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Review Article

Plant Bioactive Ingredients in Delivery Systems and Nanocarriers for the Treatment of Leishmaniasis: An Evidence-Based Review

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Abstract

Background: This study was designed considering the challenges of leishmaniasis treatment and the benefits of carriers of drug delivery systems to review plant bioactive ingredients in delivery systems and nanocarriers for the treatment of leishmaniasis.

Methods: The methodology of this review investigation followed the 06-PRISMA recommendations. The searches were carried out up to January 30, 2022, in the central English databases SCOPUS, WEB OF SCIENCE, EMBASE, PUBMED, and GOOGLE SCHOLAR using the search terms "ţ", "leishmaniasis", "herbal medicines", "drug delivery", "nanocarriers", "herbal compounds", and "secondary metabolites".

Results: Out of 5731 articles, 19 publications, including 12 *in vivo* (63.15%), 3 *in vitro* (15.8%), and 4 *in vitro/ in vivo* (21.1%) up to 2022, fulfilled the criteria presence for argument in the current systematic study. Plant bioactive ingredients were curcumin, betulinic acid, artemisinin, 4-nitrobenzaldehyde thiosemicarbazone, andrographolide, pentalinonsterol, ursolic acid, amarogentin, carvacrol, 14-deoxy-11-oxo-andrographolide, quercetin, beta-lapachone, cedrol, 2´,6´-dihydroxy-4´-methoxychalcone, and oleanolic acid.

Conclusion: The high potential of plant bioactive ingredients in delivery systems due to the load on the nanocarrier for the treatment of leishmaniasis through some main mechanisms of action, e.g. changes in the fluidity and the structure of the cell wall, creation of reactive oxygen species (ROS) and mitochondrial dysfunction, inhibition of DNA topoisomerase I enzyme, minimal cytotoxicity, stimulation of cell cycle disruption, stimulation of apoptosis, enhancement of the immune system. However, further investigations, especially in the clinical setting, are required to confirm these findings.

Introduction

Leishmaniasis is an infection triggered by a protozoan of the genus *Leishmania* transmitted by various phlebotomine sandflies. Clinical signs of the disease are vary depending on the type of infectious leishmaniasis, the geographical location and the immune status of the host (1). The condition is diagnosed in three forms: cutaneous (leishmaniasis), visceral (kalazar), and mucocutaneous (spundia). The most common form of leishmaniasis is the cutaneous type, which occurs in both dry (urban) and wet (rural) forms (2).

The incidence of visceral leishmaniasis worldwide stands at 500,000 patients per year and cutaneous leishmaniasis is more than twice this number (3). The leishmaniasis mortality rate in the world is between 20,000 and 40,000 cases per year (3). So far, no adequate and reliable vaccine has been developed for this disease; the fight against this disease has always been taken into account in the health planning of other countries. International investments have failed to eradicate the disease; however, it has laways been more prevalent in different regions of the world with the emergence of new disease outbreaks (4).

In recent years, emergence of resistance to standard drugs, which are mainly five-potency antimony compounds, the treatment of leishmaniasis, has faced many challenges. Physicians' reports indicate recurrence, lack of improvement or adverse effects on patients. Moreover, these drugs are not suitable, especially in rural areas, due to their high cost and lack of access. Therefore, there has always been a need to obtain adequate and alternative compounds to conventional drugs, which has led to the use of plant compounds (5).

For the medication to have a beneficial role, it must be protected to retain its chemical and biological properties until reaching its target location. Several drugs are extremely toxic and can create unwanted side effects. If they are damaged upon release, their therapeutic effect will be reduced; it is much more effective if the drug can reach the target directly and without affecting other parts of the body (6). Nanotechnology is very effective in developing entirely new designs to raise the bioavailability of drug delivery to organs (7). The search and development of carriers of drug delivery structures aim to achieve a system with appropriate drug loading and preferred release possessions with high half-life and minimal toxicity. Carriers used in drug delivery include micelles, liposomes, nanoparticles, dendrites, liquid crystals, hydrogels, conjugates, cobosomes and hexosomes (8).

This study was designed considering the challenges of leishmaniasis treatment and the benefits of carriers of drug delivery systems to review plant bioactive ingredients in delivery systems and nanocarriers for the treatment of leishmaniasis.

Methods

Search strategy

The methodology of this review investigation followed the 06- PRISMA recommendations of the CAMARADES-NC3Rs Preclinical Systematic Review and Meta-Analysis Facility (SyRF) database. The searches were carried out from January 2000 to January 2022, in the central English databases SCOPUS, WEB OF SCIENCE, EMBASE, PUBMED, and GOOGLE SCHOLAR using the search terms "*Leishmania*", "leishmaniasis", "herbal medicines", "drug delivery", "nanocarriers", "herbal compounds", and "secondary methabolites" (Fig. 1).

Studies selection

Selected publications were carefully checked to approve eligibility and obtain data. Followed by importing the selected publication into the EndNote X9 software, repeated and

duplicate publications were ommitted. After assessing the title and summary of the publications, the appropriate publications were included for further analysis. In the next step, eligible publications with adequate inclusion criteria were included for the final analysis. Any disagreement was dissolved between authors, by the corresponding author.

Inclusion and exclusion criteria

All experimental and clinical studies evaluating plant compounds with anti-leishmanial properties in nanocarriers were included in this review. On the other hand, publications with insufficient data, studies without full text, conformity between methods and findings, incorrect descriptions of results were omitted from this study.

Data extraction

Information extracted from selected publications included: effective composition, source, molecular formula, nanocarrier, preparation method, *Leishmania* species, study type, and animal.

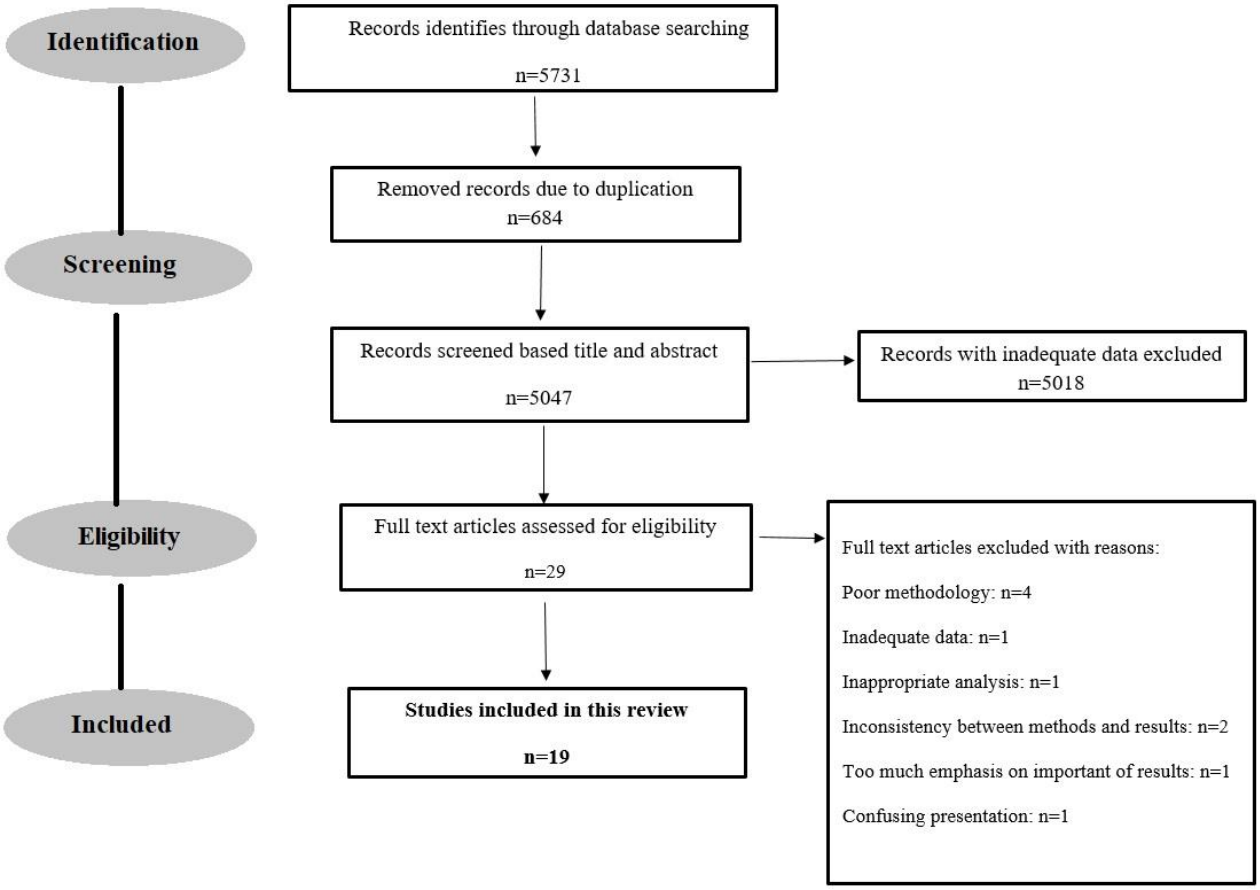

Fig. 1: Study flowchart of the current review investigation

Results and discussion

Out of 5731 articles, 19 publications, including 12 *in vivo* (63.15%), 3 *in vitro* (15.8%), and 4 *in vitro/ in vivo* (21.1%) up to 2022, fulfilled the criteria presence for argument in the current systematic study (Table 1). Plant bioactive ingredients were curcumin, betulinic acid, artemisinin, 4-nitrobenzaldehyde thiosemicarbazone, andrographolide, pentalinonsterol, ur-

solic acid, amarogentin, carvacrol, 14-deoxy-11-oxo-andrographolide, quercetin, beta-lapachone, cedrol, 2′,6′-dihydroxy-4′-methoxychalcone, and oleanolic acid.

Table 1: Plant compounds combined with nanocarriers for the treatment of leishmaniasis.

Effective composition	***Source***	***Molecular formula***	***Nanocarrier***	***Preparation method***	***Leishmania species***	***Study***	***Animal***	***Reference***
Curcumin	*Curcuma longa*	C21H20O6	Nanoliposomes/ PLGA/ Mannose-functionalized chitosan NPs	Thin-film hydration/ Emulsion solvent evaporation employing/ Response surface methodology	*L. major*/ *L. donovani*/ *L. donovani*	*In vitro*/ *In vitro & in vivo*/ *In vitro & in vivo*	-/ Hamster/ Hamster	(13-15)
Betulinic acid	Betula	C30H48O3	Nanochitosan	novel solvent and phase separation method	*L. major*	*In vivo*	Balb/c mice	(21)
Artemisinin	*Artemisia annua*	C15H22O5	PLGA	thin-film hydration	*L. donovani*	*In vivo*	Balb/c mice	(26)
4-nitrobenzaldehyde thiosemicarbazone (BZTS)	S-limonene	C8H8N4O2S	poly(ethylene oxide-b-ε-caprolactone)/ poly(ethylene oxide-b-lactide)	Different methods	*L. amazonensis*	*In vitro*	-	(29)
Andrographolide	*Paniculata andrographis*	C20H30O5	PLGA/ Nanoliposomes/	Emulsion solvent evaporation technique/ Thin-film hydration/	*L. donovani*/ *L. donovani*	*In vitro*/ *In vivo*	-/ Hamster	(32, 33)
Pentalinonsterol	*Pentalion andrieuxii*	C27H40O	Nanoliposomes	-	*L. donovani*	*In vivo*	mice	(35)
Ursolic acid	A wide range of plants	C30H48O3	Nanostructured lipid carriers (UA-	High-pressure homogeni-	*L. infantum*	*In vivo*	Golden ham-	(37)

			NLC)	zation technique			ster	
Amarogentin	*Swertia chirata*	C29H30O13	Liposome and niosome nanocarrier	-	*L. donovani*	*In vivo*	Hamster	(39)
Carvacrol	Thyme	C10H14O	Nanostructured lipid carriers (NLCs)	Warm microemulsion	*L. amazonensis*	*In vivo*	Rat	(42)
14-deoxy-11-oxo-andrographolide	*Andrographis paniculata*	-	liposome and niosome nanocarrier	-	*L. donovani*	*In vivo*	Hamster	(44)
Quercetin	A wide range of plants	C15H10O7	liposome and niosome nanocarrier	-	*L. donovani*	*In vivo*	Hamster	(46, 47)
			lipid-core nanocapsules (LNCs) of poly(ε caprolactone))	aqueous suspensions	*L. amazonensis*	*In vivo*	Balb/c mice	
Beta-lapachone	*Tabebuia avellanedae*	C15H14O3	lecithin-chitosan NPs	dissolved in an ethanolic solution	*L. major*	*In vivo*	Balb/c mice	(51)
Cedrol	*Ziziphus spina-christi*	C15H26O	nanostructured lipid carrier (NLC)	Hot-melting emulsification-ultrasonication	*L. donovani*	*In vitro & in vivo*	Balb/c mice	(53)
2′,6′-dihydroxy-4′-methoxychalcone (DMC)	*Piper aduncum*	C28H34O14	poly(D,L-lactide)	-	*L. amazonensis*	*In vitro & in vivo*	Balb/c mice	(55)
Oleanolic acid	*Calendua officinalis*	C30H48O3	PLGA	emulsion solvent evaporation technique	*L. donovani*	*In vivo*	Balb/c mice	(57)

Curcumin

Curcumin with the molecular formula $C_{21}H_{20}O_6$ (Fig. 2A) is the main active ingredient in turmeric, which belongs to the Zingiberaceae family, known for its extensive range of medicinal activities including anti-tumor, anti-diabetic, and antioxidant effects (9, 10). Additionally, this compound has gathered the attention of scientists in recent years due to its many antimicrobial properties (11). One of the disadvantages of this compound is its hydrophobic nature, which leads to its low absorption (12). Fattahi Bafghi et al. loaded curcumin onto nanoliposomes by thin-film hydration to assess its anti-leishmanial effects on *L. major*. The recorded concentrations of nanocomposite that killed 50% of the promastigote popu-

lation were 6.41, 3.8 and 2.33 µg/ml at 24, 48 and 72 h, respectively, which had a better effect than the control drug amphotericin B in all conditions (13).

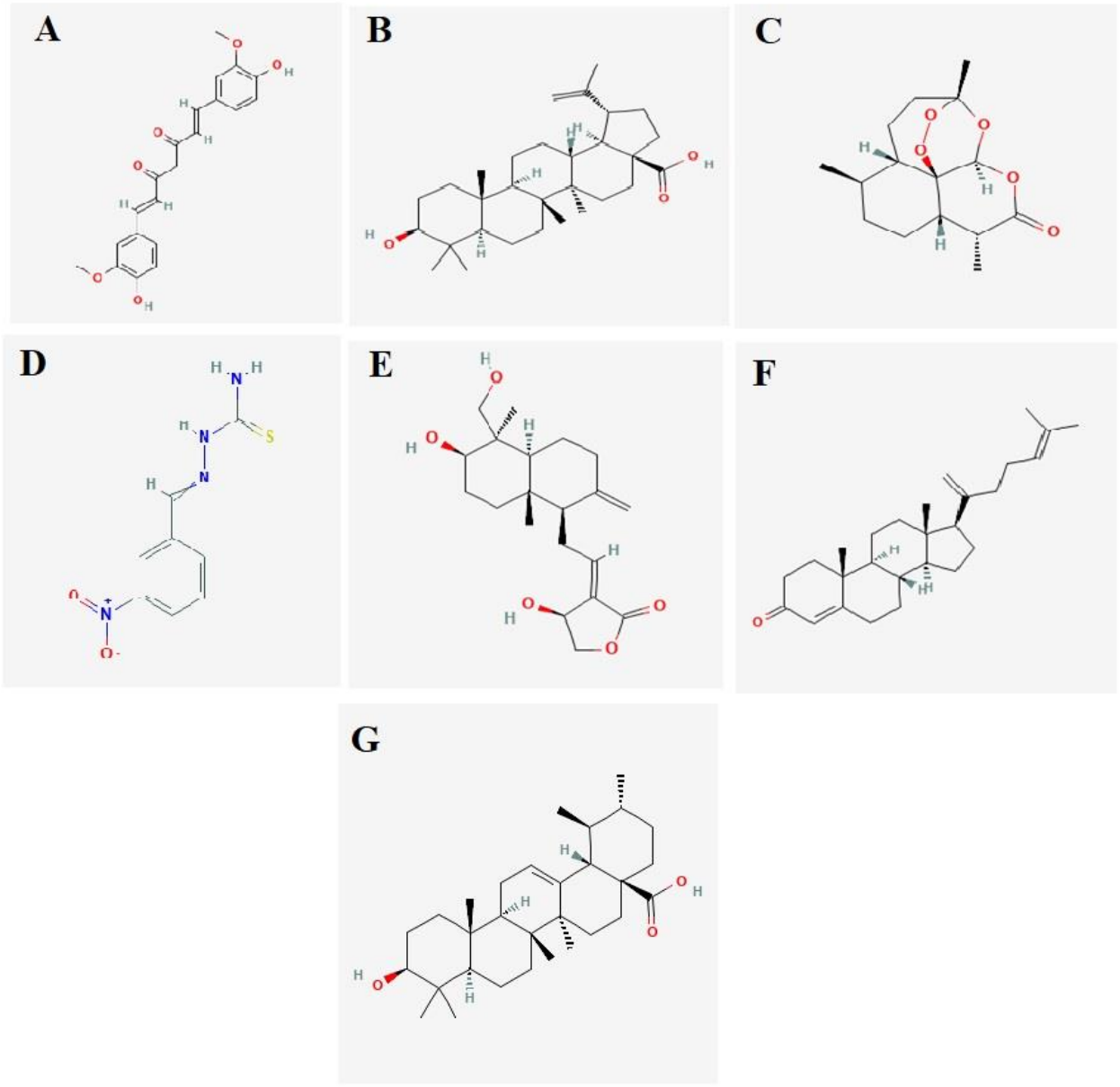

Fig. 2: Chemical structure of Curcumin (A), Betulinic acid (B), Artemisinin (C), 4-nitrobenzaldehyde thiosemicarbazone (D), Andrographolide (E), Pentalinonsterol (F), Ursolic acid (G)

Tiwari et al. emulsified curcumin by emulsion-solvent evaporation using the PLGA carrier and evaluated its efficacy alone and with miltofuscin on hamsters infected with *L. donovani*. The nanocomposition alone had significant leishmaniasis activity *in vitro* and *in vivo*; along with miltefosine, it properly inhibited promastigotes and amastigotes *in vitro*. Furthermore, under in vivo conditions, the combination of the nanoformulation with multelfosine increased the production of toxic active

oxygen/nitrogen metabolites as well as phagocytic activity (14).

Chaubey et al. used CUR-loaded mannose-functionalized chitosan nanoparticles (Cur-MCN) produced by response surface methodology in hamsters with visceral leishmaniasis as an anti-*Leishmania* drug. Cur-MCN was generated by mannose-conjugated chitosan and its efficacy and toxicity were reported on *L. donovani* compared to non-conjugated chitosan nanoparticles. The decrease of parasitic burden in the spleen of curcumin-conjugated nanocombinance hamsters was more significant in the control group. In addition, *in vitro* cytotoxicity examination against the J774A.1 cell line showed that it was nottoxic to macrophage cells. Results of the in vivo study approved the anti-leishmanial potential of the nanoformulation and indicated minimal cytotoxicity (15).

Betulinic acid

Betulinic acid has been isolated from various plant species, but its main source is the Birch tree with the scientific name Betula (16). The chemical formula of this compound is $C_{30}H_{48}O_3$ (Fig. 2B); its other name is Mairin. Betulinic acid is a more active biological form of Betulin (16). It belongs to a class of terpernoid compounds that have shown anti-inflammatory, anti-cancer, and antimicrobial properties (17). Heterocyclic derivatives of Betulin, including Betulinic acid, have been presented to have antiparasitic activity against *L. donovani* (18). In an investigation, the growth inhibitory activity of Betulinic acid had an inhibitory effect on the growth of *L. donovani* promastigotes (19). Sousa et al. found half-maximal inhibitory concentration (IC_{50}) Betulinic acid on *L. infantum* promastigotes was 50 µg / ml (20).

Zadeh Mehrizi et al. loaded nanocytosan, considering the antiparasitic effects of Betulinic acid against leishmaniasis, in order to improve the therapeutic effects and reduce its cmplications for the treatment of Balb/c mice infected with *L. major*. In vivo results demonstrated that the nano-toxicity of the betulinic acid-nanocytosan nanoparticle was nil and that its dose of 20 mg/kg could entirely cured the CL and prevent the parasite growth (21). Betulinic acid induces apoptosis by generating signals that alter mitochondrial function, unbalancing the expression level of the B-cell lymphoma-2 protein family and activating nuclear factor kappa B. Induction of apoptosis has been shown to occur by inhibiting DNA topoisomerase I and II following the use of Betulinic acid. However, low solubility and relatively short plasma half-lives have limited the clinical use of Betulinic acid; deploying nanocarriers such as chitosan can alleviate these issues (21).

In one study, this drug was used to treat *Leishmania*-infected mice compared to a control drug. Parasitic load and wound diameter measurements indicated that Betulinic acid loaded onto the produced chitosan nanoparticles was more effective than the control drug and inhibited 90% of parasite growth. One of the properties of nanoparticles was to minimize complications and elevate the efficiency of therapeutic compounds. In general, results of this study demonstrated that the nano-drug betulinic acid-nanocytosan could be effective in improving leishmaniasis. Indeed, increasing the dose of this nanodrug in the form of nanocarriers makes it possible to increase the therapeutic effects and reduce side effects. It therefore seems that this nano-formulation could be a good candidate in the future for the treatment of *L.major* lesions (21, 22).

Artemisinin

Artemisinin, with the chemical formula $C_{15}H_{22}O_5$ (Figure 2C), is a terpene lactoneandroperoxidase derivative derived from *Artemisia annua* aerial parts, widely used against the malaria parasite; moreover, it has strong anti-leishmaniasis activities (23). Studies indicate that Artemisinin can kill 50% of promastigotes up to 120 µM (24). Oral artemisinin administration is also associated with a significant decrease in parasitic number in

BALB/c mice treated with visceral leishmaniasis (25). The need to combine Artemisinin with nanocarriers is because this terzene and its derivatives, including artesunate and dihydroartemisinin, have low bioavailability and short half-lives (25).

Use of the Artemisinin nanoliposomic formulation considerably declined the *L. donovani* amastigotes and macrophage infection rate in infected mice. This formulation produced 82% inhibition in the liver and 77.6% inhibition in the spleen at 20 mg / kg body weight per dose (26). In another study, Want et al. used the nanopharmaceutical poly-lactic co-glycolic acid (PLGA)-Artemisinin to treat mice with *L. donovani*. Analysis of liver enzymes alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP), renal factors urea and creatinine indicated that this formulation was non-toxic. The loaded form of Artemisinin in PLGA polymer at a dose of 20 mg/kg declined the parasite burden by 85% in the liver and 82% in the spleen, which was more effective than the control drug (27). Experimental studies have demonstrated that Artemisinin contained in PLGA significantly inhibits the growth of amastigote forms compared to artemisinin alone (27). The action mechanism of this compound has been attributed to the induction of cell cycle interruption and apoptosis (27).

4-nitrobenzaldehyde thiosemicarbazone

The compound 4-nitrobenzaldehyde thiosemicarbazone (BZTS) with the chemical formula C8H8N4O2S (Fig. 2D) is derived from S-limonene, which can be extracted from the peel of most citrus fruits. The anti-tumor, anti-bacterial, anti-viral and anti-parasitic properties of this plant compound have been studied and proven in various studies. However, its hydrophobicity has limited the use of this compound (28).

Britta et al. aimed to synthesize nanoparticle-based block copolymers that could improve the bioavailability of BZTS. In this investigation, the activity of BZTS nanoparticle suspensions against *L. amazonsensis* amastigotes was studied *in vitro*. Results indicated the significant inhibitory activity of the nanocomposition, which was directly related to concentration. Moreover, weak cytotoxic activity against macrophages was another promising result in terms of obtaining an effective anti-leishmanial agent (29).

Andrographolide

Andrographolide with the chemical formula $C_{20}H_{30}O_5$ (Fig 2E) is a two-ring diterpene compound and the most effective substance purified from the leaves of *Andrographis paniculata* (30). This compound, which is known as a potent anti-leishmaniasis agent with low toxicity, has disadvantages, e.g., minimal bioavailability, short plasma half-life and poor organ localization (31). Roy et al. found Andrographolide was loaded onto a poly (DL-lactide-co-glycolic acid) nanocarrier and assessed its effect on the inhibition of *Leishmania* amastigotes. Based on the results, the IC_{50} of nanodrugs compared to Andrographolide alone was 34 and 160 µM, respectively. The action mechanism of this compound in the inhibition of the *Leishmania* parasite by cytostatic mechanism was also introduced (32).

Sinha et al. reported that the Andrographolide nanoformulation in combination with liposomes is more effective in delivering anti-leishmaniasis agents to phagocytic cells. The nanodrug reduced the parasitic load by 67% in the spleen of *L. donovani* infected hamsters. Moreover, positive tissue changes in spleen tissue were observed in the tested group in comparison with the control group (33).

Pentalinonsterol

Pentalinonsterol with the chemical formula $C_{27}H_{40}O$ (Fig. 2F) is a sterol extracted from the plant Pentalion andrieuxii (34). *L. donovani* infected mice were cured with a pentalinon-

sterol liposomal nanoformulation. The nanodrugs were found to reduce more than half of the parasitic burden on the liver and spleen. These nanoliposomes have demonstrated good potential to encapsulate and stabilize pentalinonsterol molecules against a variety of environmental conditions and protect them from degradation (35).

Ursolic acid

Ursolic acid with the chemical formula $C_{30}H_{48}O_3$ (Fig. 2G) is a 5-ring lipid-friendly triterpenoid found in a wide range of plants and fruits (36). This compound has many pharmacological activities in the treatment of diabetes, antitumor and antimicrobial activity; and its anti-leishmanial activity on various species has been proven in a number of studies. However, the limited solubility of this compound has prevented its widespread use (36).

Jesus et al. observed that the compound was loaded into nanostructured lipid carriers (UA-NLC), and *L. infantum* infected golden hamsters cured with the nanocomposition, which showed no morphological alterations in their visceral tissues (37). AST, ALT, urea and creatinine were not abnormal. In addition, the reduction of parasitic load in UA-NLC-treated hamsters was more significant than the ursolic acid-free group and the amphotericin B group. Furthermore, the beneficial activity of UA-NLC was linked with an increased protective immune response and a high preservation level of the spleen and liver as well as the normalization of liver and kidney functions (37).

Amarogentin

Amarogentin with the chemical formula $C_{29}H_{30}O_{13}$ (Fig. 3A) is a Seco-iridoid glycoside derived from Indian herbal medicine and extracted from the plant Swertia chirata which is very bitter in taste (38). Medda et al. *L. donovani* infected hamsters were used to assess the efficacy of this compound, which was loaded onto liposome and niosome nanocarriers. The decrease of parasitic burden in the spleen after intervention with free Amarogentin, liposome nanocarriers and niosomes was 34, 69 and 90%, respectively. In addition, hepatotoxicity tests indicated no toxicity of Amarogentin in the free form and in combination with nanoparticles. Inhibiting DNA topoisomerase I enzyme has been introduced as a functional channel for this compound in the death of *Leishmania* parasite (39).

Carvacrol

Carvacrol with the chemical formula $C_{10}H_{14}O$ (Fig. 3B) is a monoterpenic phenolic compound, is one of the main constituents of the essential oil obtained from thyme and similar plants of the family (Lamiaceae) and is insoluble in water, but soluble in alcohol and ether (40). Carvacrol is cited as a compound having anti-parasitic effects, especially anti-leishmanial effect; however, its therapeutic uses are challenging due to poor solubility and oxidation as well as rapid evaporation (41). Carvacrol was loaded onto nanostructured lipid carriers (NLCs) by a warm microemulsion method (42). The nanocomposition was observed to be associated with less cytotoxicity than free carvacrol and improved its *in vitro* antipyretic activity as *L. amazonensis* amastigote. Finally, the in vivo pharmacokinetics of carvacrol following bolus intravenous inoculation in rats indicated that the compound underwent hepatic circulation and had a extensive half-life and minimal clearance (42).

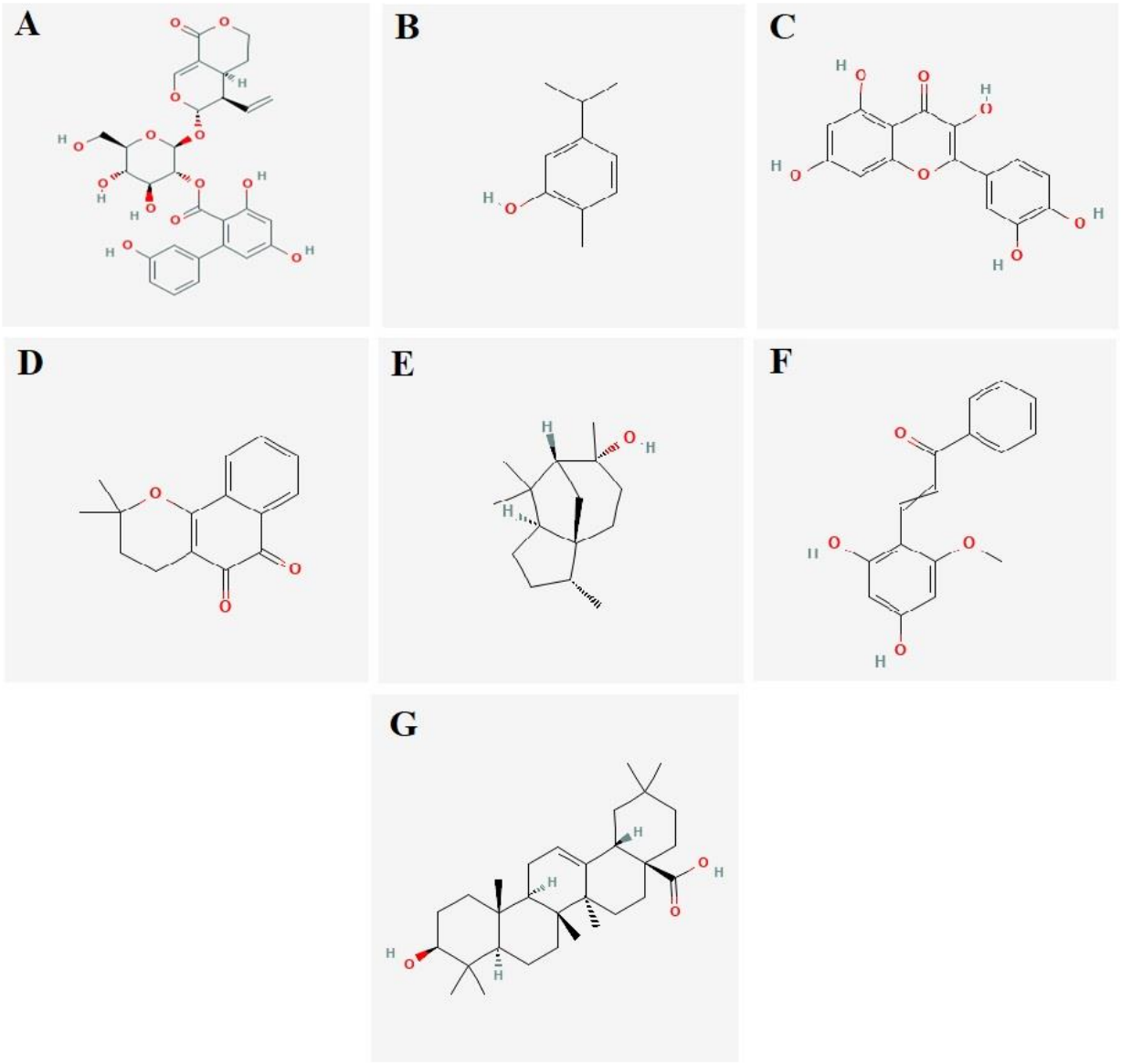

Fig. 3: Chemical structure of Amarogentin (A), Carvacrol (B), Quercetin (C), 4 Beta-lapachone (D), Cedrol (E), 2′, 6′-dihydroxy-4′-methoxychalcone (F), Oleanolic acid (G)

14-deoxy-11-oxo-andrographolide

14-deoxy-11-oxo-andrographolide is derived from a native plant in India called Andrographis paniculata (43). The compound was loaded onto two nanocarriers of liposomes and niosomes in the study by Lala et al. to investigate its anti-leishmaniasis effects on hamsters treated with *L. donovani*.

Splenic parasitic load after the intervention with the liposomal, nosomal and free drug nanocomposition was reduced by 72, 78 and 39%, respectively. Measurement of liver enzymes and renal factors indicated that loading plant composition into liposome or niosome nanoparticles significantly reduced liver and kidney toxicity as well as spleen toxicity compared to its free state (44).

Quercetin

Quercetin with the chemical formula $C_{15}H_{10}O_7$ (. 3C) is a powerful flavonoid from the Allium family that has a wide range of benefits for human health (45). Sarkar et al. compared the inhibitory effects of free quercetin in combination with the nanocarriers of liposomes and niosomes on hamsters with *L.donovani*. Reduction of splenic parasitic load after the intervention with free quercetin and liposome- and nosome-loaded nanoparticles was estimated to be 26, 51 and 68%, respectively. In addition, the measurement of blood factors demonstrated an increase in toxicity in the presence of free quercetin and a decrease in toxicity after intervention with nanodrugs. Moreover, the spleen tissue was examined histologically, which indicated a reduction in histotoxicity in the group receiving nanoformulations compared to the free drug. This study highlighted that quercetin exerts its inhibitory role through reactive oxygen species (ROS) creation and mitochondrial dysfunction (46).

Sousa-Batista et al. found quercetin was loaded into lipid-core nanocapsules (LNCs) of poly (ε caprolactone). Mice infected with *L. amazonensis* received the oral dose of free quercetin (16 mg / kg) or synthesized nanomedicine (0.4 mg/kg). Lesion size after receiving free quercetin and quercetin loaded in LNCs was reduced by 38 and 64%, respectively. Parasitic load was reduced by 71 and 91%, respectively. None of the treatments in this study resulted in increased toxicity indices (47).

Beta-lapachone

Beta-lapachone with the chemical formula $C_{15}H_{14}O_3$ (. 3D) is a natural ortho-naphthoquinone product attained from *Tabebuia avellanedae* barks, native to South America (48).

The anti-tumor, anti-malarial and anti-*Trypanosoma cruzi* effects of this compound have been proven in previous research (49). In addition, *in vitro* studies have demonstrated potent anti-leishmaniasis effects of beta-lapachone on *L. infantum* and *L. amazonensis* (50). Beta-lapachone was loaded onto lecithin-chitosan nanoparticles to evaluate its effectiveness in *L. major* macrophages inhibition and wound healing in Balb/c mice. Despite the not-reduction of the parasitic load, the increase of the drug in the dermis and its penetration through it reduced the wound diameter. Immunohistopathological tests in skin lesions and quantitative mRNA evaluates in depleted lymph nodes displayed that its anti-inflammatory action reduces the expression of interleukin 1 beta and cyclooxygenase-2, and decreases neutrophil penetration. The action mechanism of this compound was mediated by ROS production and apoptosis (51).

Cedrol

Cedrol with the chemical formula $C_{15}H_{26}O$ (. 3E) is a sesquiterpene alcohol in the essential oil of conifers of the Pinaceae family, especially in the families Cupressus and Juniperus (52). Pharmacological effects of this compound have been proven in the treatment of obesity. In addition, it has anti-inflammatory, antibacterial and anti-parasitic effects (52). Kar et al. loaded this compound into a NLC by the hot-melting emulsification-ultrasonication assay to evaluate the susceptibility of wild-type and drug-resistant *L. donovani* amastigotes to this nanocomposition both *in vitro* and *in vivo*. According to *in vitro* results, the IC_{50} obtained from free sedrol for wild species, sodium stibogluconate resistant, paromomycin resistant and field-resistant strains was 1.5, 2, 1.8 and 1.35 µM, respectively. Cytotoxicity was obtained in 74 µM mouse macrophage cells. The use of the nanocomposition doubled the selectivity indexes in both species. *In vivo* results indicated that loading cedar into NLC and oral administration to mice increased its bioavailability 3-5 times in drug-resistant and 2.3-3.8 times in wild species of *L. donovani*. The results were comparable to miltefosine (53).

2′, 6′-dihydroxy-4′-methoxychalcone

2′, 6′-dihydroxy-4′-methoxychalcone (DMC) (. 3F) is a substance extracted from *Piper aduncum* (54). In previous studies, DMC demonstrated relenant *in vitro* effects on *L. amazonensis* promastigotes and amastigotes with the effective doses of 0.5 and 24 µg/ml by 50%, respectively (54).

DMC was loaded onto poly (D, L-lactide) nanoparticles to study its anti-leishmanic properties in mice treated with *L. amazonensis* (55). A sixty percent decrease in wound diameter was found in the nanocomposite group in comparison to the control group. Furthermore, the decrease of load of parasites in the intervention group compared to the control group was more than 52%. This study concluded that the efficacy of this nanodrug was comparable to that of standard glucantime. The action mechanism of DMC, which destroys the parasite, was attributed to changes in the cell membrane fluidity and structure (55).

Oleanolic acid

Oleanolic acid with the chemical formula $C_{30}H_{48}O_3$ (3G) is a triterpenoid extracted from the flower of *Calendua officinalis* with antibacterial, antiviral, and anti-tumor effects. Additionally, this compound kills the *Leishmania* parasite by causing apoptosis (56). Ghosh et al. loaded oleanolic acid onto PLGA nanoparticles to study its efficacy in reducing parasite load in mice infected with *L. donovani*. According to the results, the reduction in spleen parasitic load after the intervention with nanodrugs and free oleanolic acid was 78 and 67%, respectively. In addition, results of serum concentrations of ALT, AST, Blood Urea Nitrogen (BUN) and creatinine indicated low nephrotoxicity in infected mice receiving an Oleanolic acid formulation (57).

As the strength points of the eligible studies, we can mention the high *in vitro* and *in vivo* efficacy of these compounds on different forms of *Leishmania* parasites at low concentrations, as well as the lack of high toxicity. However, the lack of evaluation of these compounds in clinical phases as well as the use of commercial forms of these compounds instead of the main compounds isolated from plants can be considered as limitations and weaknesses of these studies.

Conclusion

The high potential of plant bioactive ingredients in delivery systems due to the load on the nanocarrier for the treatment of leishmaniasis through some main mechanisms of action e.g., changes in the fluidity and the structure of the cell wall, creation of ROS and mitochondrial dysfunction, inhibition of DNA topoisomerase I enzyme, minimal cytotoxicity, stimulation of cell cycle disruption, stimulation of apoptosis, enhancement of the immune system. However, further investigations, ecpecially in the clininal setting, are required to confirm these findings.

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Competing interests

None.

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None

References

1. Ibarra-Meneses AV, Corbeil A, Wagner V, Onwuchekwa C, Fernandez-Prada C. Identification of asymptomatic *Leishmania* infections: a scoping review. Parasites Vectors. 2022;15(1):5.

2. Mann S, Frasca K, Scherrer S, Henao-Martínez AF, Newman S, Ramanan P, Suarez JA. A Review of leishmaniasis: current knowledge and future directions. Curr Trop Med Rep. 2021;8(2):121-32.
3. Alanazi AD, Alyousif MS, Saifi MA, Alanazi IO. Epidemiological studies on cutaneous leishmaniasis in Ad-Dawadimi District, Saudi Arabia. Trop J Pharm Res. 2016;15(12):2709-12.
4. AlMohammed HI, Khudair Khalaf A, E Albalawi A, et al. Chitosan-Based Nanomaterials as Valuable Sources of Anti-Leishmanial Agents: A Systematic Review. Nanomaterials. 2021;11(3):689.
5. Nafari A, Cheraghipour K, Sepahvand M, Shahrokhi G, Gabal E, Mahmoudvand H. Nanoparticles: New agents toward treatment of leishmaniasis. Parasite Epidemiol Control. 2020;10:e00156.
6. Albalawi AE, Alanazi AD, Sharifi I, Ezzatkhah F. A systematic review of curcumin and its derivatives as valuable sources of antileishmanial agents. Acta Parasitol. 2021;66(3):797-811.
7. Albalawi AE, Khalaf AK, Alyousif MS, et al. Fe3O4@ piroctone olamine magnetic nanoparticles: Synthesize and therapeutic potential in cutaneous leishmaniasis. Biomed Pharmacother. 2021;139:111566.
8. Albalawi AE, Abdel-Shafy S, Khudair Khalaf A, et al. Therapeutic potential of green synthesized copper nanoparticles alone or combined with meglumine antimoniate (glucantime®) in cutaneous leishmaniasis. Nanomaterials. 2021;31;11(4):891.
9. Cheraghipour K, Ezatpour B, Masoori L, et al. Anti-Candida activity of curcumin: A systematic review. Curr Drug Discov Technol. 2021;18(3):379-90.
10. Cheraghipour K, Marzban A, Ezatpour B, Khanizadeh S, Koshki J. Antiparasitic properties of curcumin: A review. AIMS Agric Food. 2018;3(4):561-78
11. Shakib P, Ali AS, Javanmard E, et al, Cheraghipour K. Anti-trichophyton effects of curcumin: A systematic review. Anti-Infect Agents. 2021;19(4):29-34.
12. Zhai K, Brockmüller A, Kubatka P, Shakibaei M, Büsselberg D. Curcumin's beneficial effects on neuroblastoma: Mechanisms, challenges, and potential solutions. Biomolecules. 2020;10(11):1469.
13. Fattahi Bafghi A, Haghirosadat BF, Yazdian F, et al. A novel delivery of curcumin by the efficient nanoliposomal approach against *Leishmania major*. Prep Biochem Biotechnol. 2021;51(10):990-7.
14. Tiwari B, Pahuja R, Kumar P, Rath SK, Gupta KC, Goyal N. Nanotized curcumin and miltefosine, a potential combination for treatment of experimental visceral leishmaniasis. Antimicrob Agents Chemother. 2017;61(3):e01169-16.
15. Chaubey P, Mishra B, Mudavath SL, et al, Monteiro M. Mannose-conjugated curcumin-chitosan nanoparticles: efficacy and toxicity assessments against Leishmania donovani. Int J Biol Macromol. 2018;111:109-20.
16. Yogeeswari P, Sriram D. Betulinic acid and its derivatives: a review on their biological properties. Curr Med Chem. 2005;12(6):657-66.
17. Moghaddam MG, Ahmad FB, Samzadeh-Kermani A. Biological activity of betulinic acid: a review. Pharm Pharmacol. 2012;3:119-123
18. Halder A, Shukla D, Das S, Roy P, Mukherjee A, Saha B. Lactoferrin-modified Betulinic Acid-loaded PLGA nanoparticles are strong antileishmanials. Cytokine. 2018;110:412-5.
19. Roy Chowdhury A, Mandal S, Goswami A, et al. Dihydrobetulinic acid induces apoptosis in *Leishmania donovani* by targeting DNA topoisomerase I and II: implications in antileishmanial therapy. Mol Med. 2003;9(1):26-36.
20. Sousa MC, Varandas R, Santos RC, Santos-Rosa M, Alves V, Salvador JA. Antileishmanial activity of semisynthetic lupane triterpenoids betulin and betulinic acid derivatives: synergistic effects with miltefosine. PloS One. 2014;9(3):e89939.
21. Zadeh Mehrizi T, Shafiee Ardestani M, Haji Molla Hoseini M, Khamesipour A, Mosaffa N, Ramezani A. Novel nanosized chitosan-betulinic acid against resistant *Leishmania major* and first clinical observation of such parasite in kidney. Sci Rep. 2018;8(1):11759.
22. Zadeh Mehrizi T, Khamesipour A, Ardestani MS, et al. Comparative analysis between four model nanoformulations of amphotericin B-chitosan, amphotericin B-dendrimer, betulinic acid-chitosan and betulinic acid-dendrimer for

treatment of *Leishmania major*: real-time PCR assay plus. Int J Nanomedicine. 2019;14:7593.
23. Meshnick SR. Artemisinin: mechanisms of action, resistance and toxicity. Int J Parasitol. 2002;32(13):1655-60.
24. Ghaffarifar F, Heydari FE, Dalimi A, Hassan ZM, Delavari M, Mikaeiloo H. Evaluation of apoptotic and antileishmanial activities of Artemisinin on promastigotes and BALB/C mice infected with *Leishmania major*. Iran J Parasitol. 2015;10(2):258.
25. Sen R, Bandyopadhyay S, Dutta A, Mandal G, Ganguly S, Saha P, Chatterjee M. Artemisinin triggers induction of cell-cycle arrest and apoptosis in *Leishmania donovani* promastigotes. J Med Microbiol. 2007;56(9):1213-8.
26. Want MY, Islammudin M, Chouhan G, Ozbak HA, Hemeg HA, Chattopadhyay AP, Afrin F. Nanoliposomal artemisinin for the treatment of murine visceral leishmaniasis. Int J Nanomedicine. 2017;12:2189-2204.
27. Want MY, Islamuddin M, Chouhan G, Dasgupta AK, Chattopadhyay AP, Afrin F. A new approach for the delivery of artemisinin: formulation, characterization, and ex-vivo antileishmanial studies. J Colloid Interface Sci. 2014;432:258-69.
28. Siddiqui EJ, Azad I, Khan AR, Khan T. Thiosemicarbazone complexes as versatile medicinal chemistry agents: a review. J Drug Deliv Ther. 2019;9(3):689-703.
29. Britta EA, Scariot DB, Falzirolli H, Ueda-Nakamura T, Silva CC, Borsali R, Nakamura CV. Cell death and ultrastructural alterations in *Leishmania amazonensis* caused by new compound 4-Nitrobenzaldehyde thiosemicarbazone derived from S-limonene. BMC Microbiol. 2014;14:236.
30. Kumar G, Singh D, Tali JA, Dheer D, Shankar R. Andrographolide: Chemical modification and its effect on biological activities. Bioorg Chem. 2020;95:103511.
31. Das S, Halder A, Mandal S, Mazumder MA, Bera T, Mukherjee A, Roy P. Andrographolide engineered gold nanoparticle to overcome drug resistant visceral leishmaniasis. Artif Cells Nanomed Biotechnol. 2018;46(sup1):751-62.
32. Roy P, Das S, Bera T, Mondol S, Mukherjee A. Andrographolide nanoparticles in leishmaniasis: characterization and *in vitro* evaluations. Int J Nanomedicine. 2010;5:1113.
33. Sinha J, Mukhopadhyay S, Das N, Basu MK. Targeting of liposomal andrographolide to *L. donovani*-infected macrophages in vivo. Drug Delivery. 2000;7(4):209-13.
34. Oghumu S, Varikuti S, Saljoughian N, et al. Pentalinonsterol, a constituent of pentalinon andrieuxii, possesses potent immunomodulatory activity and primes t cell immune responses. J Nat Prod. 2017;80(9):2515-23.
35. Gupta G, Peine KJ, Abdelhamid D, et al. A novel sterol isolated from a plant used by Mayan traditional healers is effective in treatment of visceral leishmaniasis caused by *Leishmania donovani*. ACS Infect Dis. 2015;1(10):497-506.
36. Seo DY, Lee SR, Heo JW, et al. Ursolic acid in health and disease. Korean J Physiol Pharmacol. 2018;22(3):235-48.
37. Jesus JA, Sousa IM, da Silva TN, et al. Preclinical assessment of ursolic acid loaded into nanostructured lipid carriers in experimental visceral leishmaniasis. Pharmaceutics. 2021;13(6):908.
38. Keil M, Härtle B, Guillaume A, Psiorz M. Production of amarogentin in root cultures of Swertia chirata. Planta Med. 2000;66(05):452-7.
39. Medda S, Mukhopadhyay S, Basu MK. Evaluation of the in-vivo activity and toxicity of amarogentin, an antileishmanial agent, in both liposomal and niosomal forms. J Antimicrob Chemother. 1999;44(6):791-4.
40. Cheraghipour K, Masoori L, Zivdari M, et al. A systematic appraisal of the use of carvacrol-rich plants to treat hydatid cysts. J Parasit Dis. 2022; 46(3):916-922.
41. Suntres ZE, Coccimiglio J, Alipour M. The bioactivity and toxicological actions of carvacrol. Crit Rev Food Sci Nutr. 2015;55(3):304-18.
42. Galvão JG, Santos RL, Silva AR, et al. Carvacrol loaded nanostructured lipid carriers as a promising parenteral formulation for leishmaniasis treatment. Eur J Pharm Sci. 2020;150:105335.
43. Rashid PT, Ahmed M, Rahaman MM, Muhit MA. 14-Deoxyandrographolide isolated from *Andrographis paniculata* (Burm. f) Nees growing in Bangladesh and its antimicrobial properties. Dhaka Univ J Pharm Sci. 2018;17(2):265-7.
44. Lala S, Nandy AK, Mahato SB, Basu MK. Delivery in vivo of 14-deoxy-11-oxoandrographolide, an antileishmanial agent,

by different drug carriers. Indian J Biochem Biophys. 2003;40(3):169-74.
45. Kheirandish F, Delfan B, Mahmoudvand H, et al. Antileishmanial, antioxidant, and cytotoxic activities of *Quercus infectoria* Olivier extract. Biomed Pharmacother. 2016;82:208-15.
46. Sarkar S, Mandal S, Sinha J, Mukhopadhyay S, Das N, Basu MK. Quercetin: critical evaluation as an antileishmanial agent *in vivo* in hamsters using different vesicular delivery modes. J Drug Target. 2002;10(8):573-8.
47. Sousa-Batista AJ, Poletto FS, Philipon CI, Guterres SS, Pohlmann AR, Rossi-Bergmann B. Lipid-core nanocapsules increase the oral efficacy of quercetin in cutaneous leishmaniasis. Parasitology. 2017;144(13):1769-74.
48. Almeida ER. Preclinical and clinical studies of lapachol and beta-lapachone. Open Nat Prod J. 2009;2(1):٤٢-٤٧ .
49. Boveris AL, Docampo RO, Turrens JF, Stoppani AO. Effect of β-lapachone on superoxide anion and hydrogen peroxide production in *Trypanosoma cruzi*. Biochem J. 1978;175(2):431-9.
50. Ramos-Milaré ÁC, Oyama J, Murase LS, et al. The anti-*Leishmania* potential of bioactive compounds derived from naphthoquinones and their possible applications. A systematic review of animal studies. Parasitol Res. 2022;121(5):1247-1280.
51. Moreno E, Schwartz J, Larrea E, et al. Assessment of β-lapachone loaded in lecithin-chitosan nanoparticles for the topical treatment of cutaneous leishmaniasis in *L. major* infected BALB/c mice. Nanomedicine. 2015;11(8):2003-12.
52. Dayawansa S, Umeno K, Takakura H, et al. Autonomic responses during inhalation of natural fragrance of "Cedrol" in humans. Auton Neurosci. 2003;108(1-2):79-86.
53. Kar N, Chakraborty S, De AK, Ghosh S, Bera T. Development and evaluation of a cedrol-loaded nanostructured lipid carrier system for in vitro and in vivo susceptibilities of wild and drug resistant *Leishmania donovani* amastigotes. Eur J Pharm Sci. 2017;104:196-211.
54. Zadeh Mehrizi T, Pirali Hamedani M, Ebrahimi Shahmabadi H, et al. Effective materials of medicinal plants for *Leishmania* treatment *in vivo* environment. J Med Plant. 2020;19(74):39-62.
55. Torres-Santos EC, Rodrigues Jr JM, Moreira DL, Kaplan MA, Rossi-Bergmann B. Improvement of in vitro and in vivo antileishmanial activities of 2', 6'-dihydroxy-4'-methoxychalcone by entrapment in poly (D, L-lactide) nanoparticles. Antimicrob Agents Chemother. 1999; 43(7):1776-8.
56. Pollier J, Goossens A. Oleanolic acid. Phytochemistry. 2012; 77:10-5.
57. Ghosh S, Kar N, Bera T. Oleanolic acid loaded poly lactic co-glycolic acid-vitamin E TPGS nanoparticles for the treatment of *Leishmania donovani* infected visceral leishmaniasis. Int J Biol Macromol. 2016; 93:961-70.