Abstract
Diterpene lactones have been identified as active compounds in several medicinal plants, including *Andrographis paniculata* (Burm. f.) Nees, which is a medicinal plant that has been used for centuries across the world. Andrographolide is the major diterpene from *A. paniculata* and the main bioactive constituent of this species. The effectiveness of diterpenes can be affected by factors that limit their oral bioavailability, such as their poor water solubility, slow dissolution rates, low gastrointestinal absorption, high chemical and metabolic instability, and rapid excretion. In this context, the purpose of the present review is to compile and compare literature data on the bioavailability of diterpene lactones from *A. paniculata* after oral administration in medicinal plant extracts or in their free forms and to highlight strategies that have been used to improve their oral bioavailability. Considering that medicinal plant extracts are commonly used as dried powder that is reconstituted in water before oral administration, novel pharmaceutical formulation strategies that are used to overcome difficulties with diterpene solubility are also compiled in this review. The use of self-microemulsifying drug delivery systems is a good strategy to enhance the dissolution and consequently the bioavailability of andrographolide after oral administration of *A. paniculata* extract formulations. On the other hand, herbal medicine technology, pH-sensitive nanoparticles, nanosuspensions, nanoemulsions, nanocrystal suspensions, nanocrystal-based solid dispersions, and solid dispersion systems are useful to formulate andrographolide in its free form and increase its oral bioavailability. The use of a suitable andrographolide delivery system is essential to achieve its therapeutic potential.

1 Introduction
Diterpenes are, by definition, C_{20} compounds based on four isoprene (C_{5}H_{8}) units that may be derived by the mevalonate and deoxyxylulose phosphate pathways [1]. Extensive modifications of the diterpene skeleton are responsible for the generation of highly oxidized diterpenes bearing one or more lactone moieties [1].

Diterpenes exhibit enormous variation in their physicochemical properties due to their great structural diversity [2]. They can have acid or basic character, and present different solubility profiles, dissolution rates, and molecular weights, which can interfere with their absorption, distribution, metabolism, and elimination.

The lipophilicity and the dissolution rate, expressed as log*P* (the partition coefficient of a nonionic compound between the hydrophilic and lipophilic phases in an octanol/water system) and log*D* (the partition coefficient of ionized compounds, normally weak acids and bases) are recognized as key properties in transport processes, including intestinal absorption and distribution to different tissues [3]. Several of the diterpenes that have been
identified as active compounds in medicinal plants used in traditional medicine are poorly soluble in water and show slow dissolution rates [4–8]. This solubility behavior is a major challenge when attempting to achieve a pharmacological effect.

Although isolated bioactive constituents are preferred when researching and developing new drugs, medicinal plant extracts are used in traditional medicine as the only therapeutic option in many countries [9]. Therefore, the development of formulations of medicinal plant extracts or isolated compounds to enhance the solubility, dissolution, and oral absorption of diterpenes is important for improving the bioavailability of these poorly water-soluble compounds, regardless of how they will be used.

Medicinal plant extracts are commonly used as dried powder that is reconstituted in water before oral administration, and novel pharmaceutical formulation strategies that are used to overcome difficulties with diterpene solubility are compiled in this review. Many of the studies in this field are very promising and have resulted in formulations that also guarantee the stability of diterpenes during the digestion processes.

It should be highlighted that bioactive compounds need to be released from matrices to become bioaccessible and available for absorption by the gastrointestinal tract [10]. Under gastrointestinal conditions, including the occurrence of enzymatic processes with pH variations, interactions with food, and residence times, bioactive compounds are susceptible to degradation or biotransformation that modifies their biological activity.

There are many examples of medicinal plants that contain diterpenes among their bioactive constituents, such as Andrographis paniculata (Burm. f.) Nees. However, this species shows significant variation in diterpene bioavailability depending on the formulation of complex extracts or even isolated diterpenes considered. Therefore, this review presents relevant formulations of A. paniculata extracts used globally in traditional medicine, with a focus on the bioavailability of the diterpene lactones identified as active constituents, and it compares literature data on the bioavailability of diterpenes after oral administration in medicinal plant extracts or in their free forms, highlighting strategies that have been used to improve their oral bioavailability.

2 Literature Search Strategy

A comprehensive literature search of the PubMed, Scopus, SciFinder Scholar, and Web of Science databases for articles up to July 31, 2021 was performed. Combinations of the following keywords were used: “Andrographis paniculata,” “andrographolide,” “bioavailability,” “formulations,” “preparations,” “products,” and “pharmaceutical technologies.”

No limitations were placed on the publication date, but only relevant literature data on the bioavailability of diterpene lactones from A. paniculata after oral administration in medicinal plant extracts or in their free forms that were published in English were included.

The references cited in the selected articles were reviewed to identify further relevant studies.

3 Therapeutic Potential and Bioavailability of Diterpenes from Andrographis paniculata Extracts and Extract Formulations

Andrographis paniculata (Burm. f.) Nees is a medicinal plant that has been used for centuries across the world, including in countries such as India, Bangladesh, Pakistan, China, the Philippines, Malaysia, Indonesia, and Thailand, to treat diseases such as gastrointestinal tract disorders, upper respiratory infections, cancer, diabetes, thrombosis, hypertension, fever, and herpes [11–13]. This species is also one of the most used medicinal plants in Ayurvedic medicine, and has been reported to have antioxidant and anti-inflammatory activities, among others [14–18]. Recently, Sa-ngiamonsutorn et al. [19] reported anti-SARS-CoV-2 activity of an Andrographis paniculata extract and andrographolide, its major constituent, in human lung epithelial cells.

There are many products of this plant on the market, but the preparations are not yet well standardized to offer the expected effectiveness. The aerial parts of this species are the most used, but its roots have also been used as a folk remedy in Asia and Europe [20, 21]. Previous phychemical studies carried out with aerial parts (leaves and stems) and roots from A. paniculata have resulted in the isolation of more than 55 ent-labdane diterpenoids, 30 flavonoids, eight quinic acids, four xanthones [11, 13, 22], and five noriridoids [13, 23].

The major diterpene of A. paniculata, known as andrographolide (Fig. 1), has been reported to be the main bioactive constituent of this species. 14-Deoxy-11,12-didehydroandrographolide, neoandrographolide, and 14-deoxyandrographolide (Fig. 1) have also been reported to be important bioactive labdane diterpene lactones [12, 13].

Andrographolide was reported to have anti-inflammatory [24, 25], anti-cancer [26, 27] and anti-platelet [28] activities, while 14-deoxy-11,12-didehydroandrographolide has shown a potent hypotensive effect [29] and anti-platelet activity that is higher than that of andrographolide [28]. Neoandrographolide showed anti-inflammatory activity by inhibiting nitric oxide (NO) production both in vitro and ex vivo more strongly than andrographolide [30], and 14-deoxyandrographolide showed platelet-activating factor antagonistic activity in bovine neutrophils [31].
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The available literature on pharmacokinetic studies performed after oral dose administration of *A. paniculata* extracts or extract formulations only provide pharmacokinetic parameters for the diterpene andrographolide, except for the study performed in humans by Pholphana et al. [12], in which the authors determined the pharmacokinetic parameters for the four active diterpenes shown in Fig. 1. In that study, the authors also investigated the dissolution behaviors of these diterpenes under simulated gastrointestinal tract conditions at pH values of 1.2, 4.5, and 6.8.

Pholphana et al. [12] used an aerial part of *A. paniculata* from the Chulabhorn Research Institute (Sakaew Province, Thailand) to prepare capsules containing 350 mg of powdered raw material. According to the authors, each capsule contained 8.16 ± 0.28 mg of andrographolide (diterpene 1, Fig. 1), 1.35 ± 0.04 mg of 14-deoxy-11,12-didehydroandrographolide (diterpene 2, Fig. 1), 0.90 ± 0.03 mg of neoandrographolide (diterpene 3, Fig. 1), and 0.96 ± 0.04 mg of 14-deoxyandrographolide (diterpene 4, Fig. 1).

The pharmacokinetic study was carried out using 20 healthy Thai volunteers (10 female and 10 male) with an average age of 34.10 ± 6.03 years, who each received four capsules with 200 mL of water before meals for three consecutive days. On day 4, after an overnight fast, all volunteers again received four capsules with 200 mL of water, and blood samples were collected before drug administration and after predefined time points to determine the concentrations of the four major active diterpenes in plasma using a validated LC-MS/MS method. The pharmacokinetic parameters of the four diterpenes were compared between females and males, and the authors also calculated the relative systemic exposure, represented by the dose-normalized AUC of each diterpene.

Regarding dissolution testing, all the diterpenes showed similar dissolution profiles in all of the tested media, and their dissolution profiles were highest at pH 6.8 (86.92%, 78.74%, 93.74%, and 96.69% at 100 min for diterpenes 1–4, respectively) and lowest at pH 1.2 (58.19%, 21.06%, and 56.10% at 100 min for diterpenes 2–4, respectively). Diterpene 1 was least soluble at pH 4.5 at 100 min (75.31%), but the % dissolution of each diterpene reached a plateau state within 60 min of dissolution.

According to the authors, these results indicated that the four diterpenes were entirely released from the capsules containing crude powder of *A. paniculata*. However, it should be noted that diterpene 3 is a glycoside derivative of diterpene lactone that is acid hydrolyzed to form aglycone. This explains the lower solubility of diterpene 3 at pH 1.2. The results of the pharmacokinetic study (Table 1) indicated that, although the dose of diterpene 1 given to each subject was higher than that of diterpene 2, the plasma concentrations of 2 were higher than those of 1, resulting in a higher AUC of 2 than 1.

According to the authors, there were some factors that could justify these results: (1) the greater solubility of diterpene 1 than diterpene 2 may have caused greater absorption of diterpene 1 by passive diffusion, but literature data point to the occurrence of active transport of diterpene 1 (efflux) in the gastrointestinal tract [32, 33] but only passive transport of diterpene 2 [34]; (2) studies carried out by other authors in rats [35] showed that the renal excretion of diterpene 1 was only 8.75% and that more than 90% of this diterpene is presumably eliminated by metabolic transformations; and (3) previous studies carried out by the same group to investigate the stability of diterpenes at room temperature during storage [12] indicated that the content of diterpene 1 in the samples decreased while the content of diterpene 2 increased by more than 50% after 15 months of storage.

It should be pointed out that higher solubility does not always mean higher absorption. Diterpene 1 is reported to have an efflux transporter-mediated absorption mechanism [32, 33], which may have contributed to the detection of a

\[ \text{R: glucose moiety} \]

Fig. 1 Chemical structures of four major active diterpenes from *Andrographis paniculata*: andrographolide (1), 14-deoxy-11,12-didehydroandrographolide (2), neoandrographolide (3), and 14-deoxyandrographolide (4)
lower concentration of diterpene 1 in plasma when compared to diterpene 2.

The authors further suggest that diterpene 1 may have been converted to diterpene 2 in the body as well. Tran et al. [36] reported that 14-deoxy-11,12-didehydroandrographolide (diterpene 2) was one of the phase I metabolites of andrographolide.

A. paniculata preparations are commonly used in the treatment of cold, fever, and noninfectious diarrhea. Given its anti-inflammatory activity, diterpene 1 is one of the most important constituents for demonstrating the effectiveness of these medicinal preparations [13]. Therefore, the results obtained in the pharmacokinetic studies carried out by Pholphana et al. [12] are very relevant because they demonstrate that although diterpene 1 is the major active constituent in the medicinal preparations, the amount of diterpene 2 that reaches the systemic circulation is about 14 times greater than that of diterpene 1. As diterpene 2 presents a potent hypotensive effect [29] and exhibits stronger anti-platelet activity than diterpene 1 [28], the blood pressure of the patient should be monitored and observed carefully during their treatment period to avoid hypotension.

Pholphana et al. [12] also reported that the pharmacokinetic parameters of all four major active diterpenes were significantly different for females as compared to males. However, after dose normalization to 1 mg/kg, no statistically significant difference between females and males was found (Table 1). According to the authors, the differences in pharmacokinetic parameters between the sexes were mainly attributable to the lower body weights of females as compared to males. The authors suggested that dose adjustment should be considered when this plant product is used in a patient with a very low body weight.

Panossian et al. [35] reported the pharmacokinetic and oral bioavailability of andrographolide after oral administration of a 60% ethanolic water soluble extract of A. paniculata (containing 5% of andrographolide) and Kan Jang tablets (containing 4.25 mg of andrographolide), as formulated and supplied by the Swedish Herbal Institute, Gothenburg. Kan Jang tablets are formulated with a fixed combination of A. paniculata and Acanthopanax senticosus extracts. First, the authors investigated the bioavailability of andrographolide in 139 time-mated male Wistar rats after oral administration of A. paniculata extract dissolved in distilled water at two levels of dosage, 20 and 200 mg/kg. Bioavailability data on andrographolide were then obtained from 16 healthy volunteers (average age of 39.8 years; seven males and nine females) who received four tablets of Kan Jang with 200 ml of water. Blood samples were taken before and after oral administration at intervals of 30 min up to 8.0 h. The main pharmacokinetic parameters of andrographolide in rats, as reported by Panossian et al. [35], are presented in Table 2.

Panossian et al. [35] reported that andrographolide was rapidly absorbed into the blood after oral administration of A. paniculata extract at 20 mg/kg. Increasing the dose tenfold did not increase the concentration of andrographolide in the blood proportionately, and the pharmacokinetics of andrographolide in rats showed similar characteristics whether a dose of 20 mg/kg or a dose of 200 mg/kg of A. paniculata extract was administered. The authors also reported that the concentration–time profiles of andrographolide obtained with these doses were exponentially related and were well described by a one-compartment model. This means that, in each time period, a constant proportion of andrographolide was eliminated; that is, the elimination rate was proportional to the concentration. According to Panossian et al. [35], the bioavailability of andrographolide in rats after oral administration of A. paniculata extract dissolved in distilled water at a dosage of 20 mg/kg was 0.91%; at a dosage of 200 mg/kg it was even smaller, 0.21%. Regarding the bioavailability

\[ \Delta \text{Adis} \]

| Diterpene | Dose of each diterpene in A. paniculata capsules (mg) | Sex | AUC_0–t (ng·h/mL) | AUC_0–t/dose (dose normalized) (ng·h/mL)/(mg/kg) | T_max (h) | C_max (ng/mL) | C_max/dose (dose normalized) (ng/mL)/(mg/kg) |
|-----------|-----------------------------------------------------|-----|-------------------|-----------------------------------------------|---------|---------------|-----------------------------------------------|
| 1         | 8.16 ± 0.28                                         | Male | 47.58 ± 8.24     | 94.64 ± 15.12                                | 0.80 ± 0.15 | 30.08 ± 5.55  | 60.05 ± 11.49                               |
|           |                                                     | Female| 62.87 ± 10.63    | 99.52 ± 14.66                                | 0.75 ± 0.08 | 34.74 ± 4.02  | 56.17 ± 6.33                               |
| 2         | 1.35 ± 0.04                                         | Male | 108.77 ± 8.82    | 1318.26 ± 116.04                             | 0.90 ± 0.07 | 36.31 ± 4.29  | 434.13 ± 45.98                             |
|           |                                                     | Female| 147.56 ± 9.94    | 1452.53 ± 110.62                             | 1.00 ± 0.00 | 53.48 ± 4.87  | 529.50 ± 55.46                             |
| 3         | 0.90 ± 0.03                                         | Male | 8.59 ± 0.74      | 155.83 ± 14.36                               | 0.70 ± 0.08 | 8.70 ± 0.82   | 156.46 ± 13.09                             |
|           |                                                     | Female| 8.70 ± 0.54      | 128.19 ± 8.10                                | 0.83 ± 0.08 | 7.66 ± 0.62   | 113.10 ± 9.40                              |
| 4         | 0.96 ± 0.04                                         | Male | 10.07 ± 0.07     | 168.83 ± 15.57                               | 0.75 ± 0.08 | 9.65 ± 1.20   | 160.02 ± 15.57                             |
|           |                                                     | Female| 10.65 ± 0.78     | 147.68 ± 12.48                               | 0.80 ± 0.08 | 9.82 ± 0.62   | 135.96 ± 10.27                             |

\[ AUC \text{ area under the concentration-time curve from time zero to time } t, C_{\text{max}} \text{ maximum concentration, } T_{\text{max}} \text{ time to reach } C_{\text{max}} \]
data for andrographolide obtained from the 16 healthy volunteers, the pharmacokinetic parameters of andrographolide were highly variable and were explained well by an open two-compartment model.

In that study, andrographolide was absorbed quickly after oral administration of Kan Jang. The maximal concentration of andrographolide in the blood (393 ng/mL) was found to occur at 1.5–2.0 h. However, andrographolide distribution was quite variable, occurring quickly in four volunteers and slowly in body organs and tissues of 12 volunteers.

The authors also simulated the steady-state andrographolide plasma concentration following multiple doses of Kan Jang (four tablets given three times a day) using single-dose data in humans. The calculated steady-state plasma concentration of andrographolide was approximately 660 ng/mL, which, according to the authors, is enough to noticeably inhibit the PAF-mediated inflammatory response.

Sermkaew et al. [37] developed self-microemulsifying formulations of an A. paniculata extract in liquid and pellet forms for improving the oral bioavailability of andrographolide. The A. paniculata used in that study was collected from U-thong hospital, Suphanburi Province, Thailand. The liquid self-microemulsifying drug delivery system (SMEDDS) was composed of A. paniculata extract (11.1%), Capryol 90 (40%), Cremophor RH 40 (40%), and Labrasol (8.9%). This liquid SMEDDS was further adsorbed onto colloidal silicon dioxide and microcrystalline cellulose and converted to SMEDDS pellets by the extrusion/spheronization technique. The microemulsion droplet sizes of the liquid and pellet formulations after dilution with water were in the ranges of 23.4 and 30.3 nm, respectively.

The oral absorption of andrographolide was determined in male New Zealand white rabbits that were divided into four groups with three rabbits per group. Either an aqueous suspension of A. paniculata extract (equivalent to 35 mg/kg andrographolide), A. paniculata extract liquid SMEDDS (equivalent to 17.5 and 35 mg/kg andrographolide), or A. paniculata extract SMEDDS pellets (equivalent to 17.5 mg/kg andrographolide) were dispersed in 15 mL of distilled water and mixed homogeneously prior to oral administration. Blood samples were collected via the auricular artery at times ranging from 0 to 720 min after oral administration, and the pharmacokinetic parameters of andrographolide reported by Sermkaw et al. [37] are presented in Table 2.

Analysis of the pharmacokinetic results of the new SMEDDS in liquid and pellet forms showed increases in the AUC_{0-∞} and C_{max} of andrographolide when compared with those achieved after oral administration of an aqueous suspension of A. paniculata extract. Liquid SMEDDS and SMEDDS pellets given at a dose of 17.5 mg/kg showed 15- and 13-fold greater absorption of andrographolide, respectively, compared to the andrographolide absorption observed when a 35 mg/kg dose of the unformulated extract was given. However, the T_{max} values of andrographolide obtained with the liquid SMEDDS and the SMEDDS pellets were no different from the T_{max} value of andrographolide obtained with A. paniculata extract.

Based on the studies presented so far, which were carried out with unformulated crude extracts of A. paniculata and self-microemulsifying formulations of A. paniculata extracts, it can be said that the use of self-microemulsifying drug delivery systems is a good strategy for enhancing the dissolution and consequently the oral bioavailability of

### Table 2 Pharmacokinetic parameters (mean ± SD) of andrographolide after oral administration of Andrographis paniculata extract preparations

| A. paniculata preparation | Dose of andrographolide | Species | AUC (ng·h/mL) | T\textsubscript{max} (h) | C\textsubscript{max} (ng/mL) | Reference |
|---------------------------|-------------------------|--------|--------------|----------------|-----------------|-----------|
| 60% ethanolic water       | 20 mg/kg of extract     | Male Wistar rats | 7090 ± 1550\textsuperscript{a} | 2.41 ± 0.15 | 1273 ± 200 | [35] |
| soluble extract of A.     | (5% andrographolide)    |        |              |                |                 |           |
| paniculata                 |                         |        |              |                |                 |           |
| 60% ethanolic water       | 200 mg/kg of extract    | Male Wistar rats | 15070 ± 2000\textsuperscript{a} | 1.67 ± 0.3 | 3000 ± 600 | [35] |
| soluble extract of A.     | (5% andrographolide)    |        |              |                |                 |           |
| paniculata                 |                         |        |              |                |                 |           |
| A. paniculata extract     | 35 mg/kg andro-         | Rabbits | 109.75 ± 4.07\textsuperscript{b} | 1.5  | 90 ± 20  | [37] |
| liquid SMEDDS             | grapholide              |        |              |                |                 |           |
| A. paniculata extract     | 35 mg/kg andro-         | Rabbits | 2882.8 ± 123.5\textsuperscript{b} | 1.5 | 800 ± 50 | [37] |
| liquid SMEDDS             | grapholide              |        |              |                |                 |           |
| A. paniculata extract     | 17.5 mg/kg andro-       | Rabbits | 1707.24 ± 145.8\textsuperscript{b} | 1.5 | 540 ± 70 | [37] |
| SMEDDS pellets            | grapholide              |        |              |                |                 |           |
| A. paniculata extract     | 17.5 mg/kg andro-       | Rabbits | 1480.85 ± 321.29\textsuperscript{b} | 1.5 | 440 ± 60 | [37] |
| SMEDDS pellets            | grapholide              |        |              |                |                 |           |

\textsuperscript{a}AUC_{0-∞}, \textsuperscript{b}AUC_{0-12h}

\(AUC\) area under the concentration-time curve, \(C_{\text{max}}\) maximum concentration, SMEDDS self-microemulsifying drug delivery system, \(T_{\text{max}}\) time to reach \(C_{\text{max}}\).
andrographolide. Although there was an improvement in the pharmacokinetic parameters of andrographolide with the use of suitable pharmaceutical technology for developing extract formulations, the use of an isolated bioactive compound in therapy instead of a plant extract is very advantageous for ensuring consistency of composition and avoiding side effects. In the case of *A. paniculata*, given that andrographolide is the main bioactive constituent that displays anti-inflammatory, anti-cancer, and hepatoprotective activities, among others [13, 24, 38–40], this option becomes very relevant, since significant variations in the andrographolide contents of wild and cultivated *A. paniculata* populations are observed [41–43].

4 Andrographolide as a Pure Compound: Properties, Stability, and Bioavailability in Different Formulations

Andrographolide is a class II drug in the Biopharmaceutical Classification System, as it suffers from poor water solubility and low bioavailability [8]. Indeed, its experimental log*P* value is 2.632 (SD 0.135) and its aqueous solubility is 3.29 µg/mL (SD 0.73) at 25 °C [44].

*A. paniculata* contains approximately 1% andrographolide by dry weight [45], but according to Dechatiwonse et al. [46], the total diterpene lactone content decreases by 26% after 1 year of storage of the dried and powdered aerial part of this plant in dry ambient conditions. These results are very close to those obtained by Pholphana et al. [43], who reported that the total diterpene lactone content decreased by 5.3% after 3 months, 7.6% after 6 months, and 32.6% after 9 months. Nevertheless, the same authors [43] reported that the content of diterpene lactones before storage was 10.67%, so the total lactone content after 1 year will still comply with the minimum content of diterpene lactones required to use this plant in Thailand (6%).

Lomlim et al. [47] determined the stability of andrographolide in its crystalline and amorphous forms at elevated temperatures, and reported that its crystalline form was stable at 70 °C for 3 months. On the other hand, its amorphous form decomposed after 2 months under the same conditions. The major degradation product was identified as 14-deoxy-11,12-didehydroandrographolide (diterpene 2, Fig. 1), which was also reported to be a bioactive constituent of *A. paniculata* extracts [47]. The same degradation product was detected at a comparable rate when the powdered aerial part of *A. paniculata* was submitted to a heat-accelerated degradation experiment [48].

Pharmaceutical technologies are powerful tools for not only improving the pharmacokinetic properties of a compound but also avoiding the undesired degradation or transformation of the compound during manufacture and storage [49]. The work reported by Yen et al. [50] is an example of the use of these technologies to avoid the degradation of andrographolide during the storage period. Those authors developed and optimized an andrographolide-loaded nanoeulsion formulation and evaluated the stability of andrographolide in this formulation over 90 days at 4 and 25 °C. The andrographolide content remained at approximately 96% throughout the storage period.

Bioactive compounds are also susceptible to degradation or biotransformation during digestion processes and metabolism. Instability of andrographolide in gastrointestinal acidic and alkaline environments was reported by some authors [51, 52]. Investigations of andrographolide metabolites in humans resulted in the identification of a sulfate [53], cysteine S-conjugates [53], glucuronide conjugates [54], and urea adducts [55] of andrographolide (Fig. 2).

Studies of andrographolide metabolites in rats enabled the identification of one metabolite (compound RM, Fig. 2) that was identical to the compound clinically used in Lianbizhi injections in China to treat upper respiratory tract infections [56, 57]. This injection has also been used clinically to treat pneumonia, and yielded better results than those achieved with azithromycin [58]. There are no reports in the consulted literature of the biological activities of other andrographolide metabolites identified in humans.

Andrographolide was also reported to be a substrate of efflux transporters [32, 33], and this fact, associated with its rapid biotransformation, makes it essential to use pharmaceutical technology to improve its pharmacokinetic properties and increase its bioavailability.

Maiti et al. [59] formulated a herbosome of andrographolide with a naturally occurring phospholipid (1,2-distearoyl-sn-glycero-3-phosphocholine) purchased from Lipoid (Germany) in order to enhance the bioavailability and hepatoprotective activity of andrographolide in rats. Andrographolide was obtained from Natural Remedies Pvt. Ltd (Bangalore, India), and the developed method for preparing the herbosome was patented by this research group [40]. Regarding pharmacokinetic studies, 12 male albino Wistar rats were divided into two equal groups that received the andrographolide in distilled water or carboxymethyl cellulose orally at a dose of 25 mg/kg body weight or the andrographolide herbosome orally at a dose equivalent to 25 mg/kg andrographolide. Blood samples were collected 0.5 h post administration from the jugular vein of each animal under ether anesthesia at different time points, and the concentration of andrographolide in rat plasma was determined using the method of Panossian et al. [35] with a slight modification. The peak concentrations of andrographolide (6.79 µg/mL) and andrographolide herbosome (9.64 µg/mL) were achieved after 2.5 and 4 h, respectively. The effect of the
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andrographolide herbosome persisted for a longer period of time and the herbosome gave a higher relative bioavailability of 104.24%.

The pharmacokinetic parameters reported by Maiti et al. [59] are presented in Table 3.

Chellampillai and Pawar [60] reported an improvement in the bioavailability of andrographolide with the use of a pH-sensitive nanoparticle formulation of this diterpene prepared by a nan precipitation technique using Eudragit® EPO (cationic polymethacrylate copolymer). Andrographolide (purity ≥ 96%) was isolated from powdered leaves of Andrographis paniculata according to a method reported by Bothiraja et al. [44, 61].

The bioavailability of andrographolide from optimized nanoparticles (particle size of 255 ± 9 nm) and from pure andrographolide (particle size of 49,461 ± 9 nm) suspended

R: glucuronic acid moiety

Fig. 2 Chemical structures of andrographolide (AG) and some of its metabolites identified in humans (HM) and in rats (RM)
in 0.3% carboxymethyl cellulose was assessed in male Wistar albino rats at a dose of 10 mg/kg. After mild ether anesthetization, blood samples were collected using a retro-orbital puncture technique at predetermined time intervals. The serum samples were analyzed by HPLC according to the method reported by Suo et al. [62] with slight modifications. The pharmacokinetic parameters were calculated using a noncompartmental approach and are presented in Table 3. The authors reported that the andrographolide from the nanoparticle suspension with pH-dependent solubility

Table 3 Pharmacokinetic parameters (mean ± SD) of andrographolide after oral administration of different formulations of this diterpene

| Andrographolide formulation | Dose of diterpene | Species                | AUC_{0-∞} (ng·h/mL) | T_{max} (h) | C_{max} (ng/mL) | Reference |
|----------------------------|-------------------|------------------------|---------------------|-------------|----------------|-----------|
| Andrographolide solid dispersion formulation | 100 mg/kg | Male Sprague Dawley rats | 928.2 ± 181.1 | 0.4 ± 0.3 | 254.0 ± 59.7 | [8] |
| Andrographolide suspension | 300 mg/kg | Male Sprague Dawley rats | 953.3 ± 130.3 | 2.1 ± 1.8 | 206.6 ± 57.6 | [8] |
| Andrographolide-loaded nanoemulsion formulation | 100 mg/kg | Male Sprague Dawley rats | 3700 ± 240 | 0.08 | 3780 ± 880 | [50] |
| Andrographolide suspension | 300 mg/kg | Male Sprague Dawley rats | 1870 ± 170 | 0.75 ± 0.18 | 730 ± 80 | [50] |
| Andrographolide in distilled water with 0.3 g/kg carboxymethyl cellulose | 25 mg/kg | Male albino Wistar rats | 26740 ± 1420 | 2.50 | 6790 ± 540 | [59] |
| Andrographolide herbosome | 25 mg/kg | Male albino Wistar rats | 87300 ± 2350 | 4.0 | 9640 ± 720 | [59] |
| Pure andrographolide in 0.3 % carboxymethyl cellulose | 10 mg/kg | Male albino Wistar rats | 2169 ± 143 | 1.00 ± 0.06 | 830 ± 50 | [60] |
| Andrographolide nanoparticles | 10 mg/kg | Male albino Wistar rats | 4807 ± 261 | 0.25 ± 0.02 | 2670 ± 130 | [60] |
| Andrographolide coarse powder | 40 mg/kg | Male Sprague Dawley rats | 94.44 ± 27.57 | 28.75 ± 12.50 min | 77.74 ± 22.17 | [63] |
| Physical mixture of andrographolide coarse powder, TGPS, and sodium lauryl sulfate | 40 mg/kg | Male Sprague Dawley rats | 152.00 ± 35.52 | 22.00 ± 9.48 min | 80.25 ± 17.35 | [63] |
| Andrographolide dripping pills | 40 mg/kg | Male Sprague Dawley rats | 172.30 ± 27.45 | 22.50 ± 5.57 min | 168.17 ± 47.28 | [63] |
| Freeze-dried andrographolide nanosuspensions with TGPS | 40 mg/kg | Male Sprague Dawley rats | 367.82 ± 91.40 | 12.50 ± 8.80 min | 235.91 ± 53.73 | [63] |
| Freeze-dried andrographolide nanosuspensions without TGPS | 40 mg/kg | Male Sprague Dawley rats | 253.19 ± 27.34 | 22.00 ± 10.00 min | 200.49 ± 18.91 | [63] |
| Andrographolide nanocrystal-based solid dispersion | 20 mg/kg | Male Wistar rats | 1,794.783 ± 311.213 | 0.44 ± 0.14 | 323.423 ± 43.527 | [64] |
| Andrographolide nanocrystal suspension | 20 mg/kg | Male Wistar rats | 1,564.784 ± 416.853 | 0.36 ± 0.17 | 346.741 ± 38.163 | [64] |
| Water-dispersed crude andrographolide | 20 mg/kg | Male Wistar rats | 379.521 ± 124.233 | 1.11 ± 0.23 | 52.506 ± 10.652 | [64] |

AUC area under the concentration-time curve, C_{max} maximum concentration, T_{max} time to reach C_{max} in hours unless specified otherwise

*Mean ± SD, unless specified otherwise

Δ Adis
up to pH 5.5 was selected for site-specific release in the upper part of the gastrointestinal tract to avoid the degradation of this diterpene in the distal segments of the gastrointestinal tract. The values of AUC$_{0-\infty}$ and C$_{max}$ achieved after the administration of andrographolide nanoparticles were almost 2.2-fold and 3.2-fold higher than those obtained with the pure andrographolide, and the bioavailability was increased by 121.53%. According to the authors, the results indicated the potential of pH-sensitive nanoparticles for the oral delivery of low-bioavailability phytoconstituents such as andrographolide.

Qiao et al. [63] developed amorphous andrographolide nanosuspensions using a wet media milling technique followed by freeze drying. D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS), a surfactant that inhibits P-glycoprotein function, and sodium lauryl sulfate were used as surface stabilizers. The andrographolide was purchased from Shanghai YuanYe Bio-Technology Co., Ltd. (Shanghai, China) and had a mass purity of > 98.0%. Liquid andrographolide nanosuspensions (particle size of 215.6 ± 3.3 nm) were rapidly cooled with 1% (w/v) mannitol as a cryoprotectant, kept at −80 °C for 12 h, and transferred to a 6-L freeze dryer (Labconco Corporation, Kansas City, KS, USA), where they were dried at −30, −10, 0, and 25 °C for 10, 5, 2, and 2 h, respectively, at 1 psi pressure to obtain a dried andrographolide nanosuspension powder (particle size of 244.6 ± 3.0 nm). The authors also produced freeze-dried andrographolide nanosuspensions without TPGS. Included among the samples to be evaluated were andrographolide coarse powder (particle size of 10–200 µm); a physical mixture of the andrographolide coarse powder, TPGS, and sodium lauryl sulfate; and andrographolide dripping pills that were purchased from Tasly Pharmaceutical Group Co., Ltd. (Tianjin, China).

Pharmacokinetic studies were performed with 30 male Sprague Dawley rats divided into five groups that received andrographolide coarse powder; a physical mixture of the andrographolide coarse powder, TPGS, and sodium lauryl sulfate; andrographolide dripping pills; a freeze-dried andrographolide nanosuspension with TPGS; or a freeze-dried andrographolide nanosuspension without TPGS. All formulations were dispersed homogeneously in a 0.5% sodium carboxyl methyl cellulose aqueous solution to form suspensions, and the same dose of andrographide (equivalent to 40 mg of andrographolide/kg) was given by gavage to the rats in these five groups. Blood samples were collected from the vein of the eyeball from 5 to 720 min after oral administration. The concentration of andrographolide in the plasma was measured by an LC–MS/MS method. Noncompartmen-
tal pharmacokinetic analysis was used to determine the pharmacokinetic parameters, which are presented in Table 3.

Compared to the andrographolide coarse powder, the dripping pills and nanosuspensions with or without TPGS were more easily absorbed in vivo. In addition, the freeze-dried andrographolide nanosuspension with TPGS exhibited significantly higher plasma exposure when compared to the same formulation without TPGS. According to the authors, this result was mainly ascribable to the inhibitory effects of TPGS on P-glycoprotein efflux function. The authors also mentioned that the results indicated that nanosuspensions can act as effective delivery devices for andrographolide to enhance its oral bioavailability and biological efficacy.

Yen et al. [50] developed and optimized an andro-
grapholide-loaded nanoemulsion formulation by using high-pressure homogenization to improve diterpene oral bioavailability and its protective effects against inflammatory bowel disease. Andrographolide (purity ≥ 98%) was obtained from Tokyo Chemical Industry (Tokyo, Japan). Pharmacokinetic studies of the andrographolide nanoe-
mulsion and andrographolide suspension were performed after oral administration to male Sprague Dawley rats that were divided into three groups: (1) six rats that received an intravenous injection of the andrographolide solution at a dosage of 5 mg/kg (the diterpene was dissolved in normal saline and DMSO; 1:1, v/v); (2) six rats that received the andrographolide suspension orally at a dosage of 300 mg/kg (the diterpene was suspended in 2% w/v carboxymethyl cellulose aqueous solution); and (3) six rats that received the optimized andrographolide-loaded nanoemulsion formulation orally at a dosage of 100 mg/kg. Blood samples were collected via a jugular vein catheter before drug administration and at predetermined time points after administration.

Pharmacokinetic parameters reported by Yen et al. [50] were calculated using noncompartmental analysis and are presented in Table 3. The authors demonstrated that the relative bioavailability of the diterpene administered in the nanoemulsion formulation (particle size of 122 nm) was 594.3% greater than that of the diterpene in the suspension administered through the same route. According to the authors, this increase can be attributed to the enhanced andrographolide solubility and intestinal permeability and reduced droplet size in the formulation.

Ma et al. [64] developed an andrographolide nanocrystal suspension and converted it into an andrographolide nanocrystal-based solid dispersion by spray drying. The andrographolide (purity > 95%) was purchased from Zel-
ang Medical Technology (Nanjing, China). According to the authors, this new formulation has advantages associated with the use of nanocrystals and solid dispersions, including excellent dissolution and high drug loading of poorly soluble drugs. Pharmacokinetic studies were performed with 18 male Wistar rats divided into three groups of six animals. The groups received an andrographolide nanocrystal-based solid dispersion, an andrographolide nanocrystal suspension, and crude andrographolide dispersed in water, respectively, via oral administration at a dose of 20 mg/kg. Blood samples
were taken by retro-orbital puncture at predetermined intervals during the period from 0.25 to 24 h post administration. Andrographolide plasma concentrations were determined by a validated HPLC–tandem mass-spectrometry method [63].

The pharmacokinetic parameters reported by Ma et al. [64] are presented in Table 3. The AUC_{0–∞} of the andrographolide nanocrystal-based solid dispersion was 4.72 times larger than that of crude andrographolide, but the main pharmacokinetic parameters of the andrographolide nanocrystal-based solid dispersion were not significantly different from those of the andrographolide nanocrystal suspension. The authors concluded that this new formulation—which combined nanocrystals and a solid dispersion using surfactant-free dispersants as a matrix and was successfully prepared via homogenization and spray-drying technology—enhanced drug loading and improved the speed of drug dissolution.

Yen et al. [8] developed an andrographolide solid dispersion formulation composed of andrographolide (purity ≥ 99.0%), polyvinylpyrrolidone (PVP K30, Mw 40,000 g/mol), and Kolliphor EL (1:7:1, w/w/w). This formulation and andrographolide powder were suspended in pure water and administered orally to two groups each comprising five male Sprague Dawley rats. One group received andrographolide powder at a dose of 300 mg/kg and the other group received the andrographolide solid dispersion formulation at a dose of 100 mg/kg. Blood samples were taken using a catheter at intervals during the period from 0.083 to 8 h post administration. Andrographolide plasma concentrations were determined by a validated HPLC method [50]. Oral bioavailability was calculated by comparing the AUC values for the andrographolide suspension and the andrographolide solid dispersion formulation after dosage normalization. The pharmacokinetic parameters obtained in this study are presented in Table 3. According to the authors, the dose of 300 mg/kg was selected to appropriately reflect the pharmacokinetic profile of the andrographolide suspension, and Kolliphor EL was chosen to prevent the efflux of andrographolide by P-glycoprotein and thus increase andrographolide oral absorption. The authors concluded that the andrographolide solid dispersion formulation increased the relative bioavailability of andrographolide approximately threefold.

The results of the studies presented in this section demonstrate that pharmaceutical technologies are useful to improve the dissolution profile and oral bioavailability of andrographolide. Comparison of the AUC values for the unformulated andrographolide and the formulations of this diterpene in each study indicates that herbosomes, nanoe-mulsions, pH-sensitive nanoparticles, nanosuspensions with TGPS, and nanocrystal technologies are all excellent strategies for improving andrographolide oral bioavailability. The inclusion of andrographolide in a formulation system is also advantageous as it allows the degradation of this diterpene during the storage period and under gastrointestinal conditions to be avoided. The use of delivery systems to formulate andrographolide can simultaneously increase the efficacy and decrease the dose needed to achieve the required therapeutic effect, avoiding side effects. The therapeutic potential of unformulated andrographolide is restricted by its low bioavailability, and the use of pharmaceutical technologies is an innovative means to convert this natural product or its analogs into a clinically useful oral drug.

5 Conclusions

The studies included in this review show that the use of pharmaceutical technologies to develop formulations of Andrographis paniculata extracts or its isolated diterpene lactone improves the solubility, dissolution, stability during the digestion process, and bioavailability of the major bioactive diterpene lactone. Liquid self-microemulsifying drug delivery systems, herbosomes, pH-sensitive nanoparticle formulations, nanosuspensions, nanoemulsions, and nanocrystal-based solid dispersions have already had a significant impact on the oral bioavailability of allopathic drugs. The use of a suitable drug delivery system can also enable a substantial dose reduction of plant medicinal extracts or compounds without compromising the expected therapeutic efficacy, avoiding side effects. This review also provides ideas and strategies for further research into and development of medicinal plant formulations.

Declarations

Author contributions JPLD, HSR, and LSA performed the literature search and wrote the draft. NAJCF was responsible for conceptualization, reviewing the manuscript and editing.

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