

Title:

Plants with promising antileishmanial activity in Colombia: a systematic review and meta-analysis

Short title:

Antileishmanial Plants in Colombia: A Systematic Review

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Abstract

Introduction. Leishmaniasis remains a major public health challenge in Colombia, driven by high incidence, *Leishmania* species diversity, and drug resistance. Colombian medicinal plants, rooted in rich ethnobotanical traditions and supported by the country's exceptional biodiversity, represent a promising yet underexplored resource for the development of novel antileishmanial therapies.

Aim of the study. To systematically assess Colombian medicinal plants with reported *in vitro* antileishmanial activity, to estimate pooled IC₅₀ values through meta-analysis, and to identify extracts with favorable selectivity indices (SI) as potential antileishmanial candidates.

Materials and methods. A systematic search (2000–April 2025) of PubMed, EMBASE, and LILACS identified *in vitro* studies reporting IC₅₀ values of Colombian plant extracts against *Leishmania* spp. A random-effects meta-analysis was used to estimate pooled IC₅₀ values. Risk of bias was assessed using a modified QUIN tool. Subgroup analyses explored methodological and biological factors, such as plant part, extraction solvent, and taxonomic family.

Results. Twenty-five studies were included, covering 110 records on 24 plant species. Thirteen studies with complete IC₅₀ ± SD data were eligible for meta-analysis. The pooled mean IC₅₀ was 41.25 µg/mL (95% CI: 37.95–44.55), with substantial heterogeneity (I² = 100%). Lower IC₅₀ values were associated with bark/wood extracts, methanol or dichloromethane solvents, and species from the Chrysobalanaceae and Bignoniaceae families. *Xylopia discreta* (Annonaceae) showed the highest SI (up to 110). Additional species from the Lamiaceae, Lauraceae, Picramniaceae, and Rutaceae families also demonstrated favorable SI values. However, methodological variability limited the ability to make direct comparisons across studies.

Conclusion. Several Colombian plant species showed promising *in vitro* antileishmanial activity, with selected extracts combining high potency and notably high selectivity. The

inclusion of SI analysis in this review provides a more meaningful assessment of therapeutic potential beyond IC₅₀ values alone. These findings underscore the value of Colombia's plant biodiversity as a source of candidate compounds for antileishmanial drug development. However, standardized *in vitro* protocols, consistent cytotoxicity evaluation, and *in vivo* validation remain essential to ensure comparability and guide the selection and advancement of the most promising extracts toward therapeutic application.

Introduction

Leishmaniasis is a neglected tropical disease caused by protozoan parasites of the genus *Leishmania*, transmitted to humans through the bite of infected female *Phlebotomine* sandflies (*Phlebotomus* spp. in the Old World and *Lutzomyia* spp. in the New World) [1]. This vector-borne disease presents a wide clinical spectrum, with three main manifestations: cutaneous leishmaniasis (CL), the most prevalent form, characterized by ulcerative skin lesions that may result in disfiguring scars; mucocutaneous leishmaniasis (MCL), a severe and potentially mutilating progression of CL that affects the mucous membranes of the nasopharyngeal region; and visceral leishmaniasis (VL), the most severe form, which involves internal organs such as the spleen, liver, and bone marrow, leading to systemic complications and high mortality if left untreated [2, 3].

In Colombia, nine *Leishmania* species have been historically reported: *L. panamensis*, *L. braziliensis*, *L. guyanensis*, *L. equatoriensis*, *L. lainsoni*, *L. colombiensis*, *L. mexicana*, *L. amazonensis*, and *L. infantum*. All except *L. infantum*, which is associated with VL, have been linked to CL. Furthermore, *L. panamensis*, *L. braziliensis*, and *L. guyanensis* have also been associated with MCL [4]. In addition, *L. naiffi* and *L. lindenbergi* were identified more recently in three Colombian soldiers, representing the first reports of these species in the country [5]. Compared to 2023, an overall increase of 25.5% in reported cases of leishmaniasis in Colombia was observed in 2024, from 5,486 to 6,886 notifications [6, 7]. This increase was mainly driven by a 26.2% increase in CL, which continues to represent the most frequent clinical manifestation. In addition, a moderate increase of 6.6% was observed in cases of MCL,

while cases of VL decreased by 15% [7]. The persistent epidemiological burden of leishmaniasis in Colombia, driven by its endemicity, high incidence rates, broad geographical distribution, and the diversity of *Leishmania* species, highlights the urgent need for improved disease management

Conventional treatment strategies for leishmaniasis in Colombia rely primarily on the use of pentavalent antimonials, amphotericin B, miltefosine, and other pharmacological agents [8]. Despite their widespread use, these therapies are associated with significant limitations, including severe toxicity, high cost, complex administration protocols, and, most critically, the increasing emergence of drug-resistant *Leishmania* strains. A recent systematic review addressing treatment failure and clinical relapse in leishmaniasis identified Latin America as one of the regions with the highest number of cases, with Brazil, Colombia, and French Guiana reporting the highest rates of relapse [9]. The study also highlighted the impact of treatment failure across clinical forms of the disease, reporting failure or relapse rates of 47.6% for CL and 45.2% for VL. These findings underscore the urgent need for novel therapeutic strategies capable of overcoming the challenges posed by drug-resistant *Leishmania* strains.

Within the repertoire of pharmacological approaches, increasing attention is being paid to the reuse of existing drugs and the identification of new compounds with anti-leishmanial activity. In this context, natural products have emerged as a particularly promising area of exploration. Medicinal plants offer a rich source of bioactive molecules that may lead to the development of safer, more effective, and affordable therapeutic options, addressing key gaps in current pharmacological interventions.

Colombia's exceptional biodiversity presents a valuable opportunity for bioprospecting efforts. As one of the most biodiverse countries in the world, Colombia hosts a wide range of plant species, many of which produce secondary metabolites with antiparasitic activity [10]. This phytochemical diversity constitutes a largely untapped resource for the discovery of new leishmanicidal compounds. Recent pharmacological and ethnobotanical studies have

identified several species with activity against *Leishmania* spp. [11], further supporting the need for a systematic evaluation of the available evidence.

This systematic review and meta-analysis aims to comprehensively analyze research conducted between January 1, 2000, and April 30, 2025, on Colombian medicinal plants with documented antileishmanial activity. By synthesizing the available evidence, the study aims to identify candidate species and their bioactive constituents with potential for the development of safer and more effective treatments for leishmaniasis. Additionally, it aims to identify current knowledge gaps and propose future research priorities to support the advancement of these natural products toward clinical application.

Methodology

Reporting System and Registration

This systematic review and meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [12]. The study protocol was prospectively registered in the International Prospective Register of Systematic Reviews (PROSPERO) under the registration number CRD420251076470, accessible at <https://www.crd.york.ac.uk/PROSPERO/view/CRD420251076470>.

Data Sources

A comprehensive literature search was conducted in the PubMed, EMBASE, and LILACS databases. These platforms were selected for their complementary coverage of biomedical, pharmacological, and regional scientific literature. The search was restricted to articles published between January 1, 2000, and April 30, 2025, in English, Spanish, or Portuguese.

Search Strategy

Search queries combined Medical Subject Headings (MeSH) and free-text terms, linked with Boolean operators (“AND” and “OR”) to optimize sensitivity and specificity. The full search strategies for each database are provided in the Supporting information (S1 Table). An

information specialist contributed to the development and refinement of search terms. We followed the PRISMA 2020 checklist for reporting (S2 Table).

Eligibility criteria

Studies were included if they (i) reported *in vitro* leishmanicidal activity of Colombian plant materials, (ii) provided IC₅₀ values for antiparasitic activity, (iii) were published as full-text articles, and (iv) were written in English, Spanish, or Portuguese. Studies were excluded if they (i) lacked IC₅₀ data, (ii) did not clearly confirm the Colombian origin of the plant material or failed to cite a primary source verifying it, (iii) were not available in full-text format, (iv) consisted of publication types such as conference abstracts, reviews, letters to the editor, posters, infographics, or any other format lacking complete data, (v) relied exclusively on *in silico* approaches, or (vi) evaluated only synthetic derivatives.

Study Selection and Quality Assessment

Study selection was conducted by three independent reviewers who screened titles, abstracts, and full texts according to the predefined eligibility criteria. Discrepancies were resolved by consensus. For quality assessment, a modified version of the quality assessment tool for *in vitro* Studies (QUIN tool) [13] was applied. Each study was evaluated based on specific methodological components, scoring 2 points for clearly and adequately reported elements, 1 point for insufficiently specified ones, and 0 points for those not reported. The total score was expressed as a percentage of the maximum possible, and studies were classified into three quality levels: poor (<50%), moderate (50–75%), or high (>75%).

Statistical analysis

To estimate the pooled inhibitory effect of plant extracts and isolated compounds against *Leishmania* spp., a meta-analysis was conducted by calculating the mean IC₅₀ values and their corresponding 95% confidence intervals (CIs) from the included studies. Given the anticipated heterogeneity across studies, a random-effects model was applied. Forest plots were generated to visualize both individual and pooled IC₅₀ estimates along with their CIs.

Between-study heterogeneity was assessed using Cochran's Q test (with $p < 0.1$ indicating significant heterogeneity) and the I^2 statistic, interpreted as low (25%–49%), moderate (50%–74%), or high ($\geq 75\%$). Potential publication bias was evaluated using funnel plots and Egger's test. All statistical analyses were performed using Stata 16.0 software (StataCorp, College Station, TX, USA).

Results

Study selection and data classification

A total of 115 articles were initially retrieved through database searches. After eliminating 27 duplicates, the titles and abstracts of 88 studies were examined. Of these, 55 were excluded because they were reviews, did not refer to plant species collected in Colombia, or did not focus on antileishmanial biological activity. The remaining 33 articles were evaluated in full text. Eight additional studies were excluded due to lack of access to the full text, inconsistent data, use of non-Colombian plant material, exclusive application of *in silico* models, or for focusing only on synthetic derivatives. Finally, 25 studies met the inclusion criteria and were retained for analysis (Figure 1).

Figure 1. Flowchart describing the study design process.

Although this review aimed to evaluate antileishmanial efficacy based on IC_{50} values, the included studies varied considerably in terms of data quality and completeness. Thirteen studies met the criteria for inclusion in the meta-analysis by reporting IC_{50} values along with measures of variability, such as standard deviations and the number of replicates. A second group of studies reported IC_{50} values but were excluded from the meta-analysis because the data were either outside the quantifiable range or lacked essential statistical parameters. Finally, a third group of studies did not provide quantitative IC_{50} values but presented qualitative assessments of leishmanicidal activity, classifying it as "low," "moderate," or "high," or describing it narratively. While these limitations precluded meta-analysis and the

construction of forest plots for the latter two groups, the data still contribute valuable insights for identifying promising species, informing future research priorities, and avoiding duplication of efforts. To enhance the comparability and utility of future studies, we recommend the standardized reporting of efficacy metrics alongside appropriate measures of variability.

Risk of bias assessment

To assess methodological quality and reporting standards, the included studies were evaluated using a modified QUIN tool for *in vitro* research (S3 Table). The results are summarized in Figure 2. Most of the included studies demonstrated adequate methodological clarity across most assessed domains, particularly in the presentation of results, outcome measurement methods, and the overall description of the experimental procedures. However, the domain with the most frequent shortcomings was statistical analysis. A substantial proportion of studies (about 20%) lacked sufficient detail, and nearly 30% did not specify the statistical procedures at all. This indicates that, although quantitative data were available, many studies provided limited detail on how variability, statistical significance, or effect sizes were assessed, potentially compromising the reproducibility and comparability of their findings. Additional methodological limitations were noted in the reporting of sampling techniques and the clarity of study aims or objectives. These elements were inadequately described or omitted in 25% and 20% of the studies, respectively. Incomplete reporting of sampling strategies is particularly relevant in plant-based studies, where ecological variability and harvesting protocols can influence phytochemical content and, consequently, bioactivity outcomes. In contrast, over 90% of the studies clearly described the comparison groups and methodological procedures, suggesting a generally robust experimental design and implementation, despite shortcomings in other reporting areas.

Figure 2. Methodological quality across six key domains in the included studies.

The y-axis lists the evaluated methodological domains. The x-axis represents the percentage of studies assessed. Bars are stacked and divided into three colors indicating the level of reporting: Blue = adequately reported, yellow = partially reported, and black = not

reported. Each bar sums to 100% and shows the proportion of studies falling into each reporting category per domain.

These findings highlight the need for greater adherence to standardized reporting guidelines in *in vitro* pharmacological research, to improve clarity, reproducibility, and quality assessment of preclinical evidence derived from natural products.

Characterization of bioactive extracts and experimental models

From the 13 studies included in the meta-analysis, 110 records were extracted corresponding to different extracts, fractions or metabolites from 24 plant species collected in Colombia and evaluated *in vitro* against various species of *Leishmania*. The most frequently studied species was *L. panamensis* (64/110), followed by *L. braziliensis* (25/110), *L. major* (9/110), *L. donovani* (4/110), *L. amazonensis* (4/110) and *L. guyanensis* (4/110). The promastigote stage was the most commonly used (68/110) (Table 1 & 2).

196 **Table 1. Summary of plant species, parasite strains, and reported antileishmanial activity in studies included in the meta-analysis**

197 IC₅₀ refers to the concentration required to inhibit 50% of parasite viability. In cases marked with an asterisk (*), inhibition was measured using EC₅₀. The
198 selectivity index (SI), calculated as the ratio of cytotoxic concentration to effective antiparasitic concentration, provides an estimate of therapeutic potential. SI
199 values greater than 10 are shown in bold within shaded cells, as this threshold is commonly considered a minimum criterion for preclinical evaluation, indicating
200 reduced toxicity to mammalian cells. NA, not applicable; ND, not determined; A, amastigote; P, promastigote. Superscript letters denote experimental conditions
201 as follows: a: two independent trials with triplicates; b: triplicate; c: five independent experiments; d: three independent trials with triplicates; e: duplicate; f: three
202 independent trials with duplicates; g: two independent trials with duplicates.

	Plant			Preparation				Leishmania			Antileishmanial assay			
Ref.	Botanic family	Botanic specie	Part	Extraction technique	Fractions technique	Extract	Fraction	Specie	Stage	Stain	Range (µg/mL)	IC50 (µg/mL)±SD	SI	Rep.
5	Araliaceae	Oreopanax incisus	Leaf	Solvent	NA	Dichloromethane	NA	L. panamensis	A	M/HOM/CO/87/UA-140-epir-GFP	1.78-100	24.6±1.1*	2.00	a
						Ethyl acetate						23.7±1*	2.20	
						Hexane						87.4±7.6*	>2.30	
	Chrysobalanaceae	Moquilea salicifolia				Ethyl acetate						9.8±1.2*	>20.40	
	Lauraceae	Persea ferruginea				Dichloromethane						48±7.3*	>4.20	
						Ethyl acetate						25.5±2.6*	>7.80	
	Rubiaceae	Palicourea winkleri				Dichloromethane						21.5±2.79*	3.57	
						Ethanol						29.4±0.5*	>6.81	
						Ethyl acetate						14.1±0.47*	7.75	
						Hexane						84.8±2.5*	0.14	
	28	Lauraceae				Ocotea macrophylla						Leaf	Solvent	
P			9.7±1*	10.36										
L. panamensis			A	M/HOM/CO/87/UA-140	12.67±0.11*		7.93							
			P		100.64±2.96*		1.00							
Rutaceae		Zanthoxylum schreberi	Bark/Wood	F1 - Alkaloid	L. major	A	Friedlin V1	200-450	30.135±0.685*	10.50				
						P			77.04±3.72*	4.11				
					L. panamensis	A	M/HOM/CO/87/UA-140		46.23±1.05*	6.85				
						P			61.43±3.05*	5.15				
				NA	L. major	A	Friedlin V1	12.5-150	24.95±3.15*	2.86				
						P			28.99±3.17*	2.46				
					L. panamensis	A	M/HOM/CO/87/UA-140		6.16±0.115*	11.60				
						P			17.06±1495*	4.19				
29	Bignoniaceae	Handroanthus chrysanthus	Bark/Wood	Solvent	NA	Ethanol	NA	L. braziliensis	A	M/HOM/CO/88/UA-301-EGFP	1.56-100	17.7±1.4*	3.40	b
							F1 - Methanol					7.9±0.3*	1.70	

					Membrane filtration		F2 - Methanol					11.1±1.4*	18.90	
							F3 - Methanol					6.7±1.7*	1.20	
							F4 - Methanol					3.3±0.3*	2.40	
							F5 - Methanol					23.3±4.2*	<2.57	
30	Sapindaceae	Sapindus saponaria	Fruit	Solvent	NA	Hexane-Chloroform-Methanol	NA	L. panamensis	A	M/HOM/CO/87/UA-140	0.625-10	14.5±0.8*	1.80	a
				Solid phase extraction		Chloroform-Methanol, isolation Hederagenin-3-O-(3,4-O-diacetyl-β-D-xylopiranosyl-(1-->3)-α-L-rhamnopyranosyl-(1-->2)-α-L-arabinopyranoside	L. amazonensis	P	IFLA/BR/75/PH8	23.4±0.007		ND		
			L. braziliensis				M/HOM/BR/75/M2903		32±0.14	ND				
			L. donovani				M/HOM/BR/74/PP75		35.35±3.88	ND				
			L. amazonensis				IFLA/BR/75/PH8		23±0.56	ND				
			L. braziliensis				M/HOM/BR/75/M2903	32±0.21	ND					
			L. donovani				M/HOM/BR/74/PP75	68.3±0.49	ND					
			L. panamensis				A	M/HOM/CO/87/UA-140	2.5±0.3*	0.50				
			L. amazonensis				P	IFLA/BR/75/PH8	68.4±0.35	ND				
			L. braziliensis					M/HOM/BR/75/M2903	32.4±0.28	ND				
			L. donovani					M/HOM/BR/74/PP75	68.6±0.14	ND				
			L. panamensis					A	M/HOM/CO/87/UA-140	2.7±0.6*		0.80		
			L. amazonensis				P	IFLA/BR/75/PH8	23.2±0.28	ND				
			L. braziliensis					M/HOM/BR/75/M2903	28.1±0.42	ND				
			L. donovani					M/HOM/BR/74/PP75	66.3±1.34	ND				
			L. panamensis					A	M/HOM/CO/87/UA-140	2.1±0.2*		1.80		
31	Rutaceae	Raputiarana heptaphylla	Bark/Wood				Solvent	Solid phase extraction	Ethanol	NA	L. panamensis	P	M/HOM/CO/88/CO/UA-140-Ir-GFP	0-0.118
32	Annonaceae	Xylopia discreta	Leaf	Solvent	NA	Aqueous	NA	L. panamensis	P	M/HOM/CO/87/UA-140	3.13-100	9.68±5*	32.60	d
			Ethanol			25±9.7*						>40.00		
			Ether			48.7±12*						8.00		
			Ethyl acetate			10.6±7.3*						7.40		
			Methanol			50±13*						3.82		
						30.2±8.9*						7.40		
					50±19*	2.32								
					37.5±14*	22.87								
					9.23±1.2*	64.80								
					6.25±1.1*	25.60								
					6.35±4*	110.00								
					46.1±6*	4.70								
			Seeds	NA	Chloroform	NA						3.68±0.98*	11.60	
				Ethanol									6.25±2.6*	5.66
33	Solanaceae		Flower	Solvent	NA	Ethanol	NA	L. panamensis	P	M/HOM/CO/87/UA-140	3.13-100	8.9±1*	1.70	d

		<i>Physalis peruviana</i>			Solvent		Water-insoluble					28±5*	1.25	
							Water-soluble					30±6.2*	1.04	
						Ether	Polar					102±33*	0.53	
34	Verbenaceae	<i>Lippia alba</i>	Leaf	Hydrodistillation	NA	Aqueous	NA	<i>L. brasiliensis</i>	P	M/HOM/BR/75/M2903	3.7-100	9.19±2.1*	2.90	e
		<i>Lippia origanoides</i>						<i>L. panamensis</i>	A	M/HOM/PA/71/LS94		63.87±6.2*	0.40	
								<i>L. brasiliensis</i>	P	M/HOM/BR/75/M2903		11.18±1.63*	ND	
								<i>L. panamensis</i>	A	M/HOM/PA/71/LS94		15.43±0.9*	3.40	
												42.66±2*	1.20	
												89.29±4.2*	0.50	
35	Aquifoliaceae	<i>Ilex laurina</i>	Leaf	Solvent	NA	Dichloromethane	NA	<i>L. panamensis</i>	A. axenic	M/HOM/CO/87/UA-140-epir-GFP	1.56-100	12.3±0.8*	1.40	a
									A. intracellular			20.3±3.2*	0.80	
									A. axenic			52.8±1.6*	1.10	
									A. intracellular			7.5±1.5*	7.70	
					Solid phase extraction	Ethyl acetate	Dichloromethane-methanol-acetic acid, isolation llexsaponin A,		A			5.9±0.5*	4.30	
							Dichloromethane-methanol-acetic acid, isolation Rotungenoside					41.6±1.5*	0.60	
36	Picramniaceae	<i>Picramnia gracilis</i>	Fruit	Solvent	Solid phase extraction	Ethanol	F3	<i>L. panamensis</i>	A	M/HOM/CO/87/UA-140-epir-GFP	6.25-200	17±2.8*	>2.00	a
							F4					37.5±7.6*	>11.80	
37	Lauraceae	<i>Ocotea macrophylla</i>	Leaf	Solvent	NA		NA	<i>L. panamensis</i>	P	M/HOM/CO/87/UA-140	78.125-1000	98±19.9*	2.00	f
					Membrane filtration		Isolation erythro-diarylbutane			M/HOM/CO/86/CL-250	15.625-100	26.6±2.6*	3.00	
							Isolation ocophyllal-A			M/HOM/CO/86/CL-250		58.9±32.2*	3.00	
							Isolation ocophyllal-B	<i>L. brasiliensis</i>		M/HOM/CO/87/UA-140		36.3±11.4*	4.00	
								<i>L. panamensis</i>				34±14.9*	4.00	
					NA		NA	<i>L. brasiliensis</i>		M/HOM/CO/86/CL-250	78.125-1000	85.7±22.9*	2.00	
							Isolation (+)-otobaphenol	<i>L. panamensis</i>		M/HOM/CO/87/UA-140		31.1±13.9*	6.00	
							Isolation 7,8,7',8'-didehydrootobaphenol			M/HOM/CO/86/CL-250	15.625-200	55.6±5.4*	3.00	
					Membrane filtration		Isolation dihydroflavokawin-B	<i>L. brasiliensis</i>		M/HOM/CO/86/CL-250		25±15*	2.00	
								<i>L. panamensis</i>		M/HOM/CO/87/UA-140		19.9±3.3*	2.00	
							Isolation kaurenoic acid	<i>L. brasiliensis</i>		M/HOM/CO/86/CL-250		45.4±4.8*	1.00	
								<i>L. panamensis</i>		M/HOM/CO/87/UA-140		25.3±9.2*	1.00	
38	Apiaceae	<i>Coriandrum sativum</i>	Seed					<i>L. panamensis</i>	P	M/HOM/CO/98/UA-1702	10-640	427.95±118.44*	3.00	9
	Lamiaceae	<i>Ocimum basilicum</i>	Leaf	Hydrodistillation	NA	Aqueous	NA	<i>L. guyanensis</i>		M/HOM/CO/84/CL-007		315.55±90.86*	>5.00	
		<i>Origanum vulgare</i>						<i>L. panamensis</i>		M/HOM/CO/98/UA-1702		251.59±64.18*	>6.40	
								<i>L. brasiliensis</i>		M/HOM/CO/2011/UA-3320		204.36±21.56*	2.70	
								<i>L. major</i>		Friedlin V1		171.8±20.64*	3.20	

								<i>L. panamensis</i>		M/HOM/CO/98/UA-1702		42.23±2.04*	12.90	
		<i>Thymus vulgaris</i>						M/HOM/CO/98/UA-1702		402.23±82.9*		1.10		
	<i>Poaceae</i>	<i>Cymbopogon citratus</i>						<i>L. brasiliensis</i>		M/HOM/CO/2011/UA-3320		160.06±43.49*	1.30	
								<i>L. guyanensis</i>		M/HOM/CO/84/CL-007		149.1±6.22*	1.40	
								<i>L. major</i>		Friedlin V1		194.05±29.2*	1.10	
								<i>L. panamensis</i>		M/HOM/CO/98/UA-1702		180.83±82.24*	1.20	
								<i>L. guyanensis</i>		M/HOM/CO/84/CL-007		231.4±42.43*	>6.90	
								<i>L. brasiliensis</i>		M/HOM/CO/2011/UA-3320		124.94±52.98*	1.20	
	<i>Zingiberaceae</i>	<i>Zingiber officinale</i>						<i>L. guyanensis</i>		M/HOM/CO/84/CL-007		256.95±75.17*	0.60	
								<i>L. major</i>		Friedlin V1		303±107.48*	0.50	
								<i>L. panamensis</i>		M/HOM/CO/98/UA-1702		154.83±23.86*	1.00	

39	<i>Fabaceae</i>	<i>Cabari brunnea</i>	Bark/Wood	Solvent	NA	Ethanol	NA	<i>L. brasiliensis</i>	A	M/HOM/CO/88/UA-301-EGFP	0.78-200	15.4±2.7*	0.70	a
					Solid phase extraction		F1-Methanol					88.4±25*	2.60	
							F3-Methanol, Isolation 3,5-diOMe stilbene					4.2±0.21*	<1.40	
							F3-Methanol, Isolation Pinostrobin					13.6±0.28*	3.60	
							F4-Methanol					145.9±48.2*	2.80	

203 Most extracts were obtained from species belonging to the families Lauraceae (n = 18), Sapindaceae (n = 15), and Annonaceae (n = 14).
 204 *Sapindus saponaria* was the most frequently studied species (n = 16), with reported traditional uses for treating leishmaniasis-related ulcers, as
 205 well as gastrointestinal and inflammatory disorders. *Xylopiya discreta* was also frequently cited (n = 14), although no traditional uses were
 206 mentioned in the reviewed studies. Leaves were the most commonly used plant part (n = 64), followed by bark/wood (n = 20), fruits (n = 18),
 207 seeds (n = 4), and flowers (n = 4). Regarding extraction solvents, ethanol was the most frequently used (n = 48). The characteristics of the
 208 bioactive extracts are summarized in Tables 1 and 2.

Table 2. Subgroup analyses of antileishmanial activity by plant part, extraction method, botanical family, and leishmania species

Summary of subgroup analyses from 13 studies included in the meta-analysis of plant-derived extracts with reported IC₅₀ values. Data are presented as pooled mean IC₅₀ values (µg/mL) with 95% confidence intervals (CI). Subgroups are stratified by plant part, preparation method, botanical family, and *Leishmania* species. Heterogeneity is reported as I² (%) and associated p value. n indicates the number of records contributing to each estimate. Heterogeneity statistics are not provided for subgroups represented by a single study.

Characteristics		n	IC ₅₀ (µg/mL)	95% CI		Heterogeneity [I ² (%), p value]
				Lower	Upper	
Part of plant	Fruits	18	32.07	23.24	40.89	100, <0.001
	Bark/Wood	20	23.21	20.22	26.19	100, <0.001
	Flowers	4	36.99	20.74	53.24	99, <0.001
	Leaf	64	45.66	42.44	48.87	99.8, <0.001
	Seeds	4	24.99	10.01	39.98	99.4, <0.001
Preparation	Aqueous	23	107.33	92.93	121.733	99.2, <0.001
	Chloroform	1	46.1	37.78	54.41	-
	Dichloromethane	5	24.12	16.43	31.82	98.1, <0.001
	Ethanol	48	25.57	23	28.14	99.9, <0.001
	Ether	3	48.73	7.53	89.92	92.3, <0.001
	Ethyl acetate	10	24.64	16.48	32.8	99.7, <0.001
	Hexane	2	85.05	81.76	88.34	0, 0.646
	Methanol	2	21.64	-5.85	49.14	87.6, 0.004
	Hexane-Chloroform-Methanol	16	32.67	23.31	42.03	100, <0.001
Botanic family name	Annonaceae	14	21.86	17.97	25.74	98.6, <0.001
	Apiaceae	1	427.95	311.88	544.02	-
	Aquifoliaceae	6	23.4	9.86	36.94	99.9, <0.001
	Araliaceae	3	43.58	33.66	53.51	99.5, <0.001
	Bignoniaceae	6	11.14	7.76	14.52	99.2, <0.001
	Chrysobalanaceae	1	9.8	8.84	10.76	-
	Fabaceae	5	22.13	14.96	29.29	99.9, <0.001
	Lamiaceae	6	225.69	130.9	320.47	99.1, <0.001
	Lauraceae	18	41.38	33.09	49.66	99.6, <0.001
	Picramniaceae	2	27.05	6.96	47.133	97.4, <0.001
	Poaceae	4	167.31	139.58	195.05	69.2, 0.021
	Rubiaceae	4	37.42	22.16	52.69	100, <0.001
	Rutaceae	10	34.16	29.51	38.81	100, <0.001
	Sapindaceae	15	32.68	23.32	42.03	100, <0.001
	Solanaceae	4	36.99	20.74	53.24	99, <0.001
	Verbenaceae	6	38.26	22.29	54.23	99.5, <0.001
	Zingiberaceae	4	196.03	132.65	259.42	80.7, 0.001
Leishmania specie	<i>L. amazonensis</i>	4	34.5	16.35	52.65	100, <0.001
	<i>L. braziliensis</i>	25	33.45	27.75	39.14	100, <0.001
	<i>L. donovani</i>	4	61.04	58.07	64.01	99.3, <0.001
	<i>L. guyanensis</i>	4	230.48	156.04	304.92	91.6, <0.001
	<i>L. major</i>	10	63	50.68	75.33	99.6, <0.001
	<i>L. panamensis</i>	64	31.86	29.6	34.13	99.9, <0.001

IC50 and selectivity index values

It is important to note that a low IC₅₀ indicates biological activity but does not necessarily imply selectivity. The selectivity index (SI), defined as the ratio between the cytotoxic concentration to host cells (CC₅₀) and the inhibitory concentration against *Leishmania* spp (IC₅₀), is a key parameter for evaluating how selectively a compound targets the parasite without harming

host cells. Compounds with moderate or relatively high IC₅₀ values but elevated SI values may represent safer and more selective candidates, whereas those with low IC₅₀ values but no cytotoxicity data cannot be adequately assessed. Unfortunately, a meta-analysis or comparative evaluation based on SI was not feasible in this review, as 26 of the 110 records did not report SI values. Moreover, among the studies that did report SI, only a few provided associated measures of variability.

According to the literature, extracts with SI ≥ 10 are considered selective and suitable for further studies, while values above 20 indicate high selectivity and therapeutic potential [14, 15]. Among the selective extracts identified, those tested against *L. panamensis* promastigotes exhibited the highest SI. The most selective extract was obtained from *X. discreta* (SI = 110.0), followed by *Origanum vulgare* (SI = 12.90), both exceeding the established threshold for potential further investigation. The same for the amastigote form of *L. panamensis*, for which, the most favorable selectivity was observed for extracts from *Moquilea salicifolia* (SI >20.40), *Picramnia gracilis* (SI >11.80), and *Zanthoxylum schreberi* (SI = 11.60). In the case of *L. braziliensis* amastigotes, *Handroanthus chrysanthus* demonstrated high selectivity (SI = 18.90), approaching the threshold indicative of strong therapeutic potential. For *L. major*, selectivity was observed for *Z. schreberi* (SI = 10.50, amastigote stage) and *Ocotea macrophylla* (SI = 10.36, promastigote stage), suggesting that these extracts may also merit further evaluation. Notably, *X. discreta* consistently demonstrated high selectivity indices across different extraction methods. Whether obtained through methanolic, ethanolic, aqueous, or essential oil preparations, the extracts exhibited SI values well above the threshold for further investigation, highlighting the robustness of its leishmanicidal potential. This consistency across extraction types positions *X. discreta* as a particularly promising candidate for anti-*Leishmania* drug development (Table 1).

Meta-analysis

Regarding IC₅₀ data, heterogeneity analysis revealed substantial variability among studies ($Q = 7.8 \times 10^6$, $df = 109$, $I^2 = 100\%$, $p < 0.05$), warranting the use of a random-effects model (Figure 3). The pooled mean IC₅₀ was 41.25 µg/mL (95% CI: 37.95 – 44.55). Publication bias was assessed using a funnel plot and Egger’s regression test. Visual inspection of the funnel plot (Figure 4) suggested asymmetry, with studies concentrated in the upper right quadrant and sparse in the lower left, indicative of potential reporting bias. Nevertheless, Egger’s test did not detect significant publication bias ($t = -0.25$, $p = 0.804$). Subgroup analyses revealed considerable heterogeneity in leishmanicidal activity according to the plant part used, type of extract, botanical family, and *Leishmania* species. Most subgroups showed I^2 values greater than 90% and p -values < 0.001 (Cochran’s Q test), indicating significant differences among studies. This high degree of variability can be attributed to the considerable differences in the overall experimental systems across studies. These include variation in the plant species used, the geographical location of plant collection, the specific plant part tested, and the preparation method of the extracts, ranging from crude extracts to fractions and purified metabolites. Additionally, heterogeneity arose from differences in the *Leishmania* species and strains employed for *in vitro* testing, as well as in broader methodological and experimental conditions. These findings underscore the critical need to standardize protocols in future studies to facilitate more robust and meaningful comparisons. Regarding the plant part used, leaf extracts exhibited the highest mean IC₅₀ (45.66 µg/mL; 95% CI: 42.44–48.87), whereas bark/wood extracts showed lower values (23.21 µg/mL; 95% CI: 20.22–26.19). All subgroups presented high heterogeneity ($I^2 > 99\%$; $p < 0.001$). Among solvents, aqueous extracts had the highest mean IC₅₀ (107.33 µg/mL; 95% CI: 92.93–121.73), while ethanol (25.57 µg/mL; 95% CI: 23.00 – 28.14) and dichloromethane extracts (24.12 µg/mL; 95% CI: 16.43 – 31.82) showed greater potency. Hexane extracts were the only subgroup with low heterogeneity ($I^2 = 0\%$; $p = 0.646$). The botanical families with the lowest mean IC₅₀ values were Bignoniaceae (11.14 µg/mL; 95% CI: 7.76 – 14.52) and Chrysobalanaceae (9.80 µg/mL; 95% CI: 8.84–10.76), whereas Lamiaceae (225.69 µg/mL; 95% CI: 130.90 – 320.47) and Zingiberaceae

(196.03 µg/mL; 95% CI: 132.65 – 259.42) recorded the highest values. Finally, pooled mean IC₅₀ values ranged from 31.86 µg/mL for *L. panamensis* (95% CI: 29.60 – 34.13) to 230.48 µg/mL for *L. guyanensis* (95% CI: 156.04 – 304.92), with high heterogeneity across all species subgroups ($I^2 > 99\%$; $p < 0.001$). Detailed strain-specific data are presented in Table 2.

Qualitative and semi-quantitative data

As previously noted, in addition to the quantitative analysis, records containing both qualitative and semi-quantitative data on leishmanicidal activity were included. These correspond to extracts for which exact IC₅₀ values were not reported either because they fell outside the measurable range of the assay or were assessed solely through descriptive methods. This information is summarized in figure 5, which categorizes the extracts based on their IC₅₀ values (≤ 10 µg/mL; 10–25 µg/mL; >25–50 µg/mL; >50–100 µg/mL; and >100 µg/mL). This visual representation facilitates the rapid identification of extracts with potentially relevant activity, even in the absence of complete quantitative data. Among these, promising extracts were observed from genera such as *Xylopia*, *Annona*, *Sapindus*, *Lippia*, and *Croton*, which may guide future research toward more rigorous evaluation, including toxicity and selectivity assessments as well as phytochemical characterization.

In the study by Calderón et al. (2010), ethanolic extracts from numerous plant species (collected across seven Latin American countries, including Colombia) were tested *in vitro* against *L. mexicana*. Although the authors reported that several extracts showed antileishmanial activity with IC₅₀ values ≤ 50 µg/mL, none of the extracts derived from Colombian plant species fell within this threshold (S4 Table). All tested Colombian extracts exhibited IC₅₀ values > 50 µg/mL and were therefore considered inactive or of no further interest by the authors. These findings still represent relevant preliminary background, as they demonstrate that extracts from those Colombian species were challenged in antileishmanial assays and yielded only modest activity.

Figure 3. Forest Plot from Random-Effects Meta-Analysis of the mean IC₅₀ values of medicinal plants with promising antileishmanial activity in Colombia

The pooled mean IC₅₀ was 41.25 µg/mL (95% CI: 37.95–44.55). Heterogeneity across studies was assessed using Cochran's Q test ($Q = 7.8 \times 10^6$, $df = 109$, $p < 0.05$) and the I² statistic (I² = 100.0%, $p < 0.001$), indicating substantial between-study variability.

Figure 4. Funnel Plot of Studies Included in the Meta-Analysis of IC₅₀ values of medicinal plants collected in Colombia

Funnel plot used to assess publication bias among the studies included in the meta-analysis of IC₅₀ values. Each point represents an individual study, with the x-axis indicating the reported IC₅₀ (µg/mL) and the y-axis representing the standard error of the estimate.

Figure 5. Heat Map of Leishmanicidal Activity (IC₅₀) of Plant Extracts by Plant Part and *Leishmania* Species

Each row represents a specific plant species and the part of the plant used for extract preparation, while columns correspond to different *Leishmania* species. Cell values indicate IC₅₀ values (µg/mL) obtained from in vitro assays. Gray-shaded cells indicate combinations for which no data were reported in the studies included in the meta-analysis. Abbreviations: Ld, *L. donovani*; La, *L. amazonensis*; Lb, *L. braziliensis*; Lp, *L. panamensis*; Lc, *L. chagasi*; Lg, *L. guyanensis*; Lm, *L. major*; Li, *L. infantum*.

Discussion

The results of this systematic review and meta-analysis demonstrate that several plant species collected in Colombia exhibit promising *in vitro* antileishmanial activity, as reflected by IC₅₀ values comparable to, or in some cases lower than, those reported in previous systematic reviews of plants collected in other countries. The pooled mean IC₅₀ for antileishmanial plant extracts from Colombia was 41.25 µg/mL (95% CI: 37.95–44.55), notably lower than the pooled mean reported for herbal extracts in Iran, which was 456.64 µg/mL (95% CI: 396.15–517.12) [16]. In comparison, the pooled mean IC₅₀ for Ethiopian medicinal plants was 16.80 µg/mL (95% CI: 12.44–21.16) against promastigotes and 13.81 µg/mL (95% CI: 13.12–14.50) against amastigotes [17]. While the IC₅₀ is a key parameter for estimating the biological potency of an extract or compound, it is not sufficient to determine its viability as a

pharmacological candidate. *In vitro* efficacy must be evaluated alongside toxicity and selectivity profiles. One of the main contributions of this review is the inclusion and analysis of the selectivity index (SI), which provides critical insight into the therapeutic window of a given extract. An extract with moderate IC₅₀ but high SI may, in fact, be more promising than one with a lower IC₅₀ but poor selectivity. Cytotoxicity and selectivity data are therefore essential for assessing biological specificity and guiding preclinical development of antileishmanial agents. These parameters should be considered fundamental benchmarks in future efforts to identify and validate novel therapeutic candidates.

In some notable cases, such as *X. discreta*, SI values exceeding 20 (up to 110) were reported, suggesting a highly favorable therapeutic window, particularly in the essential oil fraction tested against promastigotes of *L. panamensis*. This pattern of leishmanicidal activity combined with low cytotoxicity does not appear to be exclusive to *X. discreta* within the genus, as bioactive compounds with similar effects have been reported in other *Xylopi*a species. For example, *X. parviflora* roots collected in Limpopo Province, South Africa, and extracted with dichloromethane inhibited the growth of *L. donovani* amastigotes with an IC₅₀ of 5.01 µg/mL and an SI of 10 [18]. Likewise, a diterpene glycoside of the ent-kaurene type isolated from the leaves of *X. excellens* collected in Manaus, Brazil, exhibited *in vitro* activity against *L. amazonensis* promastigotes (IC₅₀ = 15.23 ± 0.64 µg/mL) [19]. Although the authors considered the selectivity index of 1.96 to indicate good selectivity, this interpretation relies on a less conservative threshold and lacks supporting references. Nonetheless, these findings reinforce the relevance of the *Xylopi*a genus as a promising source of antileishmanial metabolites and position the Colombian species *X. discreta*, with its consistently higher SI values, as a particularly compelling candidate for further investigation. These comparisons also underscore the importance of adopting standardized and evidence-based criteria for SI interpretation in order to facilitate the comparison of findings across studies and support the identification of truly promising therapeutic candidates.

The study by López et al. (2009) on *X. discreta* highlights the significant variability that can arise in both IC₅₀ and selectivity index (SI) values depending on the type of extract used, offering a valuable opportunity to understand how extraction methods influence bioactivity profiles. In that study, a range of solvents (water, ethanol, ether, ethyl acetate, methanol, and chloroform) were employed to obtain extracts from leaves or seeds, resulting in markedly different antileishmanial activities and selectivity indices (Table 1). For instance, among the crude extracts from leaves, SI values ranged from as low as 2.32 (ethyl acetate extract) to as high as 64.8 (methanol extract), depending on the solvent used. The essential oil fraction obtained with ethanol exhibited a SI value of 110.0, further underscoring the critical influence of extraction method and fractionation on bioactivity outcomes. This variability can be attributed to the distinct chemical affinities of each solvent. Ethanol, particularly at intermediate concentrations (70–80%), allows the solubilization of a broad spectrum of secondary metabolites with medium to high polarity. Under optimized extraction conditions—such as reflux or ultrasound-assisted extraction—ethanol significantly enhances the recovery of phenolic compounds and flavonoids, which have been widely associated with antioxidant, anti-inflammatory, antimicrobial, and antitumor activities [20, 21, 22]. In contrast, highly polar glycosylated metabolites are more efficiently extracted with water, although this non-selective solvent also dissolves hydrophilic impurities that may affect bioactivity measurements. Solvents such as dichloromethane and ethanol tend to extract mid-polarity bioactive compounds like alkaloids, terpenes, and phenolics, while ether and ethyl acetate show similar but variable affinities depending on compound structure. Methanol extracts, which concentrate moderately polar and often lipophilic secondary metabolites, have been repeatedly associated with anti-Leishmania effects [23, 24, 25]. In contrast, non-polar solvents such as hexane are more likely to extract lipophilic substances like waxes and chlorophylls, which are generally considered non-bioactive in antiprotozoal assays [26]

Taken together, these findings illustrate the critical influence of solvent selection on the phytochemical profile and biological performance of medicinal plant extracts and reinforce the importance of rational extraction strategies in antiprotozoal drug discovery.

Another noteworthy finding of this review is the high proportion of studies focused on *L. panamensis*, a species with broad geographic distribution and clear clinical relevance in Central America and along the Pacific coast of Colombia and Ecuador. Although this focus was expected given the local epidemiology, its confirmation is particularly significant in light of the global research landscape. Previous reviews have been conducted in regions such as East Africa (e.g., Ethiopia) and Asia (e.g., Iran), and have focused on *Leishmania* species that are not representative of Colombian transmission patterns: *L. tropica*, *L. major*, and *L. infantum* in Iran, and *L. aethiopica*, *L. donovani*, and *L. major* in Ethiopia. Besides *X. discreta*, we found that other Colombian plant species exhibited promising selectivity against *L. panamensis*, including *Origanum vulgare* (SI = 12.90) against promastigotes, and *Moquilea salicifolia* (SI >20.40), *Picramnia gracilis* (SI >11.80), and *Zanthoxylum schreberi* (SI = 11.60) against amastigotes. In the case of *L. braziliensis*, *Handroanthus chrysanthus* exhibited high selectivity (SI = 18.90, amastigotes), while for *L. major*, selective activity was observed in *Z. schreberi* (SI = 10.50, amastigotes) and *Ocotea macrophylla* (SI = 10.36, promastigotes). These findings support the notion that a subset of Colombian medicinal plants possess selective antileishmanial activity not only against the most prevalent local species but also against others of broader epidemiological relevance, thereby reinforcing their potential value as starting points for drug development beyond the local setting.

A major limitation of this review was the substantial methodological heterogeneity among the included studies. Some of this variability was expected, arising from differences in the plant parts used, extraction methods, presence or absence of fractionation, and the developmental stage of the parasite targeted (e.g., promastigotes, intracellular amastigotes, or axenic amastigotes). However, additional heterogeneity stemmed from the lack of standardized protocols, particularly in the methods and criteria used to evaluate antileishmanial activity. This

inconsistency hindered meaningful cross-study comparisons and contributed to the high level of statistical heterogeneity observed. Furthermore, most studies lacked *in vivo* validation, limiting the translational potential of *in vitro* findings to more advanced preclinical models. Similar challenges have been reported in reviews conducted in other geographical regions, suggesting that this is a broader, structural issue within the field. Therefore, there is a pressing need to establish harmonized experimental guidelines for antileishmanial bioassays. These should include the systematic use of standardized positive controls, generation of complete dose-response curves, and parallel evaluation of parameters such as cytotoxicity in host cells. Adoption of these practices would significantly improve the quality, reproducibility, and translational relevance of the evidence generated.

Conclusion

This systematic review and meta-analysis provides evidence that several medicinal plants collected in Colombia exhibit promising *in vitro* antileishmanial activity, with IC₅₀ and selectivity index (SI) values that support their potential for further pharmacological development. The predominance of studies targeting *L. panamensis* aligns well with Colombia's epidemiological context. *Xylopia discreta* emerged as a particularly compelling candidate against *L. panamensis*, showing consistently high SI values across multiple extraction methods. Other Colombian plant species that exhibited promising selectivity against *L. panamensis* include *Origanum vulgare*, *Moquilea salicifolia*, *Picramnia gracilis*, and *Zanthoxylum schreberi*.

Importantly, one of the key contributions of this study is the inclusion and systematic analysis of SI, an essential parameter for estimating the relative safety of plant extracts in host cells. While IC₅₀ values reflect biological potency, the SI provides critical insight into the therapeutic window of a compound. Extracts with moderate IC₅₀ but high SI may represent safer and more promising candidates than highly potent but cytotoxic ones. Therefore, incorporating SI into future evaluations is crucial for the accurate selection of leads for preclinical development.

In addition, differences in solvent polarity and extraction techniques were associated with considerable variation in both IC₅₀ and SI values. This highlights the significant impact of extraction solvents on bioactivity outcomes, reinforcing the importance of rational extraction strategies in natural product research.

Despite these promising findings, the high methodological heterogeneity among studies and the frequent absence of *in vivo* validation limit the comparability and translational applicability of the available data. Variability in plant parts used, extraction protocols, parasite developmental stages, and outcome measures all contributed to the high statistical heterogeneity observed. To address these challenges, the establishment of standardized experimental guidelines, including the use of full dose–response curves, standardized positive controls, and cytotoxicity assays in host cells, is essential. The adoption of such practices would enhance the quality, reproducibility, and translational relevance of future antileishmanial research.

CRediT authorship contribution statement

CNC: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **LM:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **GZ, ADA, ZCR, EPC, DT:** Investigation, Writing – review & editing. **JCO:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Funding

This project was funded by the Universidad El Bosque (PCI-2023-0031).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supporting information

Supplementary data to this article can be found online at

Data availability

All relevant data are within the paper and its Supporting Information files.

References

1. World Health Organization. Leishmaniasis [Internet]. Geneva: WHO; 2023 [cited 2025 Jul 7]. Available from: <https://www.who.int/news-room/fact-sheets/detail/leishmaniasis>
2. Torres-Guerrero E, Quintanilla-Cedillo MR, Ruiz-Esmenjaud J, Arenas R. Leishmaniasis: A review. F1000Res. 2017;6:750. <https://doi.org/10.12688/f1000research.11120.1>
3. Alvar J, Vélez ID, Bern C, Herrero M, Desjeux P, Cano J, et al. Leishmaniasis worldwide and global estimates of its incidence. PLoS One. 2012;7(5):e35671. <https://doi.org/10.1371/journal.pone.0035671>
4. Ovalle-Bracho C, Londoño-Barbosa D, Salgado-Almario J, González C. Evaluating the spatial distribution of Leishmania parasites in Colombia from clinical samples and human isolates (1999 to 2016). PLoS One. 2019;14(3):e0214124. <https://doi.org/10.1371/journal.pone.0214124>
5. Correa-Cárdenas CA, Pérez J, Patino LH, et al. Distribution, treatment outcome and genetic diversity of Leishmania species in military personnel from Colombia with cutaneous leishmaniasis. BMC Infect Dis. 2020;20(1):938. <https://doi.org/10.1186/s12879-020-05529-y>
6. Instituto Nacional de Salud. Informe de evento Leishmaniasis 2023. Bogotá: INS; 2024. 29 p.
7. Instituto Nacional de Salud. Informe de evento Leishmaniasis 2024. Bogotá: INS; 2025. 39 p.
8. Ministerio de Salud y Protección Social. Lineamientos de atención clínica integral para Leishmaniasis en Colombia. Versión 4. 2023.
9. Santos GA, Sousa JM, Aguiar AHBM, Torres KCS, Coelho AJS, Ferreira AL, et al. Systematic review of treatment failure and clinical relapses in leishmaniasis from a multifactorial perspective: Clinical aspects, factors associated with the parasite and host. Trop Med Infect Dis. 2023;8(9):430. <https://doi.org/10.3390/tropicalmed8090430>
10. Bravo K, Pereañez JA. Colombian biodiversity, an opportunity for the strengthening of the pharmaceutical and cosmetic industries. Vitae. 2016;23(3):163–165. <https://doi.org/10.17533/udea.vitae.v23n3a01>
11. Hassan AA, Khalid HE, Abdalla AH, Mukhtar MM, Osman WJ, Efferth T. Antileishmanial activities of medicinal herbs and phytochemicals in vitro and in vivo: An update for the

- years 2015 to 2021. *Molecules*. 2022;27(21):7579.
<https://doi.org/10.3390/molecules27217579>
12. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *Syst Rev*. 2021;10:89. <https://doi.org/10.1186/s13643-021-01626-4>
13. Sheth VH, Shah NP, Jain R, Bhanushali N, Bhatnagar V. Development and validation of a risk-of-bias tool for assessing in vitro studies conducted in dentistry: The QUIN. *J Prosthet Dent*. 2024;131(6):1038–1042. <https://doi.org/10.1016/j.prosdent.2022.05.019>
14. Peña-Morán, O. A., Villarreal, M. L., Álvarez-Berber, L., Meneses-Acosta, A., & Rodríguez-López, V. (2016). Cytotoxicity, Post-Treatment Recovery, and Selectivity Analysis of Naturally Occurring Podophyllotoxins from *Bursera fagaroides* var. *fagaroides* on Breast Cancer Cell Lines. *Molecules* (Basel, Switzerland), 21(8), 1013. <https://doi.org/10.3390/molecules21081013>
15. Indrayanto, G., Putra, G. S., & Suhud, F. (2021). Validation of in-vitro bioassay methods: Application in herbal drug research. *Profiles of drug substances, excipients, and related methodology*, 46, 273–307. <https://doi.org/10.1016/bs.podrm.2020.07.005>
16. Soosaraei M, Fakhar M, Hosseini Teshnizi S, Ziaei Hezarjaribi H, Banimostafavi ES. Medicinal plants with promising antileishmanial activity in Iran: A systematic review and meta-analysis. *Ann Med Surg (Lond)*. 2017;21:63–80. <https://doi.org/10.1016/j.amsu.2017.07.057>
17. Worku KM, Asfaw BG, Mamo DN, Haile Y, Tesfa H, Aemero M. Medicinal plants with promising antileishmanial activity in Ethiopia: A systematic review and meta-analysis. *Medicine (Baltimore)*. 2024;103(22):e38480. <https://doi.org/10.1097/MD.00000000000038480>
18. Bapela, M. J., Kaiser, M., & Meyer, J. J. M. (2017). Antileishmanial activity of selected South African plant species. *South African Journal of Botany*, 108, 342-345.
19. Volpato, H., Nakamura, C. V., Koolen, H. H., de Souza, A. D. L., & Pinheiro, M. L. B. (2018). Antileishmanial activity of a new ent-kaurene diterpene glucoside isolated from leaves of *Xylopia excellens* RE Fr.(annonaceae). *Rec. Nat. Prod.*, 12, 190-194.
20. Phanjaroen T, Boonchalaem T, Haideri M, Chaipoot S, Khiaolaongam W, Laoung-On J, et al. Effect of different extraction techniques on phenolic profile and phytochemical potential of *Gymnema inodorum* leaf extract. *Molecules*. 2024;29:5475. <https://doi.org/10.3390/molecules29225475>
21. Aibuldinov Y, Zeinuldina A, Ibrayeva M, Kolpek A, Mukazhanova Z, Nurlybayeva A, et al. Phytochemical profiling, antioxidant and antimicrobial potentials of ethanol and ethyl acetate extracts of *Chamaenerion latifolium* L. *Pharmaceutics*. 2024;17:80996. <https://doi.org/10.3390/ph17080996>
22. Anastasescu M, Badea V, Schröder V, Atkinson I, Musuc A, Popovici V, et al. Formulation and development of bioadhesive oral films containing *Usnea barbata* (L.) F.H.Wigg dry ethanol extract with antimicrobial and anticancer properties for potential use in oral cancer complementary therapy. *Pharmaceutics*. 2022;14:1808. <https://doi.org/10.3390/pharmaceutics14091808>
23. De Sousa D, Yepes L, Lopes S, Pérez-Castillo Y, Robledo S. Alkyl and aryl derivatives based on p-coumaric acid modification and inhibitory action against *Leishmania braziliensis* and *Plasmodium falciparum*. *Molecules*. 2020;25(14):3178. <https://doi.org/10.3390/molecules25143178>
24. García-Soriano J, De Lucio H, Vaquero J, Jiménez-Ruiz A, García-Marín J, Sánchez-Alonso P, et al. Pyridazino-pyrrolo-quinoxalinium salts as highly potent and selective leishmanicidal agents targeting trypanothione reductase. *Eur J Med Chem*. 2021;227:113915. <https://doi.org/10.1016/j.ejmech.2021.113915>

25. Taramelli D, Bassanini I, Basilico N, Ferrandi E, Gabriele E, Sparatore A, et al. Design, synthesis and in vitro investigation of novel basic celastrol carboxamides as bio-inspired leishmanicidal agents endowed with inhibitory activity against *Leishmania* Hsp90. *Biomolecules*. 2021;11(1):56. <https://doi.org/10.3390/biom11010056>
26. Azwanida, N. N. (2015). A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Med aromat plants*, 4(196), 2167-0412.
27. Cardona-G W, Robledo S, Alzate F, Yepes AF, Hernandez C, Velez ID, et al. Antileishmanial and cytotoxic activities of four Andean plant extracts from Colombia. *Vet World*. 2020;13(10):2178.
28. Chávez-Enciso NA, Coy-Barrera ED, Patiño OJ, Cuca LE, Delgado G. Evaluation of the leishmanicidal activity of Rutaceae and Lauraceae ethanol extracts on golden Syrian hamster (*Mesocricetus auratus*) peritoneal macrophages. *Indian J Pharm Sci*. 2014;76(3):188–97.
29. Correa E, Robledo SM, Echeverri F, Quiñones W, Arbeláez N, Murillo J, et al. In vitro and in vivo leishmanicidal and trypanocidal activities of isoflavans from *Tabebuia chrysantha* (Jacq.) G. Nicholson timber by-products. *Exp Parasitol*. 2025;270:108899. <https://doi.org/10.1016/j.exppara.2025.108899>
30. Correa E, Quiñones W, Robledo S, Carrillo L, Archbold R, Torres F, et al. Leishmanicidal and trypanocidal activity of *Sapindus saponaria*. *Bol Latinoam Caribe Plantas Med Aromat*. 2014;13(4):311–23.
31. Granados-Falla D, Gomez-Galindo A, Daza A, Robledo S, Coy-Barrera C, Cuca L, et al. Seco-limonoid derived from *Raputia heptaphylla* promotes the control of cutaneous leishmaniasis in hamsters (*Mesocricetus auratus*). *Parasitol*. 2016;143(3):289–99. <https://doi.org/10.1017/S0031182015001717>
32. López R, Cuca LE, Delgado G. Antileishmanial and immunomodulatory activity of *Xylopia discreta*. *Parasite Immunol*. 2009;31(10):623–30.
33. Martínez W, Ospina LF, Granados D, Delgado G. In vitro studies on the relationship between the anti-inflammatory activity of *Physalis peruviana* extracts and the phagocytic process. *Immunopharmacol Immunotoxicol*. 2010;32(1):63–73. <https://doi.org/10.1080/08923970903143957>
34. Neira LF, Mantilla JC, Stashenko E, Escobar P. Toxicidad, genotoxicidad y actividad anti-*Leishmania* de aceites esenciales obtenidos de cuatro quimiotipos del género *Lippia*. *Bol Latinoam Caribe Plantas Med Aromat*. 2018;17(1):68–83.
35. Pérez JM, Robledo S, Cardona W, Alzate F, Muñoz D, Herrera A. Leishmanicidal and cytotoxic activity of extracts and saponins from *Ilex laurina* (Aquifoliaceae). *Trop J Pharm Res*. 2016;15(5):973–9.
36. Robledo SM, Cardona W, Ligardo K, Henao J, Arbeláez N, Montoya A, et al. Antileishmanial effect of 5,3'-hydroxy-7,4'-dimethoxyflavanone of *Picramnia gracilis* Tul. (*Picramniaceae*) fruit: in vitro and in vivo studies. *Adv Pharmacol Pharm Sci*. 2015;2015:978379.
37. Sánchez-Suárez J, Coy-Barrera E, Cuca LE, Delgado G. Leishmanicidal and cytotoxic activities of extracts and naturally-occurring compounds from two Lauraceae species. *Nat Prod Commun*. 2011;6(2):231–4.
38. Sanchez-Suarez J, Riveros I, Delgado G. Evaluation of the leishmanicidal and cytotoxic potential of essential oils derived from ten Colombian plants. *Iran J Parasitol*. 2013;8(1):129–36.

39. Torres F, Robledo SM, Quiñones W, Escobar G, Archbold R, Correa E, et al. Exploring antiparasitic molecule sources from timber by-product industries—leishmanicidal and trypanocidal compounds from *Clathrotropis brunnea* Amshoff. *Front Pharmacol.* 2020;11:584668. <https://doi.org/10.3389/fphar.2020.584668>
40. Ruiz PG, Garavito G, Acebey CL, Arteaga L, Pinzon R, Gimenez TA. Actividad leishmanicida y tripanocida de algunas plantas reportadas como medicinales en Colombia. *Biofarbo.* 2004;13:27–30.
41. Escobar P, Leal SM, Herrera LV, Martinez JR, Stashenko E. Chemical composition and antiprotozoal activities of Colombian *Lippia* spp essential oils and their major components. *Mem Inst Oswaldo Cruz.* 2010;105:184–90.
42. Carmona D, Sáez J, Granados H, Pérez E, Blair S, Angulo A, et al. Antiprotozoal 6-substituted-5,6-dihydro- α -pyrones from *Raimondia* cf. *monoica*. *Nat Prod Res.* 2003;17(4):275–80.
43. Osorio E, Arango GJ, Jiménez N, Alzate F, Ruiz G, Gutiérrez D, et al. Antiprotozoal and cytotoxic activities in vitro of Colombian Annonaceae. *J Ethnopharmacol.* 2007;111(3):630–5. <https://doi.org/10.1016/j.jep.2007.01.015>
44. Weniger B, Robledo S, Arango GJ, Deharo E, Aragón R, Muñoz V, et al. Antiprotozoal activities of Colombian plants. *J Ethnopharmacol.* 2001;78(2–3):193–200. [https://doi.org/10.1016/s0378-8741\(01\)00346-4](https://doi.org/10.1016/s0378-8741(01)00346-4)
45. Alzate F, Jimenez N, Weniger B, Bastida J, Gimenez A. Antiprotozoal activity of ethanol extracts of some *Bomarea* species. *Pharm Biol.* 2008;46(9):575–8. <https://doi.org/10.1080/13880200801968904>
46. Arévalo Y, Robledo S, Muñoz L, Granados-Falla D, Cuca LE, Delgado G. Evaluación in vitro de la actividad de aceites esenciales de plantas colombianas sobre *Leishmania braziliensis*. *Rev Colomb Cienc Quím Farm.* 2009;38(2):131–41.
47. Neira LF, Stashenko E, Escobar P. Actividad antiparasitaria de extractos de plantas colombianas de la familia Euphorbiaceae. *Rev Univ Ind Santander Salud.* 2014;46(1):15–22.
48. Arango V, Robledo S, Séon-Ménier B, Figadère B, Cardona W, Sáez J, et al. Coumarins from *Galipea panamensis* and their activity against *Leishmania panamensis*. *J Nat Prod.* 2010;73(5):1012–4. <https://doi.org/10.1021/np100146y>
49. Coy-Barrera CA, Coy-Barrera ED, Granados-Falla DS, Delgado-Murcia G, Cuca-Suarez LE. Seco-limonoids and quinoline alkaloids from *Raputia heptaphylla* and their antileishmanial activity. *Chem Pharm Bull.* 2011;59(7):855–9. <https://doi.org/10.1248/cpb.59.855>
50. Weniger B, Vonthron-Sénécheau C, Arango GJ, Kaiser M, Brun R, Anton R. A bioactive biflavonoid from *Camptosperma panamense*. *Fitoterapia.* 2004;75(7–8):764–7.
51. Calderón AI, Romero LI, Ortega-Barría E, Solís PN, Zacchino S, Gimenez A, et al. Screening of Latin American plants for antiparasitic activities against malaria, Chagas disease, and leishmaniasis. *Pharm Biol.* 2010;48(5):545–53. <https://doi.org/10.3109/13880200903193344>

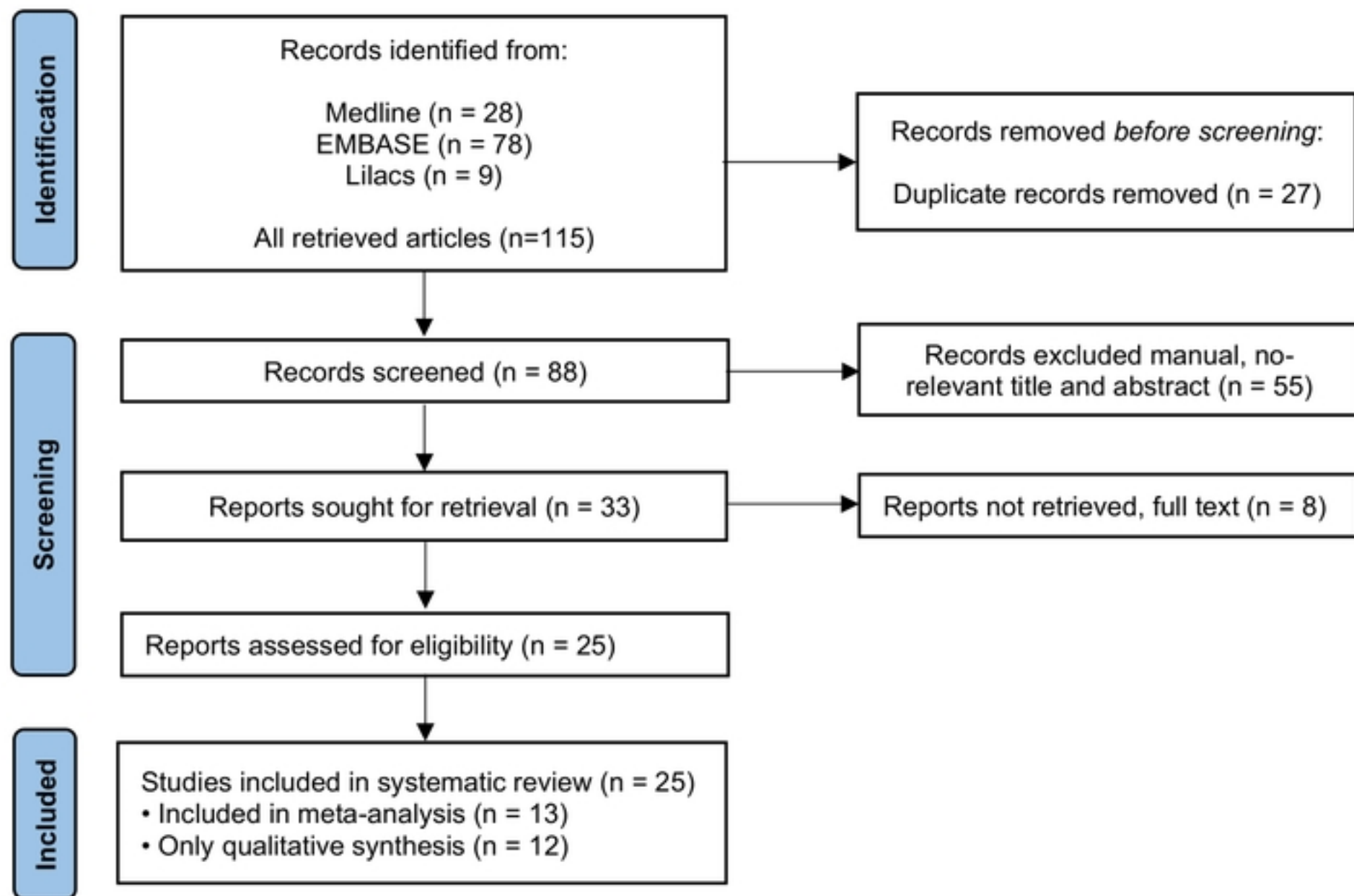


Figure1

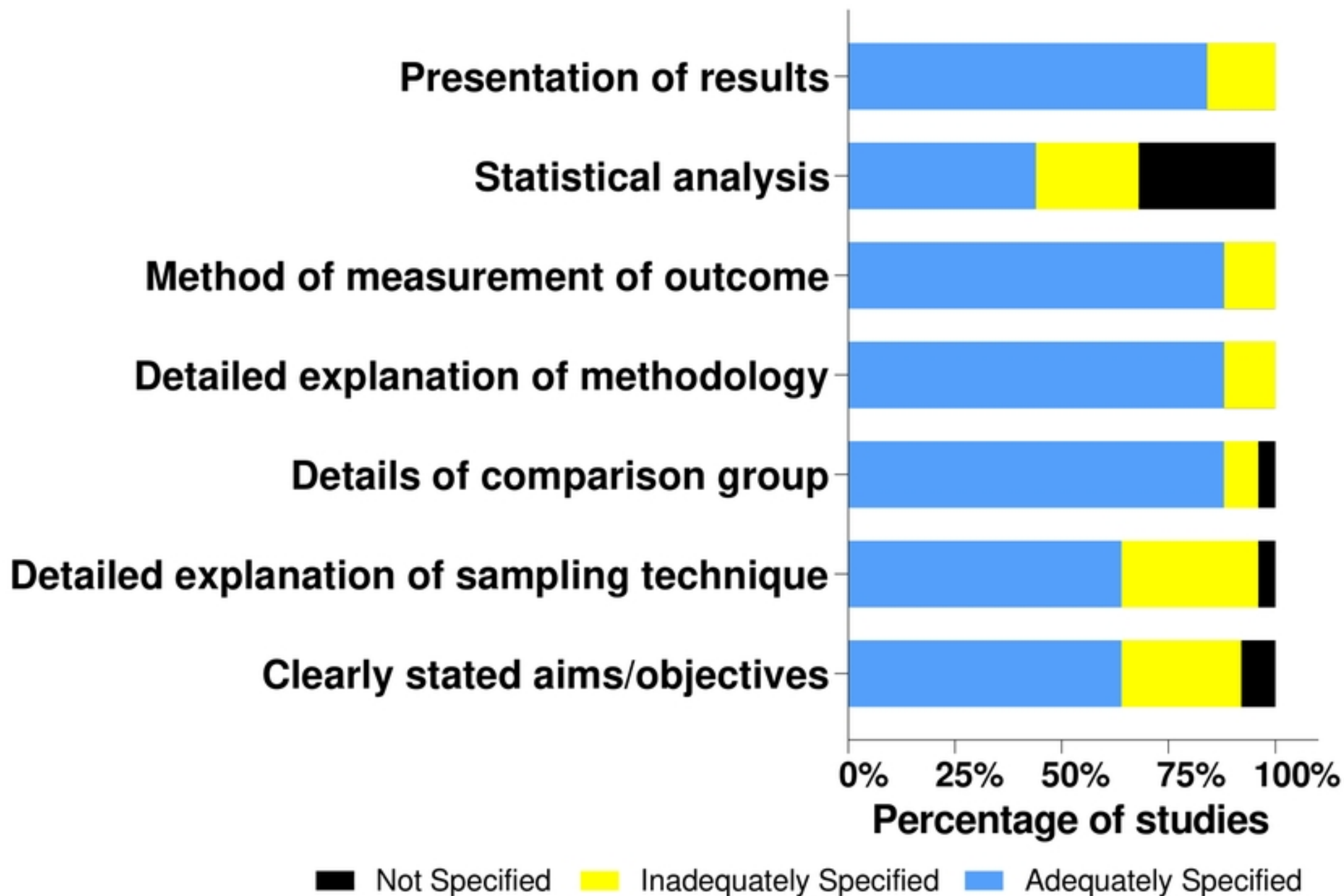
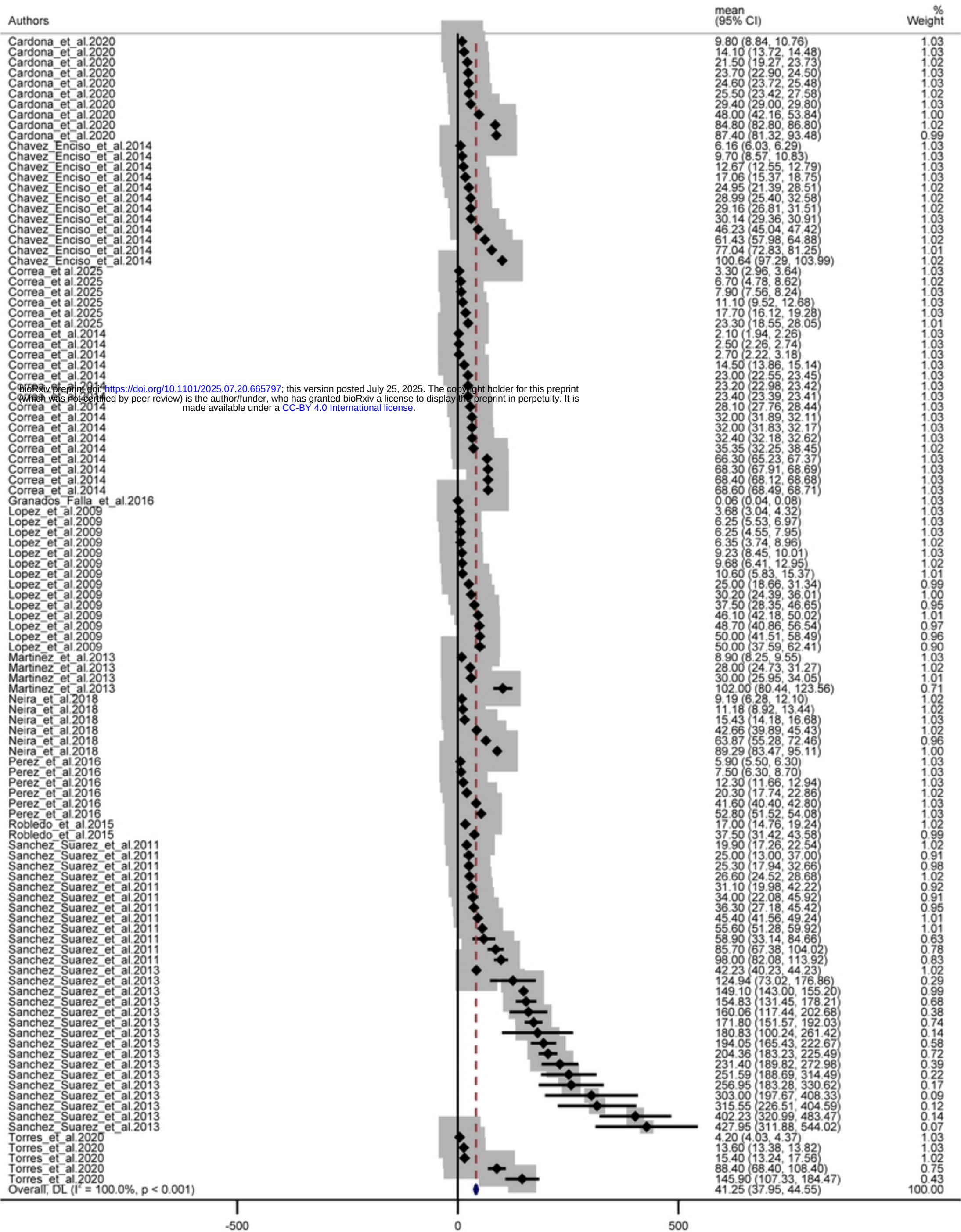


Figure2



NOTE: Weights are from random-effects model

Figure3

Funnel plot with pseudo 95% confidence limits

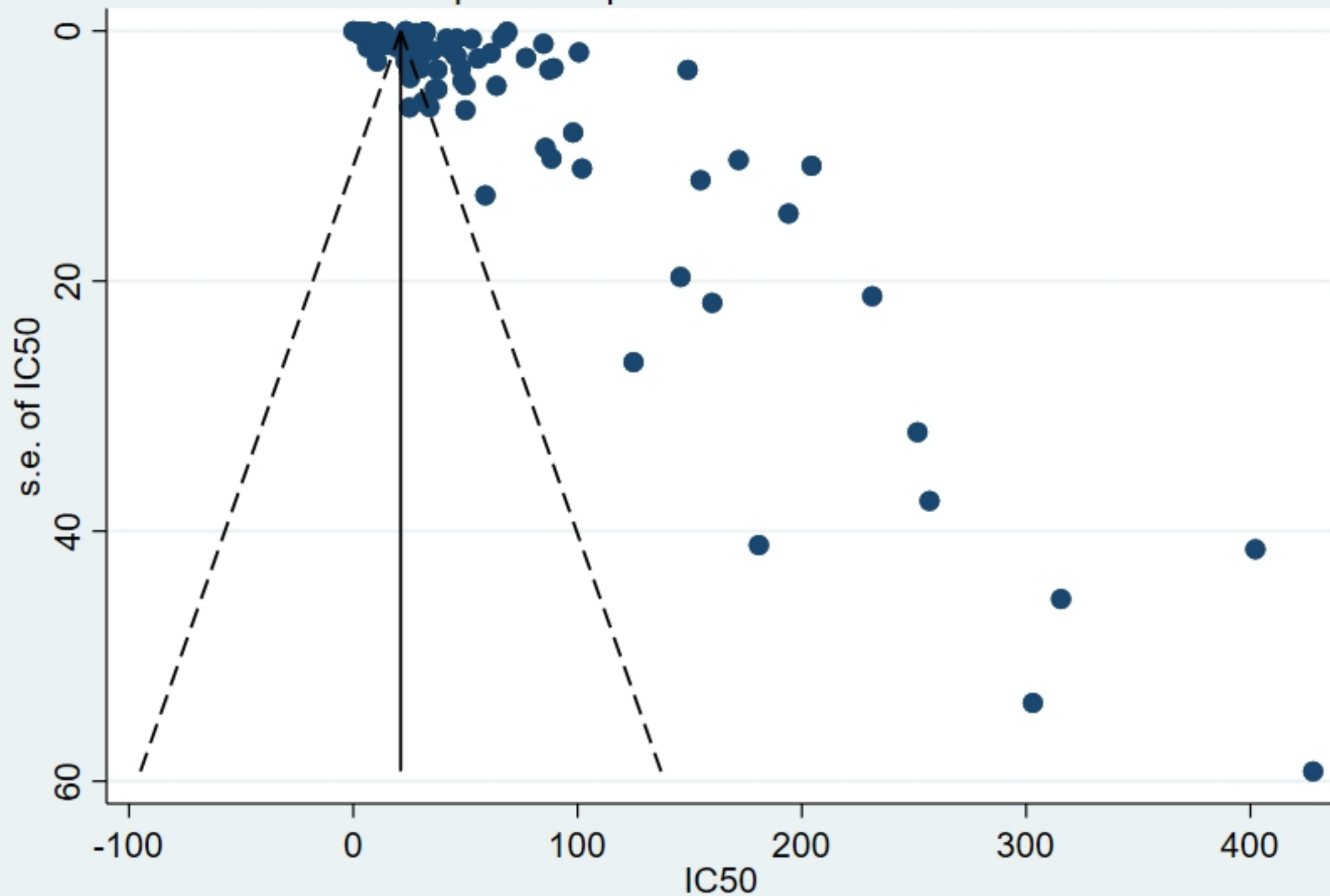


Figure4

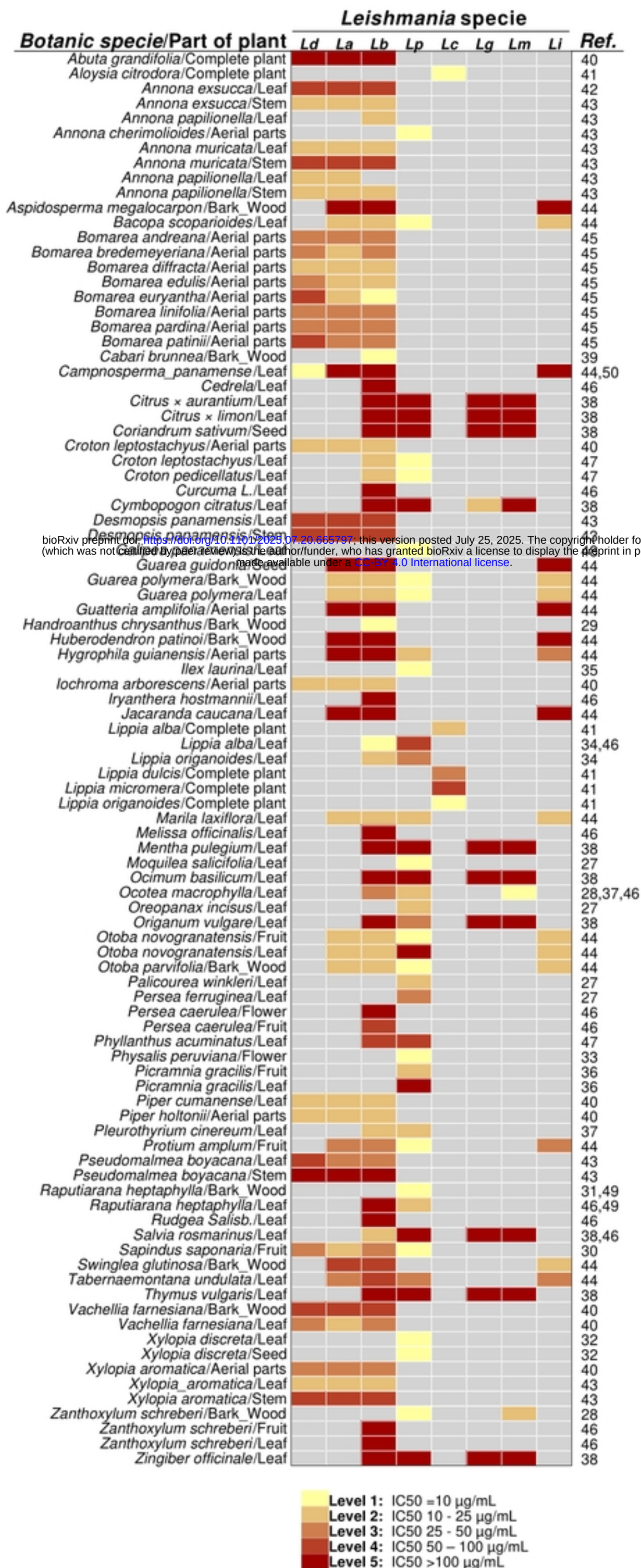


Figure5