MicroRNAs as New Bioactive Components in Medicinal Plants

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Abstract
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Herbal medicine has been used to treat diseases for centuries; however, the biological active components and the mechanistic understanding of actions of plant-derived drugs are permanently discussed. MicroRNAs are a class of small, non-coding RNAs that play crucial roles as regulators of gene expression. In recent years, an increasing number of reports showed that microRNAs not only execute biological functions within their original system, they can also be transmited from one species to another, inducing a posttranscriptional repression of protein synthesis in the recipient. This cross-kingdom regulation of microRNAs provides thrilling clues that small RNAs from medicinal plants might act as new bioactive components, interacting with the mammalian system.

In this article, we provide an overview of the cross-kingdom communication of plant-derived microRNAs. We summarize the microRNAs identified in medicinal plants, their potential targets in mammals, and discuss several recent studies concerning the therapeutic applications of plant-based microRNAs. Health regulations of herbal microRNAs in mammals are a new concept. Continuing efforts in this area will broaden our understanding of biological actions of herbal remedies, and will open the way for the development of new approaches to prevent or treat human diseases.

Supporting information available online at http://www.thieme-connect.de/products

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Introduction
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Endogenous microRNAs (miRNAs) are a class of single-stranded non-coding RNA molecules of approximately 22 nucleotides that play crucial roles in gene expression [1]. They generally bind to complimentary sequences in the 3′ untranslated region (UTR) of specific protein-coding genes, but can also interact with other regions of the gene including 5′ UTR and the coding region, inducing mRNA cleavage or translational repression [2]. miRNAs are highly pleiotropic and a single miRNA can recognize hundreds of mRNA transcripts, allowing them to regulate a diverse range of biological pathways. In mammals, an estimated 60% of all protein-coding genes may contain miRNA binding sites [3,4]. Furthermore, miRNA dysregulation is frequently associated with human diseases such as cancer, cardiovascular diseases, central nervous system diseases, and metabolic disorders [5–8], suggesting their potential as targets for the development of novel therapies for several diseases. There are currently many efforts focusing on the development of miRNA therapeutics for the treatment of human diseases. Several preclinical studies on therapeutic miRNA replacement have been initiated [9–11], and the first liposomal nanoparticle formulation of miR-34a mimic (MRX34) has entered clinical trials in patients with primary liver cancer [12], highlighting the therapeutic potential of miRNA-based novel approaches to treat diseases. However, severe on-target or off-target side effects could be caused by artificial miRNAs [13,14].

Herbal medicine has been used to treat diseases for centuries. Bioactive secondary metabolites such as polyphenols, alkaloids, saponins, and tannins have been systematically studied and used as an important source for drugs. However, synthetic supplements of phytochemicals often do not have the same efficacy as complex herb materials. The natural biological active components and mechanistic understanding of actions of plant-derived drugs are permanently discussed.

Herbal remedies may produce a beneficial effect by regulating mammalian miRNA expressions. Medicinal plant extracts and secondary metabolites such as *Dioscorea opposita* Thunb. (Dioscoreaceae) extract, ginsenoside Rh2, berberine, and waltonitone were reported to modulate disease-associated miRNA levels [15–18]. Notably, oral consumption of a plant-based diet may lead to the cross-kingdom transfer of plant-derived miRNAs in mammals. Zhang et al. [19] reported miRNAs derived from plant-based foods function as active signalling molecules to regulate mammalian genes. The authors showed that exogenous miRNAs (e.g., MIR168a and MIR156a) from rice were absorbed by cells of the gastrointestinal (GI) tract of the consuming animal, packaged into exosomes, then migrated through the plasma, and were delivered to specific organs where they bound to low-density lipoprotein receptor adapter protein 1 (LDLRAP1) mRNA and inhibited LDLRAP1 expression, thereby influencing cholesterol transport [19]. These studies provide thrilling clues that miRNAs might act as new bioactive compounds in medicinal plants. If this hypothesis can be validated, it will broaden our understanding of herbal remedies and, more importantly, will provide new insights into the development of miRNA-based therapeutics with high efficiency, reduced side effects, and low costs. This review provides current knowledge of cross-kingdom communication of plant-derived miRNAs, summarizes the miRNAs identified in medicinal plants, and discusses several recent studies concerning the therapeutic applications of medicinal plant-based miRNAs.

Evidence for the Uptake of Plant miRNAs by Mammals
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Immediately after Zhang's group discovery, numerous details of this study have been appraised. In general, follow-up studies have focused on detecting exogenous small RNAs in mammals after different diets. The results remain controversial, as summarized in ● **Table 1**, with some findings being consistent with the original publication [19–27] while others are questioning the results [28–32].

Recent reports from two groups have attracted great attention. Yang et al. [20] identified the influence of diet preference and health issues on the uptake of plant-based small RNAs. A significant increase of plant miRNA MIR2911 in both blood and urine was observed in mice that were fed with an herbal diet (*Lonicera japonica* Thunb., Caprifoliaceae; common name: honeysuckle) for several days. Damaged guts resulting from chemotherapeutic (cisplatin) treatment also enhanced plant-based miRNA uptake. In addition, a single large oral dose of exogenous miRNAs (e.g., 400 pmol of synthetic MIR2911 or MIR168 a) facilitated only the short-term detection of serum miRNAs. The following study demonstrated that the level of circulating MIR2911 was associated with the dietary intake level [33]. Zhang's group raised the critical issues for the accurate measurement of exogenous miRNAs such as RNA extraction method and internal controls [21]. These studies supply reasonable explanations on the publications reporting undetectable exogenous miRNAs. Firstly, small dosages over short time feedings of a plant diet may not induce detectable miRNAs in plasma. Secondly, natural ingredients in an herbal diet may protect plant miRNAs against degradation and enhance the uptake of miRNAs in the mammalian system, while naked miRNAs may not survive the digestion and circulation process. Thirdly, the health conditions of the GI tract may influence the uptake of plant-based miRNAs. Fourthly, not only the plant miRNAs in plasma, but the potential deposition of dietary miRNAs in other body tissues should also be taken into account. Finally, canonical methods for exogenous plant miRNA measurement are necessary to ensure the sensitivity and precision of miRNA detection.

Plant-Based miRNAs for Therapeutics
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Although the role of exogenous miRNAs is currently not well understood, the biological effects of plant miRNA in the mammalian system have been profiled. Using computational tools, 12 putative miRNAs were identified from *Curcuma longa* L. (Zingiberaceae) and found to bind with various target genes related to human diseases such as diabetes mellitus type II, cardiovascular disorders, Alzheimer's, cancer, and thalassemia [34]. Similarly, a total of six putative miRNAs were identified from *Gmelina arborea* Roxb. (Lamiaceae), while the mammalian target genes associated with cancer, blood borne disease, and other urinary infections were predicted [35].

Exosomes are one of the natural carriers of miRNA, serving as mediators in extracellular communication [36]. Using grape exosome-like nanoparticles (GELNs) as models, the biological impacts of plant exosomes have been investigated. GELNs can penetrate through the intestinal barrier, can be taken up by intestinal stem cells, and mediate intestinal tissue remodelling [37]. Oral administration of GELNs protected mice against dextran sulfate sodium-induced colitis. Apart from proteins and lipids, miRNAs, with almost 100 different kinds, were identified from GELNs [37]. Furthermore, exosome-like nanoparticles derived from edible plants, including grapes, grapefruit, ginger, and carrots, were taken up by mouse intestinal macrophages and stem cells. They were biologically active in maintaining intestinal homeostasis through activation of nuclear factor (erythroid-derived 2)-like-2 factor (Nrf-2), induction of anti-inflammatory molecules, and activation of Wnt signalling in recipient cells [38]. These studies suggested that plant miRNAs might be packaged into exosome-like nanoparticles that would be taken up by the intestine, and exert beneficial effects under both physiological and pathological conditions.

The most direct evidence of therapeutic effects of plant miRNAs has been recently published by Zhang's group [22]. A plant-derived miRNA, MIR2911, is highly stable in the decoction of a Chinese herb honeysuckle, which has been traditionally used in China for centuries to treat influenza infection. This miRNA can be absorbed by the GI tract and be delivered via the bloodstream to the lungs of the animals, which were continuously fed with a honeysuckle decoction. Importantly, this plant miRNA could directly target various influenza A viruses, suppress their replication process, and protect against influenza virus infections in mice. The region of the influenza genome that MIR2911 targeted was also identified, and validated in a mutant virus in which the sequence was modified and resistant to MIR2911 treatment [22]. This groundbreaking study suggested that plant miRNA may act as a novel bioactive ingredient that can be absorbed by mammals and reduce the risk of many diseases.

Recently, the therapeutic potential of plant-based miRNAs in cancer was experimentally confirmed [23]. A cocktail of three mammalian tumor suppressor miRNAs was synthesized with methyl groups on the 2′ position of the ribose of the 3′ nucleotide to mimic miRNAs of plant origin. Oral administration of these plant-based tumor suppressor miRNAs together with total plant RNA [isolated from *Arabidopsis thaliana* (L.) Heynh (Brassica-

Table 1 Evidences for or against uptake of plant-based miRNAs in consumers.

Evidences	Contents	miRNAs involved	Detection methods	miRNA levels	References
Contradicting evidences	Plant miRNAs were undetectable in the plasma of healthy human subjects after the ingestion/intake of fruits.	MIR156 a, MIR159 a, and MIR169 a	qRT-PCR	Undetectable.	[28]
	MiR-21 was negligibly expressed in the plasma or organ tissues of miR-21 knockout mice after intake of diets replete with endogenous miR-21.	miR-21	qRT-PCR	Undetectable in plasma; less than one copy per cell in the liver, lungs, kidneys, and stomach.	[28]
	Plant miRNAs were negligibly expressed in the plasma or organ tissues of mice after ingestion of diets replete with plant miRNAs.	MIR156 a, MIR159 a, and MIR169 a	qRT-PCR	Insignificant increase of MIR156 a in the plasma; less than one copy of MIR156 a per cell in the liver, lungs, kidneys, and stomach; undetectable levels of MIR156 a and MIR169 a in either plasma and/or organs.	[28]
	Plant miRNAs were negligible in recipient honeybee tissues.	MIR156 a, MIR159 a, and MIR169 a	qRT-PCR	Less than one copy of MIR156 a per cell in nurses and foragers; undetectable MIR156 a and MIR169 a in the abdominal tissue of bees.	[28]
	Nonhuman primates failed to uptake dietary plant miRNAs.	miR156, miR160, miR166, miR167, miR168, and miR172	qRT-PCR and droplet digital RCR	Not available.	[29]
	Little or no plant miRNAs or miR168 a were detected in the blood or liver of mice fed rice-containing diets.	miR168 a	HTS and qRT-PCR	Comparable with the control group.	[30]
	Observed plant miRNAs in animal sRNA data sets originated in the process of sequencing.	miR168	HTS	Not available.	[31]
	Transfer of plant miRNAs from guts to internal tissue was negligible in pollen recipient honeybees.	MIR156 a and miR277	qRT-PCR	Comparable with the control group.	[32]
Supporting evidences	Plant miRNAs were present in human and animal sera and organs.	MIR156 a, MIR168 a, and MIR166 a	qRT-PCR	fM Level.	[19]
	Plant miRNAs were detectable in the blood and organs of mice fed with rice.	MIR168 a	qRT-PCR	fM Level.	[19]
	Plant miRNAs existed in human plasma.	miR168 a	HTS	Ten reads per 50 000 mapped human miRNA reads.	[25]
	MiR172 was absorbed in the circulation and various organs in mice fed with cabbage.	miR172	qRT-PCR	A total of 8.6% of orally administrated miRNAs was detected in the blood, stomach, intestines, and spleen 2–6 h after feeding.	[26]
	Plant miRNAs was present in human and porcine breast milk exosomes.	35 plant miRNA species	Bioinformatics analysis HTS	Rather low (the read counts of each plant miRNA ranged from 1 to 24).	[27]
	MIR2911 was absorbed in mouse blood and lungs after continuous feeding of the honeysuckle decoction.	MIR2911	qRT-PCR	fM Level.	[22]
	MIR2911 was detectable in the sera and urine of the honeysuckle decoction-consuming mice.	MIR2911	qRT-PCR	fM Level.	[20]
	Plant miRNAs were uptaken in mice when feed together with total plant total RNA.	miR-34 a, miR-143, and miR-145	qRT-PCR	Intestinal miR-34 a was at a detectable level; detection of miR-143 and miR-145 in mouse intestines were failed.	[23]
	Plant miRNAs were detectable in human plasma from volunteers after drinking juice.	16 miRNA species such as MIR158 a, MIR159 a, MIR160 a, etc.	qRT-PCR and Northern blot	fM Level.	[21]
	Plant miRNAs were present in Western donor sera.	miR159	HTS and qRT-PCR	fM Level.	[24]

HTS: high-throughput sequencing; qRT-PCR: quantitative reverse transcriptase polymerase chain reaction

ceae)] significantly reduced the tumor burden in a well-established *Apc*^min/+ mouse model for colon cancer. Interestingly, there was a slight tumor burden reduction in the mice treated with total plant RNA alone compared with water-treated mice, although not statistically different, suggesting that plant total RNA may have a therapeutic effect [23]. This study indicated that plant engineered miRNAs that are designed for specific targets have significant potential in clinical therapeutic applications.

The cross-kingdom regulation of human cancer growth by plant miRNA has been further confirmed using *in vitro* and *in vivo* models [24]. Plant-derived miR159, presented in both raw and cooked foods (e.g., broccoli), was not only abundant in healthy human serum, but also detectable in breast cancer tissues. By targeting 3′UTR of transcription factor 7 (*TCF7*), both synthetic 2-*O*-methylated miR159 and human serum extracellular vesicles (EVs) significantly suppressed breast cancer cell proliferation. In tumor xenograft models, the mice that were continuously fed with synthetic miR159 showed significantly reduced tumor growth compared with those that received scrambled control oligonucleotides. However, no affection was observed in the xenograft tumors that stably overexpressed *TCF7* without the 3′UTR [24]. This study consolidated the assumption that circulating miRNAs absorbed from dietary plants can be transported through EVs-mediated trafficking, reach target tissue, and impact human health and disease.

It is noteworthy that reaching biologically active levels of circulating plant miRNAs through oral intake might require feeding with an herbal decoction, ground plant material, or food matrix [19, 22, 37] together, or long-term feeding regimes [22–24], suggesting that a plant-derived matrix as well as prolonged exposure might be the important factors that facilitate the high level uptake of plant miRNAs with therapeutic effects.

Stability of Plant miRNAs
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A crucial concern regarding therapeutic plant miRNAs is their stability in the preparation process as well as in mammalian circulation. The innate capacity of plant-derived miRNAs (e.g., miR166, miR167, miR168, and miR159) are degradation resistant, as evidenced that storage, processing, and cooking do not abolish the plant miRNAs present in rice, soybeans, potatos, broccoli, and cabbage [19, 24, 39]. Unlike mRNA that losses integrity during processing, plant miRNAs are robust during soaking, boiling, and homogenization processes, and even survive under unfavorable physiological conditions such as an extreme pH (e.g., simulated gastric fluid with pH of 1.2), frequent bowel movements, and potent ribonuclease (RNase) treatment [19, 39]. However, it is also reported that the majority of miRNAs were degraded in the honeysuckle decoction after boiling for 30 min, while MIR2911 possesses a specific sequence and high GC content, which makes it highly stable in this decoction and resistant to RNase treatment [22]. These observations suggested that some plant-based miRNAs are in a stable form that can withstand not only the preparation process, but also the digestion and circulation process, ensuring their regulatory activity.

Several mechanisms regarding the stability of plant miRNAs have been suggested:

1. 2′-*O*-Methylation in plant miRNAs enhances their stability by protecting them from exonucleolytic digestion and uridylation [40, 41];

2. Stabilization of circulating miRNAs is associated with RNA-binding proteins such as Argonaute proteins (AGOs) [42–44] and nucleophosmin 1 [45];

3. miRNA carriers such as exosomes, microvesicles, and high-density lipoprotein protect miRNAs from degradation [38, 46, 47];

4. The unique sequence and GC content of a plant miRNA also influences its stability [22];

5. Some plant extracts and secondary metabolitess such as extracts from *Phyllanthus reticulatus* Poir. (Phyllanthaceae), *Ananas comosus* (L.) Merr. (Bromeliaceae), *Aglaia aphanamixis* Pellegr. (Meliaceae), green tea, and polyphenols (e.g., epigallocatechin gallate, epicatechin, epicatechin gallate, and epigallocatechin) possess potent ribonuclease inhibitory activities [48–51], which may protect plant miRNAs from the enzymatic environment of the digestive tract;

6. The lipid, proteins, or polysaccharides presented in plants may protect miRNAs or miRNA carriers from degradation during preparation and processing.

Oral Delivery of Plant miRNAs
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Oral application is the dominant route of application for herbal preparations. Even if they are stable during preparation and digestion processes, could these exogenous miRNAs cross biological barriers? Despite increasing efforts, the mechanisms of functional transfer of plant miRNAs to cross the intestinal barrier remain unclear. Given the instability of naked RNA, it would be possible that miRNAs in plant materials are packaged into protein complexes and/or lipid vesicles and recognized by a mammalian transport system. Several mechanisms for the intestinal permeability of exogenous miRNAs have been proposed:

1. The RNA-containing complexes are internalized into the intestinal epithelia and become engulfed exosomes. Some of them may merge with endosomes and undergo transcytosis that transports macromolecules from one side to the other side of a biological barrier, while some other fused endosomes mature into lysosomes and undergo degradation [52, 53].

2. The RNA carriers might be transferred through M cells in Peyer's patches of the intestine to macrophages or/and dendritic cells in the gut-associated lymphoid tissue (GALT). These GALT immune cells contribute to the subsequent distribution of RNA-containing complexes throughout the body [54, 55].

3. Plant miRNAs packaged within exosome-like nanoparticles could be taken up by intestinal stem cells or macrophages where they may exert biological functions [37, 38].

4. Although naked plant miRNAs are instable and may survive only for a few hours [19], it is possible that these unprotected miRNAs are uptaken into the cytoplasm by receptor-mediated endocytosis or transmembrane miRNA transporters [52–56].

5. Additionally, altered intestinal permeability resulting from GI tract diseases, stress, malnutrition as well as folk remedies may enhance the uptake of foreign miRNAs [23, 55].

Considering that the uptake of plant miRNAs in mammals is not necessarily related to the abundance of miRNAs in the plants [19, 24], there might exist a class of plant miRNAs or miRNA carriers with specific signatures that are recognized and preferentially taken up by the mammalian GI tract. After crossing the intestinal barrier, plant miRNAs or miRNA carriers might be repacked into microvesicles and delivered to different tissues/cells where the miRNAs modulate the expression of their target genes [19, 22].

In mammalian organisms, proteins at the surface of miRNA-containing lipid vesicles take part in the target-specific internalization into cells, such as exosome surface proteins tetraspanins and lectins, while integrins, proteoglycans, and lectin receptor proteins on the surface of recipient cells may serve as receptors for the exosome uptake [57]. However, the molecules on the plant miRNA carriers responsible for the interaction with the mammalian GI tract as well as the target cells have not been elucidated.

It is necessary to mention that not all the ingested miRNAs end up in the bloodstream with potential functions. The level of exogenous miRNA matters to their biological function. It is generally considered that a threshold concentration of 100 copies per cell is required for miRNAs to execute their function [22]. From the published data, although some plant-derived miRNAs (e.g., miR160 a, miR162 a, and miR390 a) are detected in human serum [19], they may not be biologically active.

miRNAs in Medicinal Plants
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Identification of plant miRNAs is the prerequisite for the understanding of their functions. Genetic and computational approaches are usually used for the investigation of miRNAs. Based on the publically available genome sequence or expressed sequence tags (ESTs), computational methods have been used for the prediction of evolutionary conserved plant miRNAs among different species. However, except for intensively studied herbs such as *Panax ginseng* C.A.Mey (Araliaceae), *Salvia miltiorrhiza* Bunge (Lamiaceae), *Lycium chinense* Mill. (Solanaceae), and *Cynara cardunculus* L. (Asteraceae) [58–61], limited genomic data are available for medicinal plants. With the recent advent of high-throughput sequencing (HTS) technology, characterization of miRNAs through sRNA, transcriptome, or genome sequencing has become a routine tool for miRNA research.

According to our current knowledge, a total of 44 medicinal plants with identified miRNAs are available in PubMed, Science Scope, and miRase databases (● **Table 2**). With the aid of computational approaches, the functions of plant miRNA have been predicted. They are involved in various plant biological processes, including plant growth development, signal transduction, and stress responses [62–66]. Secondary metabolites have generally been considered as the pharmacological active compounds in medicinal plants, and the role of miRNAs in regulating the production of bioactive secondary metabolites has gained high attention [67–71].

It is noteworthy that even the efficacy of some herb remedies [e.g., *Viscum album* L. (Santalaceae) or *Vitex agnus-castus* L. (Lamiaceae) has been clinically demonstrated, though the scientific explanation of their pharmacological activities are not convincing from the experimental data about concentration, pharmacological activity, bioavailability, and metabolism of secondary metabolites present in the used extracts. One possibility to explain this gap might be the diversification of the bioactive substances. Besides the well-known plant secondary products, miRNAs produced by the mother plant may also serve as a mediator between botanicals and health-associated processes. A few studies demonstrated the therapeutics effects of herb-derived miRNAs in mammalian diseases and infections, such as cancer and influenza [22, 24, 34, 35].

Characterization of mammalian target genes of medicinal plant miRNAs using computational and experimental approaches might be a practical way for identifying functional miRNAs. Four features are commonly used for the computational recognition of miRNA targets [72]:

1. Base-pairing between the "seed" (the first 2–8 nucleotides starting at the 5′ end and counting toward the 3′ end of a mature miRNA) and its target gene;
2. Low free energy (a negative change in overall free energy during a reaction) is expected of the authentic miRNA target pairs;
3. An optional rule for target prediction requires cross-species conservation of the putative binding sites;
4. Site accessibility is an additional rule to measure the ease with which an miRNA can locate and hybridize with an mRNA target.

Using these criterions, it is not surprising to identify the mammalian targets of herbal miRNAs, while approximately 50 human genes were identified as putative targets of MIR168 a [19]. ● **Table 2** summarizes the currently known miRNAs from medicinal plants (more information such as traditional use/clinical importance and the main bioactive ingredients of these medicinal plants is supplied in **Table S1**, Supporting Information), and identification of their potential mammalian targets can be a promising starting point for their cross-kingdom regulatory effects studies. The following up of experimental evidence is required to validate computational predictions since the algorithms based on the physical properties of miRNA regulation may generate many false positive results [73]. On the other hand, bioinformatics tools with additional and more sophisticated analysis allowing more accurate identification of corresponding mRNA targets for these herbal miRNAs need to be developed.

Conclusions and Outlook
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Secondary metabolites serve as bioactive components in medicinal plants, however, the experimental evidence is not convincing regarding their therapeutic effects. It is now documented that the eukaryotic organism of different kingdoms could exchange miRNAs as signals affecting gene expression, indicating the possibility that miRNAs act as a new bioactive component in herbal remedies. The potential of herbal medicine-derived miRNAs in regulating human health or targeting genes associated with diseases are at a very early, exploratory stage. Conclusive validation studies are urgently needed to address this issue. It is necessary to evaluate the stability of herbal miRNAs during the preparation and digestion processes, and in what forms these miRNAs existed. If and to what extent plant-derived miRNAs are absorbed by mammals needs to be investigated. Furthermore, the mechanisms of intestinal absorption, tissue recognition, bioavailability, and function of herbal miRNAs should be established. Given the secondary metabolites presented in herbal preparations, future studies will determine if and how these secondary metabolites influence the stability and uptake of exogenous miRNAs by mammals.

Health regulation by herbal miRNAs is a new concept with a wide range of putative applications, which may include artificial synthesis of therapeutic plant-derived miRNAs that might possess lower side effects, artificially loading miRNAs into plant materials to enhance their *in vivo* efficacy, and discovery of novel therapeutic target genes. Continuing pursuit in this nascent area will not only broaden our understanding of biological actions of herbal remedies, but also open the ways for the development of new approaches to prevent or treat human diseases.

Table 2 miRNAs in medicinal plants and fungi.

Latin name	Family	microRNA discovery approaches	Number of miRNAs	Target identification/prediction	References
Antrodia cinnamomea (M. Zang & C. H. Su) Sheng H. Wu, Ryvarden & T. T. Chang	Antrodia	The genomic DNA, sRNA, and RNA sequencing, computational prediction, and experimental validation.	4 predicted conserved miRNAs and 63 novel predicted miRNA-like small RNA candidates	Targets involved in triterpenoid synthesis, mating-type recognition, chemical or physical sensory proteins, and transporters.	[74]
Oenanthe javanica (Blume) DC	Apiaceae	Transcriptome sequencing, sRNA sequencing, computational prediction, and experimental validation.	69 mature miRNAs	A total of 29 potential target genes involved in abiotic stresses including heat, cold, salinity, and drought.	[65]
Rauvolfia serpentina (L.) Benth. Ex KurzBenth. Ex Kurz.	Apocynaceae	Bioinformatics analysis.	15 conserved miRNAs belonging to 13 families	Target genes included transcription factors as well as genes related to growth and developmental processes, primary and secondary metabolism, disease resistance, and stress responses.	[75]
Catharanthus roseus (L.) G. Don	Apocynaceae	sRNA sequencing, computational prediction, and experimental validation.	81 conserved miRNAs and 7 novel miRNAs	Targets involved in diverse biological roles including secondary metabolism.	[76]
Pinellia pedatisecta Schott	Araceae	Microarray, computational prediction, and experimental validation.	101 miRNAs belonging to 22 miRNA families	Target genes involved in reproduction, transcription factor activity, and plant developmental process.	[77]
Pinellia ternata (Thunb.) Breit	Araceae	Microarray, computational prediction, and experimental validation.	54 miRNAs belonging to 23 miRNA families	Not available/done.	[78]
Panax notoginseng (Burk.) F. H. Chen	Araliaceae	sRNA sequencing, computational prediction, and experimental validation.	316 conserved miRNAs and 52 novel miRNAs	A total of 803 putative target genes involved in metabolic pathways, spliceosome, and biosynthesis of secondary metabolites.	[79]
Panax ginseng C. A. Meyer	Araliaceae	sRNA sequencing, computational prediction, and experimental validation.	101 miRNAs belonging to 42 families	Targets involved in plant development and stress defense responses.	[64]
Stevia rebaudiana Bertoni	Asteraceae	sRNA sequencing and computational prediction	100 highly conserved miRNAs families and 12 novel potential miRNAs	The predicted targets, mainly encoding enzymes, regulating essential plant metabolic and signalling pathways.	[80]
Artemisia annua L.	Asteraceae	Bioinformatics analysis.	6 potential miRNAs	A total of 8 target genes involved in artemisinin biosynthesis, signal transduction, and development.	[81]
Carthamus tinctorius L.	Asteraceae	sRNA sequencing and computational prediction.	236 known miRNAs	Target genes involved in plant growth, development, and stress responses associated with transcription, translation, ribosomal structure and biogenesis, cell cycle control, and signal transduction.	[62]
Senecio vulgaris L.	Asteraceae	Bioinformatics analysis.	10 miRNAs	Not available/done.	[82]
Cynara cardunculus L.	Asteraceae	sRNA sequencing, computational prediction, and experimental validation.	98 conserved miRNAs and 24 novel miRNAs	Targets involved in transcription and the response to various stress responses.	[61]
Helianthus tuberosus, H. annus, H. ciliaris, etc	Asteraceae	EST-based bioinformatics analysis and experimental validation.	61 novel miRNAs belonging to 34 families	Targets consisted of growth and development related transcription factors, signalling pathway kinases, stress-resistant proteins, and transport-related proteins.	[63, 83]
Humulus lupulus L.	Cannabaceae	EST-based computational prediction and experimental validation.	22 miRNAs	A total of 47 potential targets involved in plant growth and development, stress response, signal transduction, and other physiological processes.	[66]
Lonicera japonica Thunb	Caprifoliaceae	sRNA sequencing, computational prediction, and experimental validation.	148 miRNAs from honeysuckle	MIR2911 targeted at influenza virus mRNA.	[22]
Xanthium strumarium L.	Compositae	sRNA sequencing, computational prediction, and experimental validation.	1185 conserved miRNAs and 37 novel miRNAs	A total of 4187 target genes involved in signal transduction, metabolism, stress response, and those with unknown functions.	[67]
Costus pictus D. Don	Costaceae	Computational prediction and experimental validation.	42 miRNAs of belonging to 13 different families	A total of 109 potential target genes encoding transcription factors, enzymes, and various functional proteins.	[84]

continued

Table 2 *Continued*

Latin name	Family	microRNA discovery approaches	Number of miRNAs	Target identification/prediction	References
Vaccinium corybosum L.	Ericaceae	EST-based comparative genomics approach.	9 potential miRNAs	A total of 34 target involved in transcription, RNA splicing and binding, DNA duplication, signal transduction, transport and trafficking, stress response, as well as synthesis and metabolic processes.	[85]
Ricinus communis L.	Euphorbiaceae	sRNA sequencing, computational prediction and experimental validation.	86 conserved miRNAs	Target genes involved in the growth and development process.	[86]
Hypericum perforatum L.	Hypericaceae	Computational investigation prediction and experimental validation.	7 pre-miRNAs	Targets involved in metabolism, response to stress response, flower development, and plant reproduction.	[87]
Pogostemon cablin Benth	Lamiaceae	Experimental validation.	miR156 a	Targets regulated terpene synthases.	[68]
Ocimum basilicum L.	Lamiaceae	EST-based computational prediction and computational validation.	9 miRNA candidates	A total of 13 potential targets involved in stress response and secondary metabolite regulation.	[88]
Salvia miltiorrhiza Bunge	Lamiaceae	sRNA sequencing, computational prediction, and experimental validation.	452 known miRNAs and 40 novel miRNAs	A total of 69 potential targets including acetyl-CoA C-acetyltransferase and those involved in the biosynthesis of tanshinones.	[69]
Salvia sclarea L.	Lamiaceae	EST-based analysis and computational prediction.	18 conserved miRNAs	A total of 19 potential target genes involved in transcription, metabolism, or abiotic stress response and 2 targets with no known predicted function.	[89]
Linum usitatissimum L.	Linaceae	Genome-based bioinformatics analysis and experimental validation.	116 conserved miRNAs belonging to 23 families	A total of 142 putative genes involved in transcription, diverse physiological, and metabolic processes.	[90]
Dendrobium officinale Kimura et Migo	Orchidaceae	RNA sequencing, sRNA sequencing, and computational prediction.	1047 miRNA candidates	A total of 1257 potential targets involved in hormone signalling, plant development, hormone signalling, AGO1-related regulation, and secondary metabolism.	[91]
Papaver somniferum L.	Papaveraceae	sRNA sequencing, computational prediction, and experimental validation.	316 conserved miRNAs and 11 novel miRNAs	A total of 1469 target transcripts involved in stress response to stress against various factors and secondary metabolite biosynthesis processes.	[70]
Digitalis purpurea L.	Plantaginaceae	EST library construction and sequencing, computational prediction, and experimental validation.	13 miRNAs	Target genes involved in metabolism, RNA process, transcriptional regulation, signal transduction, and those with unknown functions.	[71]
Coffea arabica L.	Rubiaceae	EST-based bioinformatics analysis.	1 potential miRNA	A total of 6 potential target genes involved in biological processes such as response to chitin, cold, salt stress, and water deprivation.	[92]
Lycium chinense Mill.	Solanaceae	sRNA sequencing, computational prediction, and experimental validation.	60 conserved miRNAs belonging to 31 families and 30 putative novel miRNAs	Potential targets were involved in a wide range of metabolic and regulatory pathways including fruit maturation, lycopene biosynthesis, and signalling pathways.	[60]
Picrorhiza kurroa Royle ex Benth	Scrophulariaceae	Transcriptome sequencing, computational prediction, and experimental validation.	18 conserved miRNAs	A total of 30 potential targets involved in signal transduction, nucleic acid metabolism, disease resistance, hormonal regulation, developmental processes, and secondary metabolism.	[93]
Rehmannia glutinosa Libosch	Scrophulariaceae	Transcriptome sequencing, sRNA sequencing, computational prediction, and experimental validation.	589 conserved miRNAs and 6 novel miRNAsfamilies	A total of 165 transcript targets involved in biological regulation, response to stimuli, development, and metabolic processes.	[94]
Taxus chinensis var. *mairei*	Taxaceae	sRNA sequencing, computational prediction, and experimental validation.	871 mature miRNAsand 869 miRNA precursors	The functions of the putative target genes are largely unknown, implying that the novel miRNAs are involved in the Taxus-specific biological processes.	[95]
Camellia sinensis (L.) O. Kuntze	Theaceae	Bioinformatics analysis.	miRNAs candidates	A total of 30 potential target genes involved in transcription, physiological processes, and those with hypothetical or unknown functions.	[96]
Aquilaria sinensis (Lour.) Gilg.	Thymelaeaceae	sRNA sequencing, computational prediction, and experimental validation.	27 novel miRNAs and 74 putative conserved miRNAs	Targets involved in stress response and agarwood formation.	[97]

continued

Table 2 Continued

Latin name	Family	microRNA discovery approaches	Number of miRNAs	Target identification/prediction	References
Gmelina arborea Roxb	Verbenaceae	EST-based bioinformatics analysis.	6 putative miRNAs	Mammalian targets of signals involved in transduction and apoptosis that play a significant role in preventing diseases such as cancer, blood borne disease, and other urinary infections.	[35]
Vitis vinifera L.	Vitaceae	sRNA sequencing, computational prediction, and experimental validation.	130 conserved miRNAs belonging to 28 families	A total of 130 target genes involved relating to transcription, disease resistance, signal transduction, energy metabolism, and biosynthesis.	[98]
Curcuma longa L.	Zingiberaceae	EST-based bioinformatics analysis.	12 putative miRNAs	Mammalian targets involved in signal transduction and apoptosis that may play a significant role in preventing diseases like diabetes mellitus type 2, cardiovascular disorders, Alzheimer's, cancer, and thalassemia.	[34]
Zingiber officinale (L.) H. Karst	Zingiberaceae	Bioinformatics analysis.	16 potential miRNA families	A total of 300 target transcripts associated with/involved in cell signalling, reproduction, metabolic processes, and stress response.	[99]
Ginkgo biloba var. *epiphylla* Mak	Ginkgoaceae	sRNA sequencing, computational prediction, and experimental validation.	82 conserved miRNAs and 53 putative novel miRNAs	Target genes related to the reproductive process, metabolic process, and stimulus-response.	[100]
Paeonia lactiflora Pall.	Paeoniaceae	sRNA sequencing, computational prediction, and experimental validation.	280 and 306 miRNAs were identified from two cultivars, "Zifengyu" and "Dafugui", respectively	Target genes involved in metabolic pathways, biosynthesis of secondary metabolites, endocytosis, and ether lipid metabolism.	[101]
Momordica charantia L.	Cucurbitaceae	Bioinformatics analysis.	27 mature miRNAs	Target genes involved in the plant developmental process.	[102]
Nelumbo nucifera Gaertn	Nymphaeaceae	Computational prediction and experimental validation.	106 conserved miRNAs	A total of 847 potential targets involved in plant development and stress response.	[103]

Supporting information

A table displaying the traditional use or clinical importance and the main bioactive ingredients of medicinal plants with currently known miRNAs is available as Supporting Information.

Acknowledgments
▼

Conflict of Interest
▼

The authors declare that there are no conflicts of interest.

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