg RMME /mL, which exceeded the multi-drug resistant with inversion fold values of 2.01 and 4.56, respectively. This suggests that the RM plant acts synergistically with DXR and increases toxicity to cancer cells (24). Thus, RM could exhibit important anti-cancer properties.

Anti-acetylcholinesterase (AchE) activity

The inhibition of AchE is a strategy treatment of several neurological disorders such as Alzheimer's disease, senile dementia, ataxia, and myasthenia gravis (64,65). The RM plant has been traditionally used to appease neurological diseases (65). The results of a study showed that the stems ethanol extract had the highest inhibitory activity of AchE (IC_{50}= 52 ± 0.4 µg/mL), followed by a decoction of leaves (57 ± 1.6 µg/mL) (25).

Insecticidal and larvicidal activities

The repellent and toxic activities of Algerian RMEO against an important insect pest of stored grains *Ephestia kuehniella* (Pyralidae) revealed a median lethal concentration (LC_{50}) of 11.6 µL/L against *E. kuehniella* (30). In another work, RMEO exhibited insecticidal activity against German cockroach and *Culex pipiens* mosquitoes using anti-crawling and anti-flying tests, respectively. After 10

minutes of spraying, 100 % of German cockroaches were flipped over on their backs upon pulverization of 1 mL of RMEO. For the same dose, the mortality percentages were 97.5 % for German cockroaches after 24 h, and 99% for *Culex pipiens* mosquitoes after 30 minutes of spraying (29).

The larvicidal activity of RMEO from the aerial parts indicated that a dose of 9.6×10^{-3}% of RMEO was able to reach 100% mortality of mosquitoes larvae after 1 hour (29). Another study, 150 µL of diluted oil exhibited an attractant activity against the larvae with 76.67% of larval mortality in the first 24 hours (30).

Toxicity profile

Many plants used in traditional medicine or food have shown some toxicities. The toxicity could be induced by chemical constituents (such as tropane alkaloids and cardiac glycosides), administration mode, or the dose used (66). It was cited in an ethnobotanical study on toxic medicinal plants used in High Atlas Central of Morocco that RM may cause dermal toxicity (13). In addition, an investigation by Masri et al indicated that a 74-year-old woman was poisoned after taking leaf and stem infusion of RM for a week at a high dose (three infusions of 200 mL per day). TLC and GC-MS analysis identified some alkaloids and furanocoumarins in the patient's urine; isopimpinellin, bergapten, and xanthotoxin (67). In a report, RM intoxication began with respiratory failure and digestive disorders, including pain, vomiting, and hypersalivation, accompanied by neurological excitement and convulsion. Furanocoumarins can induce kidney and hepatic failure (67,68) and acute dermatitis through contact with RM leaves followed by exposure to the sun (69,70).

Conclusion and perspectives

Previous studies about the pharmacological and biological activities of *Ruta montana* extracts have shown the benefits of this plant on health and the environment. Research works have confirmed the traditional uses of RM plant revealing the possibility of using this species to treat many diseases involved in public health problems, such as diabetes, neurological disorders, infections, reproductive system disorders, and cancer. RM extracts could also replace chemical insecticides and fungicides since they exhibit antifungal, insecticidal, and larvicidal properties. Therefore, RM could be a potential source of new, safe, and effective antimicrobial, antidiabetic, antioxidant, anti-fertility, antihypertensive, and anticancer agent that would contribute to the control of many global health and environmental issues. Furthermore, clinical studies must be carried out to determine the pharmacodynamic and pharmacokinetic parameters in order to detect active principles, which can be used in the pharmaceutical industries. The majority of the previous works are based on *in vitro* studies and only few studies evaluated pharmacological properties *in vivo*. Most studies have investigated the essential oils and there is lack of study on crude extracts. It is also necessary to identify the phytochemicals of RM crude extracts and their mode of action in order to develop new agents. Also, further work is needed to highlight other medicinal uses of the species. Additional toxicological studies are also required to control the safety of this plant.

Several studies used classical assays to evaluate the biological activities such as disc diffusion and agar-well methods to assess the antimicrobial activities, and 2,2-diphenyl-1-picrylhydrazyl free radical, ferric reducing power, and total antioxidant capacity assays to test antioxidant effects. Although these assays are necessary for initial screening, they can be sometimes not reliable. More advanced methods may be included to support these biological activities *in vitro* followed by proper trial using human-disease based models.

Authors' contributions

NB: Study design, collection of articles, first draft manuscript writing, conceptualization, data analysis, and interpretation. SIK: Study supervision, manuscript revision. KFB: supervising, contribution to methodology, manuscript review and editing. All authors read, reviewed and approved the manuscript and English language.

Conflict of interests

The authors declared no competing interests.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication and etc.) have been completely observed by the authors.

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