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Plants and their metabolites against *Streptococcus mutans*

Landi B. O.[1], Di Pace B.[2] and Conceição A. O.[3*]

[1]Odontological Public Health Program, Una City Hall, Bahia and master student at Health Science Graduate Programe, State University of Santa Cruz, Ilhéus, Bahia, Brazil.
[2]Health Science Department, Faculty of Medicine, State University of Santa Cruz, Ilhéus, Bahia, Brazil.
[3]Biological Science Department, Faculty of Medicine, State University of Santa Cruz, Ilhéus, Bahia, Brazil.

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Oral diseases represent a major public health problem, especially for economically marginalized communities with limited access to health services. In addition, the constant increase in bacterial resistance to many of the antibiotics contributes to worsen the problem. In this context, great importance had been given to natural compounds for the discovery of new drugs that contribute to the prevention and control of oral affection. The present study proposed a systematic review of articles that used the techniques of agar diffusion and broth dilution to measure the efficiency of plant samples against *Streptococcus mutans,* one of the main agents involved in the development of dental cavities. Families and plant species most used in the study, the concentration and polarity measurements of the samples used in the tests, the disk and well variants in the agar diffusion technique, as well as the most outstanding results presented by the articles are reported. The review highlights the bacteriostatic effect of natural products against *S. mutans* and strengthened parameters that could validate the best strategy for the identification of natural products with antimicrobial action, having as object the *S. mutans*. The agar diffusion test should not be neglected as screening test but scientific measurements should be taken into consideration to obtain plant extracts which are likely to undergo clinical usage against *S. mutans*.

Key words: Medicinal plant, plant extract, antimicrobial, qualitative technique, quantitative technique.

INTRODUCTION

It has been a while since the study of natural products against microorganisms has gained interest by researchers and pharmaceutical industry. The bacterial resistance and side effects of the antimicrobial drugs available in the market have contributed to this fact (Ramakrishna et al., 2011). Moreover, the ecological awareness, traditional knowledge appreciation, and lack of access of marginalized communities to pharmaceutical medicines make the rational search for new compounds still important (Rates, 2001; Halberstein, 2005; Pelkonen et al., 2014).

Tooth decay has been considered one of the most

*Corresponding author. E-mail: aoconceicao@uesc.br.

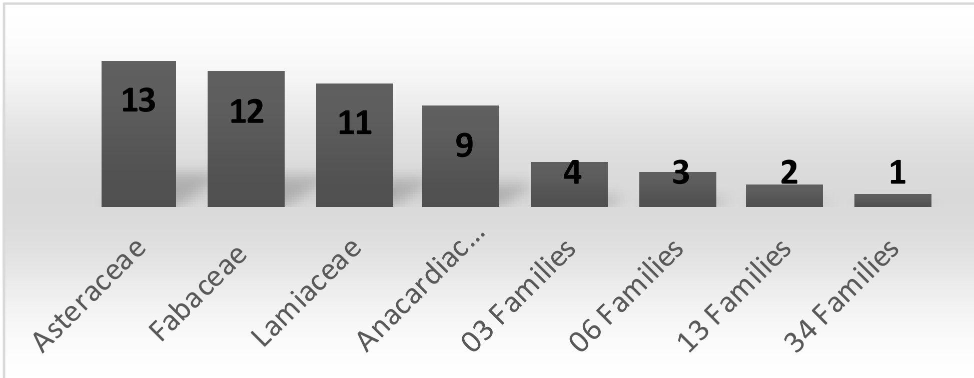

Figure 1. Number of species by family reported in peer reviewed articles found in the literature between January, 1st, 2006 to December 31th, 2016.

prevalent disease in the world (Petersen, 2003) being *S. mutans* one of the greatest risk factor for its development (Nishikawara, 2006).Trying to solve the oral diseases problem, antibacterial effect study of natural products or their derivatives has been conducted (Kouidhi et al., 2015).

Two main techniques have been used in natural products against bacteria research: The agar diffusion (Kirby Bauer) technique, considered a qualitative method, and the broth dilution technique which is defined as quantitative or semi-quantitative method depending on the objective of the studies (Valgas et al., 2007). Both techniques have been used to pre-select plant species, initial concentration to be used, extraction solvents, or isolated molecules giving support to more specific methods related to mode of action of antimicrobial substances (Balouiri et al., 2016). Although the broth dilution is considered the *gold* test for antimicrobial resistance profile (Campana et al., 2011), agar diffusion has an important role in detection of subpopulation of bacteria with resistance to antimicrobials (Matthew, 2015) and it is considered an adequate and simple tool to evaluate bacterial resistance in clinical diagnosis (Schumacher et al., 2018). Therefore, the purpose of this paper is to review and compare different methodologies used to verify the antimicrobial effect of medicinal plants and their derivatives on *S. mutans*.

MATHERIALS AND METHODS

The study is characterized by a systematic review including peer reviewed articles published in international journals using as index tool the "Periodicos CAPES" (http://www.periodicos.capes.gov.br/index.php) which gives integrated access to international indexers such as PubMed, Web of Science, LILACS, SciELO, and SCOPUS. The criteria used to select the articles included: (1) "Plant extract," "*Streptococcus mutans,*" and "medicinal plants" as key words; (2) English, Spanish, and Portuguese languages; (3) published between January, 1st 2006 and December, 31th 2016. Following selection, articles were classified by plant taxonomical classification (family and species), methodology approach (agar diffusion or broth dilution techniques), extract or substance unit measure (mg, %, etc.) used to standardize samples, solvents used for plant substances extraction such as apolar (hexane, benzene, chlorophorm, butane, and petroleum ether), aprotic polar (dichloromethane, ethyl acetate, and acetone/propane) and protic polar (ethanol, butane, methanol, and water), and isolated substances with anti-*S. mutans* activity. The criterion used to consider a "good result" for agar diffusion technique was an inhibition halo of ≥ 18 mm since from the articles selected this diametre seemed to reflect sweetable concentrations in broth dilution. In the case of broth dilution, the minimal inhibitory concentration (MIC) values ≤100 µg/mL (Cos et al., 2006) and the ratio between minimal bacterial concentrations (MBC) and MIC (MBC/MIC) ≥16 were considered "good results." MBC is ≥16 times the MIC value means that the microorganism is tolerant following criteria analyzed by Sherris (1986). Articles with description of antibacterial techniques were described but no results were excluded from the analysis. Data systemizing and graph building were done using Microsoft Excel 2010 software and results were expressed in absolute values and frequencies.

RESULTS AND DISCUSSION

Through refined search using the keywords "plant extract," "*Streptococcus mutans,*" and "medicinal plants," a total of 129 available articles were found. According to selection criteria, 28 articles were selected to this review giving 135 plant species classified in 60 botanical families tested against *S. mutans*. Asteraceae, Fabaceae, Lamiaceae, and Anacardiaceae families were the most frequent (Figure 1 and Table 1) and 30 plant families had relevant results by agar diffusion, MBC/MIC, or both (Table 2). Among them, *Rhus standley* (Anacardiaceae), *Amphipterygium adstringens* (Anacardiaceae), *Aloe vera* (Asphodelaceae), *Mikamia glomerate* (Asteraceae), *Tagetes lucida* (Asteraceae), *Bixa orellana* (Bixaceae), *Bursera simaruba* (Burseraceae), *Drymariagracilis* (Carophyllaceae), *Cnidoscolus multilobus* (Euphorbiaceae), *Glyycyrrhiza uralensis* (Fabaceae), *Liquidambar macrophylla* (Hamamelidaceae), *Cinamomum vera* (Lauraceae), *C. zeylanicum* (Lauraceae), *Persea americana* (Lauraceae), *Eysenhardti apolystachya*

Table 1. Plant species used for anti-*Streptococcus mutans* tests reported in peer reviewed articles published from January 1st, 2006 to December 31th, 2016.

S/N	Family	n	Species
1	Acanthaceae	1	*Justicia spicigera* Schlechtendal
2	Alliaceae	2	*Allium cepa; Allium sativum*
3	Amaranthaceae	2	*Achyranthes aspera; Beta vulgaris* L.
4	Anacardiaceae*	9	*Amphipterygium adstringens; Anacardium humile; Cotinus coggygria; Pistacia atlantica;Rhus coriaria; Rhus standleyi* Barkley; *Schinus terebinthifolius; Semecarpus anacardium; Spondias purpurea* L.
5	Annonaceae	2	*Annona hypoglauca; Annona senegalensis*
6	Apiaceae	1	*Trachyspermum ammi*
7	Apocynaceae	1	*Calotropis gigentica*
8	Asphodelaceae (Liliaceae)	1	*Aloe barbadensis* miller (*Aloe vera*)
9	Aspleniaceae	1	*Phyllitis scolopendrium*
10	Asteraceae (Compositae)*	13	*Cichorium intybus; Parthenium hysterophorus; Tagetes lucida; Wedelia chinensis; Calendula oficinalis L.; Helichrysum litoreum; Heterotheca inuloides* Cass; *Lostephane heterophylla* (Cav.) Benth.; *Mikania glomerata; Milleria quinqueflora L.; Senecio sessilifolius (*H. et A.) Hemsley; *Coreopsis mutica* DC.; *Cirsum mexicanum* DC.
11	Betulaceae	1	*Alnus acuminata* Kunth
12	Bixaceae	1	*Bixa Orellana* L.
13	Boraginaceae	4	*Cordia* cf. Exaltata; *Cordia nodosa; Cordia sp.; Tournefortia hartwegiana* Standley
14	Brassicaceae	1	*Eutrema japonicum*
15	Bromeliaceae	1	*Ananas comosus*
16	Burseraceae	1	*Bursera simaruba* (L.)
17	Caprifoliaceae	1	*Sambucus mexicana* Presl
18	Caryophyllaceae	1	*Drymaria gracilis* Cham. & Schehlechtendal
19	Celastraceae	1	*Celastrus paniculatus*
20	Chrysobalanaceae	1	*Parinari curatellifolia*
21	Clusiaceae (Calophyllaceae)	4	*Garcinia lancifolia; Garcinia kola; Moronobea coccinea;Mammea americana*
22	Combretaceae	1	*Terminalia chebula*
23	Convolvulaceae	1	*Ipomoea alba*
24	Crassulaceae	1	*Sedum dendroideum* Moc & Sessé
25	Cucurbitaceae	1	*Momordica charantia* L
26	Cupressaceae	1	*Juniperus communis*
27	Ebenaceae	3	*Diospyros guianensis; Euclea divinorum; Euclea natalensis*
28	Equisetaceae	2	*Equisetum arvense; Equisetum hyemale*
29	Euphorbiaceae	3	*Cnidoscolus multilobus* (Pax.) I.M. Johnston; *Croton campestris; Croton draco Schlechtendal*
30	Fabaceae (Leguminosae)*	12	*Bauhinia purpurea; Clitoria ternatea; Copaifera langsdorffii; Erythrina lysistemon; Glycyrrhiza glabra* L; *Glycyrrhiza uralensis; Libidibia ferrea* L.; *Haematoxylon brasiletto; Lysiloma candidum Brandegee; Olneya tesota; Eysenhardtia polystachya* (Ort.) Sarg.; *Prosopsis juliflora* (Swartz) DC.
31	Fagaceae	2	*Quercus elliptica; Quercus infectoria*
32	Gentianaceae	2	*Centaurium erythraea; Centaurium erythraea*
33	Geraniaceae	1	*Pelargonium peltatum*
34	Hamamelidaceae	1	*Liquidambar macrophylla*
35	Lamiaceae (Labiateae)*	11	*Hoslundia opposita; Mentha arvensis L.; Ocimum basilicum; Ocimum sanctum (tenuiflorum);* *Ossimum gratissimum; Perilla frutescens; Rosmarinus officianalis; Salvia officianalis; Thymus vulgaris L.; Mentha viridis L.; Mentha x piperita L.*
36	Lauraceae	4	*Cinnamomum cassia; Cinnamomun zeylanicum* Ness.; *Cinamonum verum; Persea americana* Mill.

Table 1. Cont'd.

37	Lythraceae (Punicaceae)	2	*Lafoensia pacari; Punica granatum*
38	Malpighiaceae	1	*Byrsonima crassifolia* (L.)
39	Meliaceae	2	*Azadirachta indica; Cedrela odorata* L.
40	Mimosaceae	1	*Stryphnodendron adstringens*
41	Mirtaceae	1	*Syzygium aromaticum*
42	Moraceae	1	*Morus alba*
43	Moringaceae	1	*Moringa oleifera*
44	Myristicaceae	1	*Myritica fragrans*
45	Myrtaceae	3	*Myrciaria dubia; Psidium guajava; Rhodomyrtus tomentosa*
46	Papaveraceae	1	*Argemone mexicana* L.
47	Pedaliaceae	1	*Dicerocaryum senecioides*
48	Phyllanthaceae	1	*Emblica offcinalis*
49	Piperaceae	2	*Piper nigrum* L.; *Piper sanctum (Miq.)*
50	Poaceae	1	*Cymbopogon citratus*
51	Rosaceae	3	*Eriobotrya japonica (Thunb.) Lindl; Prunus serotina* var. capuli Karst; *Rosa canina*
52	Rubiaceae	3	*Psychotria sp.; Zanthoxylum compactum; Zanthoxylum piperitum*
53	Salicaceae	2	*Casearia javitensis; Caseria spruceana*
54	Sapotaceae	3	*Englerophytum magalismontanum; Madhuca longifolia; Mimusops elengi*
55	Selaginellaceae	1	*Selaginella lepidophyla* (Hook. &Grev.) Spring
56	Smilacaceae	1	*Smilax sp.*
57	Solanaceae	2	*Datura stramonium* L.; *Solanum cf. lanceifolium*
58	Sterculiaceae	1	*Chiranthodendron pentadactylon* Lam.
59	Verbenaceae	2	*Lantana camara; Verbena carolina* L.
60	Zingiberaceae	2	*Zingiber mioga; Zingiber Officinale*
		135	

*Families most reported; n, number of species reported for each family.

(Leguminosae), *Haematoxylon brasiletto* (Leguminosae), *Cedrela odorata* (Meliceae), *Myrcia riadubia* (Myrtaceae), *Syzygium aromaticum* (Myrtaceae), *Argemone mexicana* (Papaveraceae), *Piper sanctum* (Piperaceae), *Punica granatum* (Punicaceae), and *Datura stramonium* (Solanaceae) results have met international journals standard requirements. It is worth noting that some "good results" reported for agar diffusion was not correlated to a "good result" in MBC/MIC.

Natural products scientific experts have been standardizing parametres to stablish sweatable antimicrobial techniques for plants extracts or substances investigation. In this case, MIC and MBC have been the most recommended strategies (Ríos and Recio, 2005; Cos et al., 2006). From the literature review, only 19 articles met the established criteria here. Results reported involved 135 plants using qualitative techniques such as agar diffusion (20%), quantitative techniques (MBC and/or MIC) (43.69%), and a combination of both (36.28%) (Figure 2). The fact that almost 80% of the results were from quantitative techniques indicates the tendency to improve the quality of results following high scientific impact journals requirements. Regarding the results reported by several of studies analyzed, a combination of both seemed to be the best choice. In this case, in the researchers' opinion, researchers can associate a technique still used in clinical diagnosis (Kirby-Bauer) to a more accurate quantitative procedure: The qualitative method to screen plant extract and the quantitative method to establish extract concentrations.

Analyzing the agar diffusion technique, different variants were reported: Disc-variant, well-variant, and cilinders-variant. They were used in 52.9, 45.43 and 1.62% of the articles reporting agar diffusion as the method of choice, respectively. Comparing the two more-frequent reported, disc-variant and well-variant, and considering the best results (inhibition zone from ≥16), no significant statistical difference ($p=0.35$) was seen between them in relation to halo size results. However, according to Valgas et al. (2007) and Silveira et al. (2009), the sensibility of diffusion method well-variant is superior to disc-variant for two reasons: (a) The presence of suspended particulate matter seems to interfere less with the diffusion of the antimicrobial substance into the agar; and (2) the precipitation of substances (that is, cationic) in the disc may prevent diffusion of antimicrobial substances into the agar. Thus, despite the restriction usage of agar diffusion by natural products researchers, based on easy execution and low coast (Silveira et al., 2009), it is believe that this approach should still be

Table 2. Plant species with relevant anti-*S.mutans* activity by agar diffusion and/or microdilution technique.

Family	Species	Part of the plant	Extract/solvent used	Technique used (unit of measurement)	Concentration (halo ≥18 mm)	MIC	MBC	Reference
Alliaceae	*Allium sativum*	Bulb	Aqueous crude extract	AD-DV (mg/g)	NI	ND	ND	OHara et al. (2008)
				AD-WV (%); MIC/MBC (mg/mL)	0.2	6.25	12.5	Jain et al. (2015)
Amaranthaceae	*Achyranthes aspera*	Root	Ethyl Acetate	AD-DV (mg/disco)	5	ND	ND	Jebashree et al. (2011)
	Pistacia atlantica	Leaf	Aqueous	AD-DV/WV - (mg/ml) MIC/MBC - (µg/mL)	40, 80, 100	60	90	Roozegar et al. (2016)
	Rhus coriaria	Peel fruit	Aqueous	AD-WV; MIC/MBC (mg/ml)	100	1.56	6.25	Vahid-Dastjerdi et al. (2014)
	Rhus standleyi Barkley	Aerial parts	Aqueous	MIC/MBC (µg/mL)	ND	32.5	125	Rosas-Piñón et al. (2012)
			Ethanolic	MIC/MBC (µg/mL)	ND	65	250	
	Schinus terebinthifolius	Leaf, stem bark	n-hexane	AD-DV; MIC (mg/mL)	20 mg/Ml (initial concentration)	3.25	ND	Pereira et al. (2011)
	Amphipterygiumadstringens Schiede ex Schlechter	Stem bark	Aqueous	MIC/MBC (µg/mL)	ND	67.5	>1000	Rosas-Piñón et al. (2012)
Apocynaceae	*Calotropis gigentica*	Leaf	Ethanol	AD-WV (%)	1.25 and 20	ND	ND	Sharma et al.(2015)
Asphodelaceae (Liliaceae)	*Aloe Vera*	Leaf	Crude (gel)	AD-WV (%) MIC (µg/mL)	50 and 100	12.5	ND	Fani and Kohanteb. (2012)
Asteraceae	*Mikania glomerata*	Certified dried aerial parts	Sequence of dichloromethane, methanol/H_2O (9:1), and n-hexane	MIC/MBC (µg/mL)	ND	6.25	12.5	Andrade et al. (2011)
	Tagetes lucida	Aerial parts	Ethanolic	MIC/MBC (µg/mL)	ND	62.5	250	Rosas-Piñón et al. (2012)
Bixaceae	*Bixa Orellana L.*	Leaf	Methanol	AD-C-PV	NC	62.50		Medina-Flores et al. (2016)
		Seeds	Methanol	MIC (µg/mL)		31.25		
Burseraceae	*Bursera simaruba* (L.) Sarg.	Stem bark	Aqueous	MIC/MBC (µg/mL)	ND	100	62.5	Rosas-Piñón et al. (2012)
			Ethanolic	MIC/MBC (µg/mL)	ND	62.5	750	
Caryophyllaceae	*Drymaria gracilis* Cham. & Schehlechtendal	Leaf	Aqueous	MIC/MBC (µg/mL)	ND	67.5	500	Rosas-Piñón et al. (2012)
Chrysobalanaceae	*Parinari curatellifolia*	Stem	Ethanol	AD-WV; MIC/MBC (mg/ml)	100	6.5	25	Oshomoh and Idu (2012)
Clusiaceae	*Garcinia lancifolia*	Fruit	Crude (juice)	AD-WV (mg/ml)	5	ND	ND	Policegoudra et al. (2012)
		Branch	Methanol	AD-WV (mg/ml)	5	ND	ND	
		Leaf	Methanol	AD-WV (mg/ml)	5	ND	ND	
		Fruit	Methanol	AD-WV (mg/ml)	5	ND	ND	
		Leaf	Dichlorometane	AD-WV (mg/ml)	5	ND	ND	

Table 2. Contd.

Combretaceae	*Terminalia chebula*	Fruit	Hexane Ethyl acetate Ethanol Methanol	MIC (mg/mL)	ND	0.76	ND	Jebashree et al. (2011)
Compositae	*Iostephane heterophylla*(Cav.) Benth.	Roots	Aqueous	MIC/MBC (µg/mL)	ND	67.5	125	Rosas-Piñón et al. (2012)
Convolvulaceae	*Ipomoea alba* L.	Aerial parts	Organic	AD-WV/MIC/MBC (mg/ml)	8.34	<0.04	≤0.04	Silva et al. (2014)
			Chloroform fraction	AD-WV/MIC/MBC (mg/ml)	10.78	ND	ND	
Euphorbiaceae	*Cnidoscolus multilobus* (Pax.) I.M. Johnston	Leaf	Aqueous	MIC/MBC (µg/mL)	ND	62.5	250	Rosas-Piñón et al. (2012)
			Ethanolic	MIC/MBC (µg/mL)	ND	15.6	<250	
Fabaceae	*Glycyrrhiza uralensis*	Root	Deglycyrrhizinated licorice root extract	MIC/MBC (µg/mL)	ND	8	8	Ahn et al. (2012)
Geraniaceae	*Pelargonium peltatum*	Leaf	Aqueous	AD-DV (mg/ml)	≥200	ND	ND	Hurtado et al. (2013)
Hamamelidaceae	*Liquidambar macrophylla*	Leaf	Ethanolic	MIC/MBC (µg/mL)	ND	67.5	500	Rosas-Piñón et al. (2012)
Lamiaceae	*Ocimum sanctum (tenuiflorum)*	Leaf	Ethanol	AD-WV (%)	5 and 10	ND	ND	Pai et al. (2015)
Lauraceae	*Cinamonum verum* J. Presl	NI	Hexane extraction	MIC/MBC (µg/mL)	200	ND	ND	OHara et al. (2008)
	Cinnamomun zeylanicum Ness.	Bark	Aqueous	MIC/MBC (µg/mL)	ND	62.5	250	Rosas-Piñón et al. (2012)
	Persea americana Mill.	Leaf	Aqueous	MIC/MBC (µg/mL)	ND	32.5	125	
			Ethanolic	MIC/MBC (µg/mL)	ND	65	500	
Leguminosae	*Eysenhardtia polystachya* (Ort.) Sarg.	Wood	Aqueous	MIC/MBC (µg/mL)	ND	78	500	Rosas-Piñón et al. (2012)
	Haematoxylon brasiletto Karst	Stem barck	Aqueous	MIC/MBC (µg/mL)	ND	10.5	125	
			Ethanolic	MIC/MBC (µg/mL)	ND	12.5	125	
Lythraceae	*Lafoensia pacari*	Leaf, roots, stem	Ethanol	AD-DV; MIC (mg/ml)	20 mg/mL initial concentration	1.0	ND	Pereira et al. (2011)
Meliaceae	*Cedrela odorata* L.	Seed	Aqueous	MIC/MBC (µg/mL)	ND	60	500	Rosas-Piñón et al. (2012)
			Ethanolic	MIC/MBC (µg/mL)	ND	32.5	250	
Myrtaceae	*Myrciaria dubia*	Seeds	Methanol	AD-WV (w/v)	1:1			Camere-Colarossi et al. (2016)
		Pulp	Methanol	MIC (µg/mL)	ND	62.5	ND	
	Psidium guajava	Leaf	Ethyl Acetate	AD-DV (mg/disco)	2.5	ND	ND	Jebashree et al.(2011)
			Ethyl acetate, hexane, etanol, and methanol	MIC (mg/mL)	ND	<0.076	ND	
	Syzygium aromaticum (L.) Merr. & Perry	Fruit	Aqueous	MIC/MBC (µg/mL)	ND	25	250	Rosas-Piñón et al. (2012)
			Ethanolic	MIC/MBC (µg/mL)	ND	62.5	125	

Table 2. Contd.

Papaveraceae	*Argemone mexicana* L.	Leaf	Aqueous	MIC/MBC (µg/mL)	ND	78	500	Rosas-Piñón et al. (2012)
Phyllanthaceae	*Emblica offcinalis*	Fruits	Crude extract	AD-WV (%)	0.2	25	50	Jain et al. (2015)
			Organic extract	MIC/MBC (mg/mL)	0.2	50	100	
			Aqueous extract		0.2	12.50	50.00	
Piperaceae	*Piper sanctum* (Miq.)	Leaf	Ethanolic	MIC/MBC (µg/mL)	ND	62.5	<500	Rosas-Piñón et al. (2012)
Punicaceae	*Punica granatum* L.	Pericarp	Aqueous	MIC/MBC (µg/mL)	ND	12.5	125	
			Ethanolic	MIC/MBC (µg/mL)	ND	62.5	250	
			Ethanol 70% Glucolic etxract	AD-CV (%)	100≥3	ND	ND	Argenta et al. (2012)
Salicaceae	*Caseria spruceana*	Leaf	Organic	AD-WV/MIC/MBC (mg/ml)	200	≥12.5	≥12.5	Silva et al. (2014)
Solanaceae	*Datura stramonium* L.	Aerial parts	Aqueous	MIC/MBC (µg/mL)	ND	100	>1000	Rosas-Piñón et al. (2012)
Zingiberaceae	*Zingiber officinale*	Rhizomes	Organic solvent	AD-WV (%) / MIC/MBC (mg/mL)	0.2	12.50	25.00	Jain et al. (2015)

AD-WV, Agar diffusion-well variant; AD-DV, agar diffusion-disc variant; AD-CP, agar diffusion - cup plate variant; ND, not done; NI, not informed; MIC, minimal inhibitory concentration; MBC, minimal bactericidal concentration; NC, information is not clear

accepted for screening of natural products taking into consideration the fact that this technique is also considered a "gold test" for clinical diagnosis (Schumacher et al., 2018).

A point of concern on medicinal plant folk usage validation is the preparation and solubilization and/or dilution of plant material. The expression of units in the analyzed results in the articles was quite varied: mg/mL (54.52%) followed by percentage (23.67%). It is important to note that 15.95% used mg/disco, 4.78% of articles did not mention any amount of measurement, and 1.06% used proportion criterion (volume/volume or weight/volume). When the best results (≥18.5 mm inhibition zone) was considered, the ones expressed in mg/mL (63.33%) were the most promising when compared to results expressed in percentage (13.33%). Furthermore, analyzing the diferences in agar diffusion technique, the more frequent was mg/mL usage on DV (62.43%) and percentage unit on WV (45.08%) (Table 3). Hence, taking into consideration these aspects, two points which deserve attention were considered: (1) the standardization of international units requested by journal editors (Cos et al., 2006) recognizing that mg/mL is still the most accurate measurement for *in vitro* tests instead of percentage, and (2) percentage seems to better represent the folk method or it is the best choice for some plant material processing (that is, resin, pasty, or gelatinous substances) and it should not be neglected.

Taking into consideration the best plants results against *S. mutans* by agar diffusion technique, three vegetal products were distinguishable in their results (≥ 30 mm inhibition zone): *Aloe vera* gel, *Garcinialancifolia* fruit juice, and *Allium sativum* bulbo juice. The *Aloe vera* gel at 50 and 100% showed inhibition zone of 30 and 54 mm, respectively, and antibacterial action was confirmed by promising results showed in MIC technique (12.5 µg/mL) (Fani and Kohanteb, 2012).The *G. lancifolia* fruit juice at 5 mg/mL gave 47 mm inhibition zone (Policegoudra et al., 2012) and the bulbo juice from *A. sativum* at 100 mg/mL showed 30 mm of inhibition zone (OHara et al., 2008). It is important to note that in the case of *A. sativum* (Jain et al., 2015), although the anti-*S. mutans* potential has been also confirmed by MIC (6,250 µg/mL) and MBC (12,500 µg/mL), the concentration showed in these two quantitative techniques are not in the parameters established by high impact publications since they use (Cos et al., 2006) reference which, unfortunately, makes uncertain the validation of these species.

From the articles selected (n=43) using broth dilution as technique for searching natural products

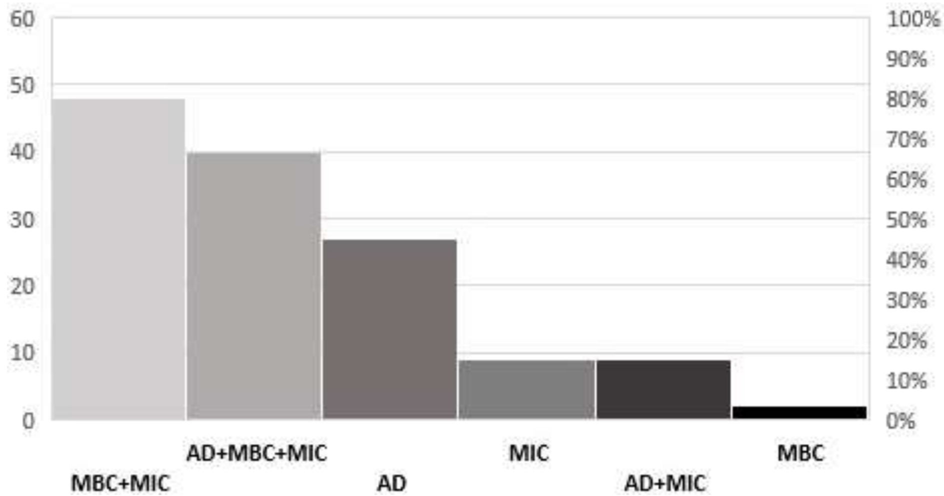

Figure 2. Qualitative and quantitative techniques reported in peer reviewed articles found in databases from January 2006 to December, 2016. AD, Agar diffusion technique; MIC, Minimal inhibitory concentration technique; MBC, Minimal bacterial concentrations technique.

Table 3. Frequency of results reported in articles using agar diffusion technique indexed in database in the period of January, 2006 to December, 2016.

AD variant	mg/mL	%	mg/disco	Proportion (v/v or w/v)	Not reported	Total
DV						
Total	123(62.43%)	14 (7.10%)	60(30.45%)	0	0	**197**
18,5-54 mm	9(75%)	2(16.66%)	1(8.33%)	0	0	**12**
WV						
Total	74(43.78%)	73(43.19%)	--	4(2.36%)	18(10.65%)	**169**
18,76-47 mm	10(55.55%)	2(11.11%)	--	2(11.11%)	4(22.22%)	**18**
Total	**216**	**91**	**61**	**6**	**22**	**396**

DV, Disc variant; WV, well variant; v/v, volume/volume; w/v, weight/volume

with anti- *S. mutans* activity, few (n=17) showed MIC and MBC in association. A ratio between MBC/MIC (r) was obtained in order to evaluate the quality of antibacterial results (Table 4). A ratio ≥16, indicating bactericidal tolerance, was found only for three species ethanolic extracts: *Cnidoscolus multilobus*, *Tournefortia hartwegiana*, and *Coreopsis mutica*. However, since MIC was considered “good results,” in the researchers’ opinion, it would be interesting to isolate molecules with antimicrobial action from these species. Microbial tolerance may be influenced by bactericidal activity such as antagonism of molecules, technical factors, or microorganism characteristics (Sherris, 1986; Traczewski et al., 2009).

Also, considering ideal MIC values, it was observed that for MIC ≥ 100 µg/mL, MBC values were 3 times higher; while for MIC ≤ 100 µg/mL, MBC values were next to 6 times higher then MIC. From the study point of view, the MIC values were higher than clinical valuable concentrations suggested in the literature (Cos et al., 2006) to reach a bactericidal effect and it means that the natural products tested had a tendency to be bacteriostatic against *S. mutans*.

Following the same criteria cited earlier (MIC ≤ 100 µg/mL and r=1) and establishing the best products reported in literature, *Glycyrrhiza uralensis* deglycyrrhizinated licorice root extract (Ahn et al., 2012), *Mikania glomerate* ent-Kaurenoic acid-rich extract (Moreira et al., 2016b), *Ipomoea alba* chloroform fraction (Silva et al., 2014), and *Pistacia atlantica* aqueous extract (Roozegar et al., 2016) showed the best results. Considering isolated substances and criteria established here, only saponin class showed important results. Saponins isolated from the seeds of *Madhuca longifolia* and *Bauhinia purpurea* were tested against two *S. mutans* strains and promising results were found for both (Jyothi and Seshagiri, 2012) (Table 5).

The polarity of plant metabolites is also a point of concern since it can interfere on substances diffusion and/or solubilization. Non-polar or other samples difficult

Table 4. MBC and MIC ratio of plant extracts with MIC ≤ 100 µg/mL tested against *S. mutans* reported in peer reviewed articles (January, 2006 to December 2016).

S/N	Species	Plant family	Part of the plant	Type of extract/solvente used	MIC µg/mL	MBC µg/mL	r	References
1	*Mikania glomerata*	Asteraceae (Compositae)	Aerial organs	ent-Kaurenoic acid-rich extract ♣	6.50	12.50	1.92	Moreira et al., 2016
2	***Glycyrrhiza uralensis***	Fabaceae	Root	deglycyrrhizinated licorice root extract♥	8.00	8.00	1.00	Ahn et al., 2012
3	*Haematoxylon brasiletto*	Fabaceae (Leguminosae)	Branch, barck	Áqueous	10.50	125.00	11.90	Rosas-Piñón et al., 2012
4	*Haematoxylon brasiletto*	Fabaceae (Leguminosae)	Branch, barck	Ethanol	12.50	125.00	10.00	
5	*Punica granatum L.*	Punicaceae	Pericarp	Áqueous	12.50	125.00	10.00	
6	*Cnidoscolus multilobus (Pax.) I.M. Johnston*	Euphorbiaceae	Leaves	Ethanol	15.60	< 250.00	16.03	
7	*Syzygium aromaticum (L.) Merr. & Perry*	Myrtaceae	Fruit	Áqueous	25.00	250.00	10.00	
8	*Rhus standleyi Barkley.*	Anacardiaceae	Aerial parts	Áqueous	32.50	125.00	3.85	
9	*Persea americana Mill.*	Lauraceae	Leaves	Áqueous	32.50	125.00	3.85	
10	*Heterotheca inuloides Cass.*	Compositae	Aerial parts	Ethanol	32.50	125.00	3.85	
11	*Cedrela odorata L.*	Meliaceae	Seed	Ethanol	32.50	250.00	7.69	
12	*Ipomoea alba*	Convolvulaceae	Aerial organs	Chloroform fraction	< 40.00*i	≤40.00*i	1.00	Silva et al., 2014
13	*Ipomoea alba*	Convolvulaceae	Aerial organs	Chloroform fraction	60.00*ii	80.00*ii	1.33	
15	*Pistacia atlantica*	Anacardiaceae	Leaves	Áqueous	60.00	90.00	1.50	Roozegar et al., 2016
16	*Cedrela odorata L.*	Meliaceae	Seed	Áqueous	60.00	500.00	8.33	Rosas-Piñón et al., 2012
17	*Syzygium aromaticum (L.) Merr. & Perry*	Myrtaceae	Fruits	Ethanol	62.50	125.00	2.00	
18	*Punica granatum L.*	Punicaceae	Pericarp	Ethanol	62.50	250.00	4.00	
19	*Cinnamomun zeylanicum Ness.*	Lauraceae	Barck	Áqueous	62.50	250.00	4.00	
20	*Cnidoscolus multilobus (Pax.) I.M. Johnston*	Euphorbiaceae	Leaves	Áqueous	62.50	250.00	4.00	
21	*Tagetes lucida*	Asteraceae	Aerial parts	Ethanol	62.50	250.00	4.00	
22	*Piper sanctum (Miq.)*	Piperaceae	Leaves	Ethanol	62.50	< 500.00	8.00	Rosas-Piñon et al., 2012
23	*Bursera simaruba (L.)*	Burseraceae	Branch, barck	Ethanol	62.50	750.00	12.00	
24	*Tournefortia hartwegiana Standley.*	Boraginaceae	Aerial organs	Ethanol	62.50	> 1000.00	16.00	
25	*Coreopsis mutica DC.*	Compositae	Aerial organs	Ethanol	62.50	> 1000.00	16.00	
26	*Rhus standleyi Barkley.*	Anacardiaceae	Aerial organs	Ethanol	65.00	250.00	3.85	
27	*Persea americana Mill.*	Lauraceae	Leaves	Ethanol	65.00	500.00	7.69	
28	*Iostephane heterophylla (Cav.) Benth.*	Compositae	Root	Áqueous	67.50	125.00	1.85	
29	*Drymaria gracilis Cham. & Schehlechtendal*	Caryophyllaceae	Leaves	Áqueous	67.50	500.00	7.41	
30	*Liquidambar macrophylla*	Hamamelidaceae	Leaves	Ethanol	67.50	500.00	7.41	
31	*Amphipterygium adstringens*	Anacardiaceae	Branch, barck	Áqueous	67.50	> 1000.00	14.81	
32	*Argemone mexicana L.*	Papaveraceae	Leaves	Áqueous	78.00	500.00	6.41	
33	*Eysenhardtia polystachya (Ort.) Sarg.*	Fabaceae (Leguminosae)	Wood	Áqueous	78.00	500.00	6.41	
35	*Ipomoea alba*	Convolvulaceae	Aerial organs	Chloroform fraction	m100.00	160.00	1.60	Silva et al., 2014
36	*Bursera simaruba (L.)*	Burseraceae	Branch, barck	Áqueous	100.00	500.00	5.00	Rosas-Piñon et al., 2012
37	*Datura stramonium L.*	Solanaceae	Aerial organs	Áqueous	100.00	1000.00	10.00	

r, Ratio between MBC and MIC; *m, Mean of reported values;♣ The final product was originated from soluble fraction of dichloromethane extraction, partitioned with n-hexane; ♥Dried roots were passed thrhough heating in distilled water (20:1 [v/w]) for 2 h, heating (78°C) in 95% ethanol (95% ethanol:residue ratio of 15:1 [v/w]) for 2 h, column (6.5 cm - 60 cm) filled with Diaion HP-20 adsorbent equilibrated with distilled water, 50% ethanol and 99% etanol. *The same extract was tested for two different bacterial concentration: i – 1×10^2; ii – 1×10^3

Table 5. Minimal inhibitory concentration and minimal bactericidal concentration of plant compounds with MIC ≤ 30 μg.mL^{-1} tested against *S. mutans*.

Species	Family	Part of the plant	Compound	*S. mutans* strain	MIC (µg/ml)	MBC (µg/ml)	MBC / MIC
Madhuca longifolia	Sapotaceae	Seed	Saponin	MTCC 890	18.30	34.40	1.88
				MTCC 497	23.60	39.60	1.68
Bauhinia purpurea	Fabaceae	Seed	Saponin	MTCC 890	26.40	43.00	1.63
				MTCC 497	26.30	38.00	1.44
			Ent-kaurenoic acid	ATCC25175	10	20	2

MIC, Minimal inhibitory concentration; MBC, Minimal bactericidal concentration.

to diffuse in the medium, should be avoided in diffusion methods (Valgas et al., 2007). In this review the most frequent category used was polar solvent but it is worth noting that a combination of solvent or crude extracts obtained directly from the plant without solvent addition was also described. By agar diffusion technique, from non-polar solvent extraction, only two showed ≥ 18 mm inhibition zone: The hexanic extract from bark of *Cinamonum verum* (OHara et al., 2008) and the hexanic extract from bark/leaves of *Schinuster ebinthifolius* (Pereira et al., 2011).

Both articles reported the use of dimetylsulfoxide as solvent to solubilize the powdered extracts. Thus, although both hexanic extracts were active at higher than clinically acceptable concentrations, the results published by Ohara et al. (2008) and Pereira et al. (2011) suggested that solvent such as dimetylsulfoxide can help some molecules to diffuse into agar medium and make possible the use of agar diffusion technique for apolar substances as suggested by Valgas et al. (2007).

The antibacterial property of a variety of natural products is documented; nonetheless, the great variability of secondary metabolites composition makes the studies in this area always laborious.For anti-*S. mutans*, it seems there is a restriction in this variety of substances. From the peer reviewed sources used in this work, the only pure substance report was saponins extracted from four species: *Madhuca longifolia, Bauhinia purpurea, Celastrus paniculatus,* and *Semecarpus anacardium* by Jyothi and Seshagiri (2012) with promising results only for saponins isolated from *M. longifolia.* The antimicrobial broad spectrum of saponins has been reported (Avato et al., 2006; Qin et al., 2016), however the amphiphilic characteristics of this class, with the diversity which it carries, plays an important role in the antimicrobial property itself or in its solubilization in aqueous based medium used in the techniques employed were considered.

Conclusion

The present systematic review showed a comprehensive examination of medicinal plants under the perspective of anti-*S. mutans* activity. Results showed that agar diffusion technique is still widely used for medicinal plants antimicrobial activity screening being either well-variant or disc-variant worthwhile as screening tests. The remarkable results from *A. vera*, *G. lancifolia* and *A. sativum* by agar diffusion or from *G. uralensis*, *M. glomerata*, *I. alba*, and *P. atlantica* by MIC/MBC show that although taxonomic criterion may be considered a leader for antimicrobial activity, ethnobotanical criterion should also be considered an excellent guide for in vitro studies. The unit of measurement used (µg/mL) is scientifically considered more accurate; however, it is worth noting that percentage as unit of measurement warrants validation since it mimics the popular usage of plants. Finally, the standardization of antimicrobial protocols for medicinal plants antimicrobial tests is needed in order to obtain more accurate results and make the comparision between natural products and controls easier.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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