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Review Article

Experimental Methodologies for the Obtainment of *Momordica charantia* L. Extracts with Anthelminth Activity: A Review

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ABSTRACT
Currently, the therapeutic alternatives available for the treatment of helminthiases are restricted to a low number of synthetic drugs that are classically used on a large scale, thus causing the resistance of several parasites. Seeking to optimize this problem, and often because it is the only option in low-income populations, the use of medicinal plants against parasites is the subject of several studies. *Momordica charantia* L. is a plant species with notorious pharmacological activities described in the literature, among which the anthelmintic activity stands out, which is mainly associated with the presence of saponins, tannins, flavonoids and alkaloids. The optimization of extractive processes through the most suitable choice of solvents and also the best methodology helps directly in obtaining rich fractions of the compounds of interest, as well as in a better yield of the extract to be used in pharmaceutical forms. Techniques that employ speed in execution, and even those that result in longer contact time between drug: solvent are reported here, as well as the use of solvents ranging from water to the most nonpolar organic compounds. The designation of a specific marker for quality control purposes is also an important factor and, as much as the plant species have a variety of constituents, the selection of a standard such as quercetin has been observed as the most common in the use of techniques such as chromatography and mass spectrometry.
Keywords: Medicinal plants, Optimization extraction, Secondary metabolites, Worm infections, Gastrointestinal parasites, Fitoterapia.

INTRODUCTION

By 2020, at least 1.5 billion people were infected with helminthiasis, equivalent to 24% of the global population. The most affected areas are in East Asia, China, the Americas and Sub-Saharan Africa, illustrating that the possibility of infection by intestinal worms transcends economic barriers. Transmission occurs basically through the ingestion of eggs contained in poorly sanitized and/or undercooked vegetables, in contaminated water and also through contact with the infected soil. This last form of transmission is the main responsible for the infection of children and animals.[1]

Agriculture is still the main form of subsistence in several countries, from a nutritional and financial point of view. Small ruminants such as sheep and goats are the most used animals by small rural farmers and, depending on how the animals are raised (as in the case of free pasture), the susceptibility to acquiring parasites is also a worrying factor. Several intestinal parasites that affect them also come from the soil, and the control of the resulting infections is still quite restricted to the use of synthetic alternatives. These, in turn, bring some concerns ranging from acquisition costs, multidrug resistance, toxicity, and even traces of chemical substances postponed to lactiferous and carnivorous inputs to be commercialized.[2-4]

There is a parallel relationship between helminthic resistance in humans and animals, especially regarding mass therapy (aiming at short-term solutions), which is a common practice in low and/or middle-income countries, especially in children and young women. This situation does not take into account an exact previous diagnosis regarding the actual stage of infections, consequently ignoring the appropriate drug and dose for each clinical condition. Today, added together, there are no more than 10 drugs available for the treatment of intestinal helminthiasis in humans or animals, some commonly used in mass treatment campaigns that often culminate in anthelmintic resistance.[5]

Therapeutic alternatives such as medicinal plants have long been reported in folk medicine, being also studied for antiparasitic applications.[6] *Momordica charantia* L., is a plant species are popularly known as "melão-de-São-Caetano", in Brazil, or "melon bitter", and has several pharmacological activities discussed

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both *in vitro* and *in vivo*, including diabetes,[7] bacterial infections,[8] cancer,[9] antioxidant activity,[10] hyperlipidemia,[11] anthelmintic activity in animals,[2,12-14] and the ethnopharmacological discussion in humans.[15-18]

The secondary metabolites contained in *M. charantia*, as in any other plant, are effectively extracted and are successful in the pharmacological application when there is adequate knowledge about which methods and solvents are ideal. In this sense, this literature review seeks to present which constituents found in bitter melon are responsible for acting against helminths, and what are the options to obtain and identify them.

CHARACTERISTICS CLASSES OF SECONDARY METABOLITES FROM *MOMORDICA CHARANTIA* L.

In general, *melão de São-Caetano* is composed of carbohydrates, phenolic compounds, essential oils, alkaloids, flavonoids, quinines, amino acids, saponins, triterpenoids and glycosides.[19] The diverse biological activities of *M. charantia* are directly related to these secondary metabolites contained in its aerial parts and roots (Figure 1). Phytochemical analyzes are the initial screening to determine the classes of these substances and thus direct the use of extracts in biological assays.

From a more focal point of view, *Momordica charantia* L. concentrates in its seeds the presence of peptides, proteins, lipids, saponins and phenolic compounds. On the other hand, its fruits contain a more complex composition, with the presence of terpenoids, lipids, saponins, phenolic compounds and steroids. In turn, the leaves have terpenoids, alkaloids, saponins, cardiotonic glycosides, flavonoids and tannins, their flowers contain fatty acids while their roots can be rich in saponins.[20-22] The type of extraction, as well as the solvent used in this process, are decisive factors for obtaining and verifying these compounds in phytochemical assays.

There are several ways in which the secondary metabolites of a plant can act, exerting biological effects of medicinal interest. In the case of anthelmintic activity, studies reveal that the saponins contained in *M. charantia* act by irritating the mucous membranes of both the digestive and respiratory systems, with greater interference, therefore, in the absorption of nutrients by the worm, culminating in his death. Tannins seem to act in a similar way when they bind to free proteins in the gastrointestinal tract, and thus somehow inhibit the utilization of nutrients.[23] Other studies also point out that these constituents can decrease the survival capacity of newborn worms. The flavonoid class is associated with neuronal degeneration mechanisms and, together with triterpene glycosides, these metabolites generate paralysis by inhibiting helminth motility. Also active in the central nervous system, inhibiting the body movements of worms by inhibiting acetylcholinesterase. Furthermore, alkaloids are capable of interfering with nitrate-dependent homeostasis mechanisms (by decreasing it and providing less synthesis of mitochondrial proteins), impairing the development of individuals, which also culminates in death. In turn, anthracene glycosides are also reported for their ability to degenerate the membrane of helminth eggs, showing the potential of the plant even in the pre-parasitic stages.[24-27]

As an example, starting from aqueous and ethanolic extracts, both obtained by dynamic maceration of the plant's leaves, Mada *et al.*[28] identified the presence of saponins, steroids, tannins, glycosides, alkaloids and flavonoids, pointing out how a single part of the plant encompasses a complexity of compounds. By the same dynamic maceration process and using water as a solvent, Wadood *et al.*[29] observed that in the leaves of *Momordica charantia* L. there are tannins, however, the absence of flavonoids was observed. Time, a highly relevant variable in an extractive process, may have been the differentiating factor when comparing the two cited works. While the first authors used a total interval of 48 hr to obtain the extracts, Wadood *et al* used a shorter extraction process. Considering that flavonoids are best extracted in the presence of organic solvents (or at least in their combination with water), the use of water

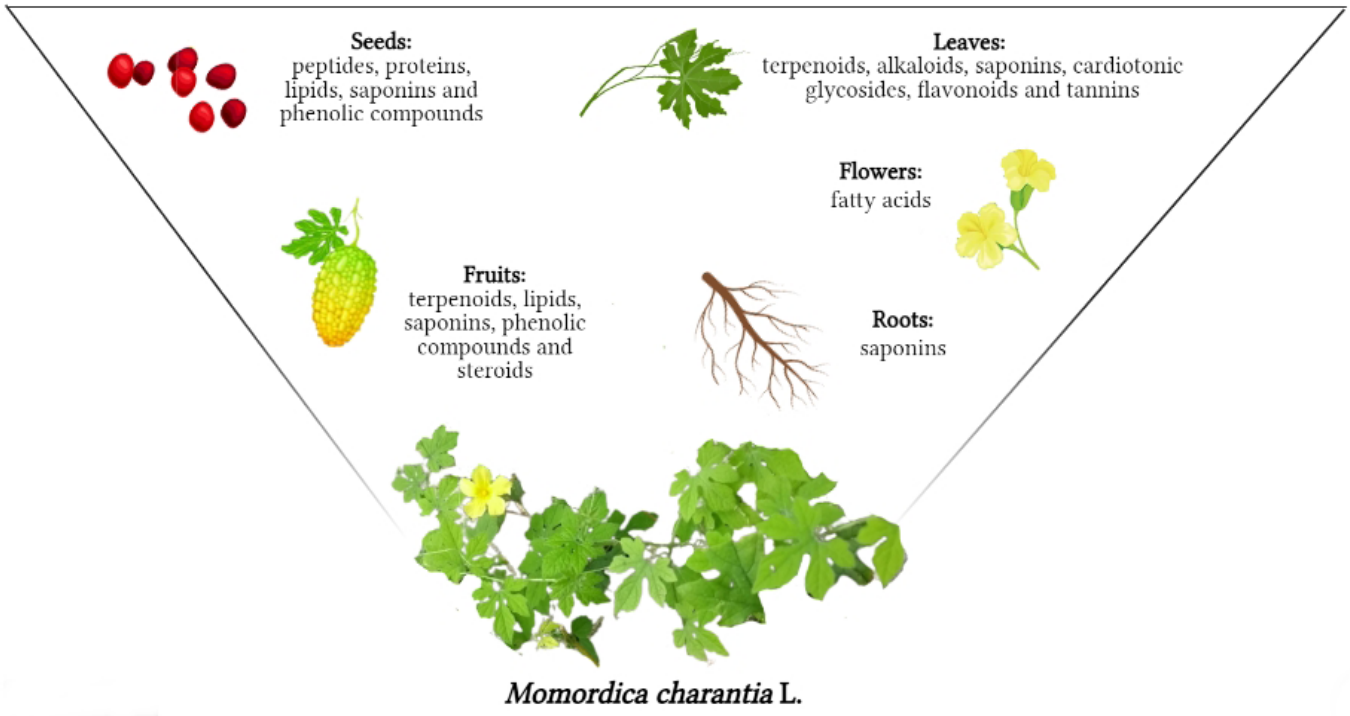

Figure 1: Class of Metabolites Contained in the Different Parts of *M. charantia* L.

only, in a short period of time, does not guarantee the obtainment of these compounds.[30]

Vinav *et al.*[27] they found that in the fruits of *M. charantia* it is possible to find alkaloids, glycosides, saponins and steroids, using extracting solvents such as methanol and water. However, the class of flavonoids and tannins were absent, even when a polarity gradient between solvents was applied, certainly, because phenolic compounds are susceptible to heat degradation, characteristic of the Soxhlet methodology used.[31,32]

In this sense, since they are molecules with different physicochemical characteristics, with different stability properties as well, the choice of extractive method and solvent (or a set of these) is a key factor in the extraction of metabolites of anthelmintic interest.

THE IMPACT OF THE CHOICE OF SOLVENT ON THE EXTRACTIVE PROCESS

Considering the anthelmintic importance of saponins, tannins, flavonoids and alkaloids, the solvent used must have the best physicochemical characteristics that provide greater yield in the extraction process of these substances. The choice of the ideal solvent should not only focus on one of the groups of metabolites but on the desired set and the largest amount of them. In addition, it is extremely important to take into account the impact that such a solvent can have on the environment, its cost, how difficult it will be to remove it, and its toxicity for the intended purpose of application.

Saponins are better extracted with more polar solvents, such as water, ethanol, methanol and hydroalcoholic solutions due to the amphiphilic character that such metabolites have. Despite this, they are difficult substances to be obtained by traditional methodologies and solvents due to the possibility of reaction between molecule groups (as is the case of acetate groups and the sugar chain) and the solvent, groups present in the aglycone portion, and polarity variation due to the size and structural differences in the sugar chain.[33]

In the case of tannins, as they are compounds that have a large number of hydroxyl groups, the extraction is ideal with mixtures composed of water and ethanol or sodium hydroxide.[34] This is due to the fact that, depending on the size, tannins can present different solubility profiles in the solvents, as well as the tannin extraction tends to decrease the pH of the medium, causing a condensation reaction between them, which generates high molecular weight precipitates that end up not being extracted, which is the reason why the medium needs to be preferably alkalized.[35]

Flavonoids, in turn, have a variety of possible solvents for their extraction, ranging from water, ethanol, methanol, acetone, and even aqueous mixtures of these organic solvents.[30] Traditionally, free flavonoids can be removed with ethanol and methanol at higher concentrations, while glycosylated flavonoids can be extracted with lower concentrations of these solvents, since the higher the concentration of water, the greater the solubilization of sugars.[36] Due to its structure composed of a central skeleton of 15 carbons and heterocyclic oxygen, different positions and amounts of groups (such as hydroxyl and carbonyl), in addition to the possibility of being linked to sugars, the choice of solvent for this class of metabolites will depend on the species profile and the extraction method used, as some flavonoids may be thermosensitive.

Alkaloids, since they have a nitrogen atom with unshared electrons, are basic in nature and can be found in free or salt form. In general, both forms are extractable with methanol or ethanol, however, in their salt form they can be better extracted using water (acidified or not at low concentrations of sulfuric or acetic acid, for instance), as salts have greater aqueous solubility. For alkaloids present in their free form, as they are substances of more lipophilic nature, the preferential extraction must be done with the use of organic solvents, such as chloroform, benzene, ether, among others.[37]

EXTRACTION METHODS FOR OBTAINING EXTRACTS WITH ANTHELMINTH ACTIVITY

To obtain pharmacologically active compounds from medicinal plants, extraction processes are used, which consist of contact between plant material and a solvent to transfer soluble and volatile solid constituents to it. Several factors can influence the extractive process: type of extraction, solvent, temperature, time, agitation, pressure, and degree of comminution of the plant drug. Regarding the ways to obtain an extract, these can be divided mainly based on the use of temperature: cold - maceration, percolation, and turbolysis; and hot - infusion, decoction, digestion, steam drag, Soxhlet, microwave extraction. Combined with the type of extraction chosen, the other aforementioned variables will determine the efficiency of the process, stability of the constituents, toxicity of the solvent (or combinations), and the overall cost according to the purpose for which it is intended.[38,39]

Table 1 briefly presents the most recent studies that correlate the anthelmintic activity of *Momordica charantia* L. and the extractive methods used.

Percolation, an exhaustive and low-cost methodology, is considered a good option when it involves the extraction of thermosensitive constituents, in addition, it is very suitable for obtaining very active metabolites in small amounts or poorly soluble metabolites.[48] Some studies used percolation to obtain extracts of *M. charantia* L. with anthelmintic action, as performed by Coêlho *et al.*[13] The study obtained extracts from the aerial parts of the plant, using 70% ethanol as solvent, performing the extraction process 3 times with the same plant material, in a fractional way, so in each of the extractions an initial fraction was separated and more than 4 or 5 final fractions. Finally, the initial fractions of the 3 extractions were mixed and concentrated in a rotary evaporator. The obtained extract had its activity tested *in vivo* in contaminated sheep and, in 5 days of treatment with a dose of 5mg/kg, promising antiparasitic activity results were obtained, as they induce a 79.5% reduction in helminth eggs per gram of animal faeces. The authors also hypothesize that the use of solvents with higher polarities could help in the isolation of some groups of more restricted active substances, promoting the maximization of this activity. In general, there is a correlation that extracts obtained by percolation in 70% ethanol are able to extract flavonoids, tannins and saponins, precisely markers of anthelmintic activity.[49-52]

Pereira *et al.*,[40] used the same extractive method, but using only the leaves of *M. charantia* L. and using 95% ethanol to obtain the crude extract and subfractions. The crude extract was concentrated in a rotary evaporator and dried by lyophilization. Part of the extract went through liquid-liquid partition using solvents of different polarities (hexane, dichloromethane, butanol and water) and after obtaining the subfractions, the solvents were evaporated. The anthelmintic activity of the crude extract and subfractions was evaluated *in vitro* using Fasciola hepatica miracidia. All extracts affected the embryonic development of the worm eggs, with the butanol subfraction being the most prominent, due to its additional action in inhibiting the hatching of eggs due to the flavonoid quercetin. Jaramillo, Trujillo and Rodriguez,[25] also carried out a percolation of *M. charantia* leaves, but with a solvent of greater polarity, methanol, thus making it possible to extract other active constituents such as alkaloids, triterpenes and anthracene glycosides. The methanolic extract inhibited the viability of sheep nematode eggs *in vitro*, due to the presence of secondary metabolites. Coincidentally, studies that used percolation evaluated the biological activity of the extract against eggs

Table 1: Anthelmintic Activity of *Momordica charantia* L. Based on Different Extracts

Reference	Year	Part of the plant	Solvent	Drug:solvent ratio	Extractive method	Species of worm	Kind of study
[13]	2020	Aerial parts	70% Ethanol	Miscellaneous	Percolation	*Intestinal nematode eggs (unspecified)*	*In vivo*
[40]	2016	Leaves	Ethanol Partitioned in distilled water, hexane, dichloromethane and butanol	Uninformed	Percolation	*Fasciola hepatica*	*In vitro*
[25]	2021	Leaves	98% methanol	Uninformed	Percolation	*Haemonchus sp.*	*In vitro*
[41]	2016	Green fruits	Distilled water	1:5 (g/mL)	Decoction	*Pheretima posthuma*	*In vitro*
[42]	2019	Fruit peel	Distilled water	3:10 (g/mL)	Decoction	*Pheretima posthuma*	*In vitro*
[12]	2010	Aerial parts	Ethanol	5:14 (g/mL)	Maceration	*Haemonchus sp. Trichostrongylus sp. Oesophagostum sp.*	*In vitro*
[43]	2011	Leaves	Ethanol	1:6 (g/mL)	Maceration	*Bunostomun, Haemonchus, Oesophagostomun, Trichostrongylus*	*In vivo*
[44]	2016	Leaves and fruits	Hexane, ethyl acetate, distilled water and methanol (sequential)	1:2 (v/v)	Maceration	*Haemonchus contortus*	*In vitro*
[45]	2015	Seeds	Petroleum ether Chloroform Ethanol Distilled water (sequential)	Uninformed	Maceration	*Pheretima posthuma*	*In vitro*
[46]	2015	Leaves	70% Ethanol	Uninformed	Maceration	*Ascarim suum*	*In vitro*
[27]	2016	Fruits	Methanol or water Sequential extraction in petroleum ether, chloroform, ethyl acetate and 50% ethanol	Uninformed	Soxhlet	*Eisenia foetida*	*In vitro*
[47]	2015	Leaves and fruits	Distilled water and methanol	Miscellaneous	Aqueous by decoction Dynamic Maceration + Maceration	*Haemonchus contortus*	*In vitro*

and larvae in embryonic stages, illustrating the potential activity of *M. charantia* in the early stages of helminth life.

Also presenting low cost and easy execution, the decoction is a widely used extractive technique, being indicated, for example, for rigid parts of the plant, where the use of temperature can be differential. The boiling of plant material together with water (solvent used), however, makes it a restricted methodology for obtaining substances with greater thermoresistance.[48] Rashid *et al*.[41] prepared by decoction, for 30 min, an extract of the fruits of *M. charantia* L. for subsequent synthesis of polyaniline-coated silver nanoparticles (AgNPs). The system (extract + AgNPs) showed strong *in vitro* anthelmintic activity against worms due to their synergistic activities. Following the same line, a decoction of the fruit peels was prepared by Shelar and Collaborators,[42] and the resulting aqueous extract was also conveyed in silver nanoparticles (AgNPs). The AgNPs in association with the extract showed remarkable anthelmintic activity when compared to the isolated extract, both causing complete death of the worms at doses of 50 mg/mL and 2 mg/mL, respectively, which may be associated with the improvement of the activity of saponins/glycosides.

Also using the decoction method, Akther and Collaborators,[47] obtained an aqueous extract of leaves and fruits at 1%, 5%, and 10% (mass of plant material/volume of solvent), which later had their effectiveness evaluated *in vitro* against adults Haemonchus contortus, noting that the proportion at 10% was the most effective against the worms and that the extracts from the fruits (80%) had better performance in killing the parasites than the extracts from the leaves (60%). These results can be related to secondary metabolites that can be extracted by water, such as saponins, glycosides, and some phenolic compounds.[28,47,53]

Maceration is one of the oldest and most used types of extraction, both in terms of the traditional use of medicinal plants and at an industrial level. This is due to the simplicity of the method and its advantages.[54] Considering the simplicity and economy of the process, maceration is also advantageous because it is carried out at room temperature, protecting thermosensitive substances and reducing costs. Despite this, it can take a long time for the ideal amount of extractable substances to be reached, and some care is required depending on the chosen solvent.[55]

Two different studies evaluated the activity of ethanolic macerates (absolute ethanol) against eggs, larvae and adult helminths of goats and found that even using the same solvent, the drug: solvent ratio can be decisive in obtaining significant pharmacological results. The study conducted by Gomes *et al*.[12] used macerate prepared over three days of aerial parts of *São Caetano* melon, in tests against eggs and larvae,

and the results showed that *São Caetano* melon extract at 25% and 50% reduced the number of viable eggs to 64.53% and 62.86%, while the positive control, 5% albendazole, reduced to 84.21% within the three-day incubation period. Regarding larvicidal activity, the extract showed a statistically similar result to the positive control. The study indicates that triterpene glycosides (saponin class) present in the plant, such as momordicin I and momordicin II, in addition to tannins, are responsible for such actions since they are extractable by ethanol. Brito-Junior *et al*.,[43] in turn, using the leaves and in a smaller proportion than the previous study, based on *in vivo* studies in goats, found that when compared to the negative control (distilled water), there was a little significant decrease in the number of eggs per gram of faeces.

Another study evaluated the activity of *M. charantia* against helminths of goats through successive macerations, with different solvents of increasing polarity (hexane, ethyl acetate, methanol and water), of leaves or fruits and maceration time between each solvent of 24 hr.[44] The tests were carried out *in vitro* models with the powder obtained by lyophilizing the aqueous extract at 20 mg/mL of the leaves or fruits, the latter showing better results after 72 hr of testing on infective lavas of *H. contortus*. The authors attribute the activity to the presence of tannins, saponins and alkaloids. As a polar solvent, water has the ability to extract tannins and saponins in greater amounts, while flavonoids and alkaloids in smaller amounts, due to the factors mentioned in the previous section.

Melon seeds were also subjected to maceration in the work of Vedamurthy *et al*.[45] The method used different solvents with increasing polarity (petroleum ether, chloroform, ethanol and distilled water) in successive 7-day macerations against *Pheretima posthuma*. The results showed that all extracts had better results than the positive control (Albendazole) for the time of paralysis and time of death in adult worms. Chloroform was the most effective extractive solvent, precisely because it is an ideal solvent for obtaining substances of the alkaloid class.

Aiming to test the anthelmintic activity of *M. charantia*, a study carried out in 2015 compared the effect of macerating the leaves of the plant (obtained in 70% ethanol for 4 days) *versus* pyrantel pamoate, against *Ascaris suum*, a species worm very similar to the one that affects humans. The extracts in 40 and 80% were effective in the generation of paralysis of adult worms (but with less intensity compared to the positive control), this being associated with the presence of saponins, tannins, flavonoids and triterpene glycosides.[46]

Completing the compilation of extractive techniques, Soxhlet extraction, which is also an exhaustive process in relation to the raw material,[56] is advantageous because it can extract large amounts of substances using a specific volume of solvent, not requiring further filtration and can be coupled in a series system. Despite this, it makes it impossible to extract thermosensitive compounds and requires a high amount of solvent, which requires a subsequent concentration step, and requires considerable time for extraction.[57]

Gandhi, Vadalia and Behzad,[27] used the Soxhlet apparatus using *São-Caetano* melon fruit powder with water and methanol (separately) and then carried out a scheme of successive extractions with solvents with increasing polarity (petroleum ether, chloroform, ethyl acetate, methanol, and 50% hydroalcoholic solution. They evaluated the anthelmintic activity against *Eisenia foetida* and found that the best extracts were those of methanol and water (both without participating in the successive extraction), with phytochemical analysis indicating the presence of saponins, a very relevant marker for anthelmintic activity,[58] and extractable in these solvents due to their high polarity. Among those tested in the successive extraction, chloroform was the best solvent, evidencing the presence of alkaloids, probably in its molecular and free form, which also justifies its relevant anthelmintic activity. In terms of comparison between non-exhaustive and cold techniques, it is important to emphasize that the Soxhlet method can have a higher yield than non-exhaustive techniques, since the solvent is able to continuously leach substances from the matrix, even with the total volume remaining the same.

MARKERS AND IDENTIFICATION

Much is known about the complexity of existing compounds in *Momordica charantia* and how the selection of a suitable extractive method as well as the solvent (or combinations) can provide specific substances for different purposes. Among some of the compounds that can be identified in isolation, there are within the saponin class of this plant, steroidal glycosides such as charantin; triterpene glycosides such as momordicins, momordina, momordicosides, karavilagenin, karavilosideos, kuguacinas, goyasaponins (in addition to residues from the enzymatic breakdown of saponins themselves, such as diosgenin); proteins such as momorcharins; sterols such as momordenol; triterpenoids such as momordicillin, momordicinins, curcubitans, cycloartenols, charantosides; carotenoids such as cryptoxanthin; terpenes such as cucurbitacins; flavonoids like quercetin, apigenin, luteolin and phenolic compounds like gallic acid.[19,28,29,40,58]

The identification of markers can be a key piece when looking for the use of plant derivatives in a more targeted way in the treatment of pathological and related manifestations. Analytically speaking, for this purpose, the techniques of High-Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC) are normally used, combined with Mass Spectrometry (MS), for example. In addition, Hydrogen (NMR^{1}H) Carbon (NMR^{13}C) and Infrared (IR) Nuclear Magnetic Resonance have been considered auxiliary methodologies of great relevance.[59-61]

Findings from the NMR^{1}H spectrum performed on an ethanolic extract isolate 95.4°GL from the leaves of *M. charantia* by Guarniz *et al*.[62] show the presence of signals that correspond to the distribution of hydrogens in the chemical structure of the marker momordicin II (a saponin), which are the hydrogens of five methyl groups attached to quaternary carbons, two hydrogens of the methyl groups that are attached to an olefinic carbon, three hydrogens attached to carbons with hydroxyls, and one hydrogen of a formyl group (Figure 2).

Furthermore, in this same study,[62] it was also evaluated the carbon distribution profile by NMR^{13}C, in which it was possible to observe six quaternary carbons, ten methyl carbons, one aldehyde carbon, seven methylene carbons and carbons referring to the structure of the β-glucopyranosyl group, present in the structure of momordicin II. The flavonoid quercetin was identified by Pereira *et al*.[40] in the 95% ethanol extract through mass spectrometry combined with QIT and TOF

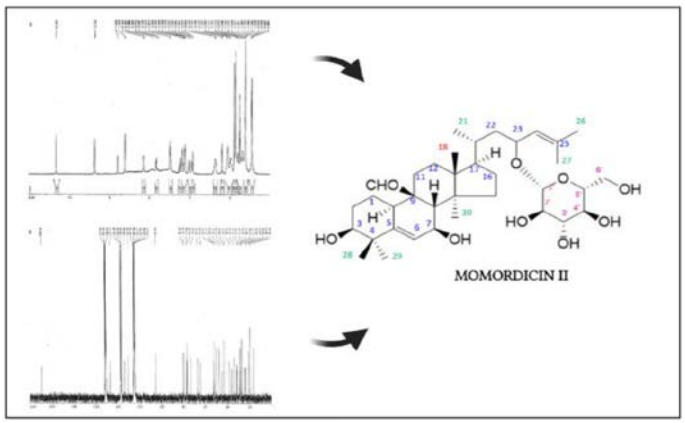

Figure 2: Identification of Momordicin II, Based on NMR^{1}H and NMR^{13}C.[62]

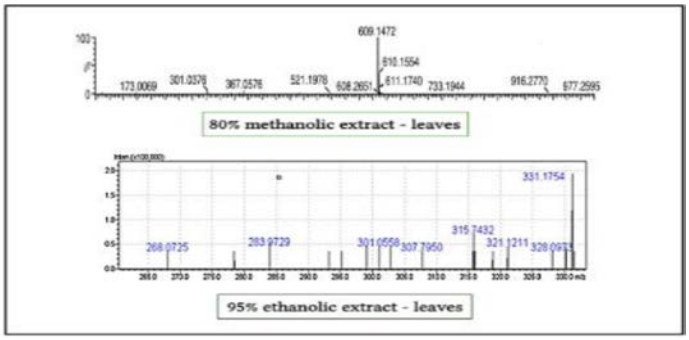

Figure 3: Mass Spectrum in the Negative Mode Showing that Both 80% and 95% Ethanol Extracts are Able to Extract Quercetin.[40,64]

Table 2: Identification of Some Markers from *M. charantia* by Mass Spectrometry.

Marker	Mass-to-charge ratio (m/z)	Reference
Quercetin	301.0558	[40,64]
Isoharmetin-3-O-glucoside	316.045	[63,64]
Kaempferol-3-O-rutinoside	285.025	[63,64]
Kaempferol-3-O-rutinoside	285.40	[63,64]
Charantina	204.09 (base peak); 395.34 (sterol fragment); 451.10 (glycosidic fragment)	[65]
β-sitosterol	575.19	[66]

technology, in the ESI ionization mode. After elucidating the presence of flavonoids in the extract by HPLC, the mass spectrum in negative mode revealed the presence of quercetin in the ion *m/z* 301.0558 (Figure 3; Table 2). Quercetin isomers were also identified by Madala *et al*.[63] in the methanolic extract of 80% of *M. charantia* leaves through base ion peak (BIP) UHPLC-qTOF-MS chromatograms. Such isomers were quercetin-3,7-O-diglucopyranoside, quercetin-3-O-sambubioside, quercetin-3-O-rutinoside, quercetin-3-O-arabnoside-rhamnopyranoside and quercetin-3,7-di-rhamnopyranoside, which appear at *m/z* 300.021/301.029. In addition to these, the isorhamnetin-3-O-glucoside isomer and the kaempferol-3-O-rutinoside and kaempferol-3-O-glucoside isomers were elucidated at *m/z* 316,045 and 285,025/285,040, respectively.

Svobodova *et al*.[64] also identified isomers of quercetin, kaempferol and isorhamnetin in 80% ethanol extract of aerial parts of *M. charantia* through the association of UPLC and MS. Quercetin derivatives appear at λ_{max} 354 nm and ionic fragment *m/z* 301. Kaempferol derivatives at λ_{max} 348 nm and *m/z* 285. While isorhamnetin derivatives show λ_{max} around 356 and *m/z* 317 (Table 2).

In another study carried out in 2019, which sought to identify the metabolite charantine (another saponin) in the alcoholic extract of the aerial parts of *M. charantia* through gas chromatography combined with mass spectrometry, a retention time of 73.84 min was observed. The mass spectrum of the retained compound at 73.84 min revealed the charantine through a base peak at *m/z* 204.09, a sterol fragment peak at *m/z* 395.34 and a glycoside fragment peak at *m/z* 451.10, in addition to ions belonging to the carbohydrate moiety, at *m/z* 147, 204, 217, 305 and 361. Peaks referring to the estradiol moiety were also identified at *m/z* 129 and 155.[65] The molecular peak was *m/z* 861.43. The charantine was also identified in the ethanolic extract of the fruits of *M. charantia* through the HPLC technique.[66] In this research, the ethanol extract was washed with potassium hydroxide, diluted in water and extracted with diethyl ether. The ether extract, in turn, after treatment with hydrochloric acid, provided a precipitate rich in the charantine metabolite, which was purified by HPLC and characterized from NMR and mass spectroscopy. Charantine appears like a mixture of stigmasterol glycoside (STG) and β-sitosterol glucoside (BSG) on HPLC, with retention times of 10.691 min and 11.977 min, respectively. The mass spectrum reveals BSG with M^+-H ions at 575.19 (*m/z*) and M^+-glucose-2H at 397.22 (*m/z*), while STG could not be obtained in pure form and, therefore, spectral studies could not be properly applied.[66]

CONCLUSION

Several studies indicate that both *in vitro* and *in vivo*, there is a potential anthelmintic activity related to secondary metabolites of *Momordica charantia*. Such compounds have proven action from the early stages of the worms' life to their adult forms, making this plant species a therapeutic option in places where there is no access to traditional synthetic drugs and as an alternative in environments where helminthic resistance is a reality (either in humans or in the veterinary field). It is known that tannins, saponins, alkaloids and flavonoids are the main markers related to anthelmintic activity and, in common, basically all of them can be extracted in solvents such as ethanol, methanol or hydroalcoholic mixtures, which leads to the use of one or at least few solvents capable of obtaining them in a single process. In addition, the extractive techniques of maceration (low-cost methodology) or percolation (exhaustive method without the use of heat), among the observed studies, seem to be the ones that best provide compounds related to anthelmintic activity, especially when there is the use of ethanol as solvent and aerial parts of *M. charantia*. As for the proof that extract samples can actually fulfil their objective against helminths, there are several options of markers to be analyzed, however, it is observed that quercetin is a standard of easy acquisition and identification through techniques such as chromatography and mass spectrometry, gaining notable space among the studies.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

AgNPs: Silver Nanoparticles; **HPLC:** High-Performance Liquid Chromatography; **GS:** Gas Chromatography; **MS:** Mass Spectrometry; **NMR:** Nuclear Magnetic Resonance; **NMRC:** Carbon Nuclear Magnetic Resonance; **NMRH:** Hidrogen Nuclear Magnetic Resonance; **QIT:** HYPERLINK "http://www.chm.bris.ac.uk/ms/qit.xhtml" Quadrupole Ion Trap; **ESI:** Electrospray ionization; **TOF:** Time of Flight; **STG:** Stigmasterol Glycoside; **BSG:** β-Sitosterol Glucoside; **BIP:** Base Ion Peak; **IR:** Infrared; **UHPLC:** Ultra-high Pressure Liquid Chromatography; **UPLC:** Ultra Performance Liquid Chromatography; **CNPq:** National Council for Scientificand Technological Development; **FACEPE:** Fundação de Amparo a Ciência e Tecnologia do Estado de Pernambuco.

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