Development of effervescent medical powder containing *Maytenus ilicifolia* extract Mart. ex Reissek for treatment of gastric disorders

Niraldo Paulino¹,²*, Gisele Fernanda Vieira¹, Minoru Ikekawa da Silva Braga¹, Luana Beatriz de Lima Carvalho¹, Isaias Pompeu Bernardo¹, Vanessa Franquini Nogueira³

¹Faculdade de Guarulhos, Grupo Universidade Brasil, Av. Guarulhos, 1844 - Vila Augusta, Guarulhos, SP, 07025-000
²Medical Lex Gestão de Informações e Cursos Ltda, Av. Desemb. Vitor Lima, 260, sala 908, Trindade, Florianópolis, SC, 88040-400

Abstract

Brazil is the country with the biggest biodiversity on the planet, but it does not avail itself enough of this potential to produce herbal medicines. *Maytenus ilicifolia* Mart. Ex Reissek is popularly known as “Espinheira-Santa” and in Brazil is widely used in popular medicine to treat a number of diseases such as gastritis and dyspepsia, and also displays laxative, diuretic, analgesic, antiseptic and healing properties and antineoplastic and antimicrobial activities. Among the reasons that hinder a better exploration of such biodiversity, the absence of an innovative culture draws a lot of attention, besides the inherent difficulties in the characteristics of the research and the development of this product modality. The aim of this study was developing an effervescent powder containing medicinal extract of *M. ilicifolia*. The methodologies to determine quality of the plant and extract including the organoleptic characteristics, the moisture content and volatiles, total ashes, extractive content, pH, and the bulk density. In our experiments, we demonstrated that the plant has been identified and has displayed the appropriate organoleptic characteristics to the preparation of the extract; we also demonstrated that the extract has been incorporated into a formulation that maintained its organoleptic characteristics, pH and content, as well as the formation of stable gases. With these experiments, we demonstrate for the first time that the developed formulation can serve as a prototype for the development of pharmaceutics effervescent product containing standardized extract of *M. ilicifolia*.

Keywords: Effervescent salt, *Maytenus ilicifolia*, gastritis.

Introduction

Gastric disorders are a major complaint of patients in the healthcare system worldwide, especially those changes related to the stomach and/or duodenum, indicating a proportion of two out of 10 people affected by problems ranging from transient malaise to gastritis, ulcer, and tumors.¹

Factors such as stress, *Helicobacter pylori* infection, smoking, poor nutrition, alcohol, continuous use of anti-inflammatory drugs, genetic predisposition, and endogenous aggressors (acid, pepsin, and bile) are associated with the etiology of this disease. It is generally accepted that gastric ulcer is established when the balance between aggressive and mucosal defending factors is disrupted.²

The Ulcer is a deep lesion of the gastric mucosa that affects the components of epithelial and connective tissue, including subepithelial myofibroblasts, smooth muscle cells, vessels, and nerves. Such ulcerative lesions can be acute or chronic and most often appear in any portion of the gastrointestinal tract exposed to the aggressive action of peptic acid.³-⁵

Several drugs have been used in the treatment of gastric and duodenal ulcers, with gastric antisecretory agents such as H₂-type histaminergic receptor antagonists being of great importance protons such as omeprazole and lansoprazole.⁶

*Maytenus ilicifolia* belongs to the Celastraceae family, contains 98 genus and approximately 1264 species found mainly in tropical regions (Figure 1). Popularly known as “maideno, espinheira-santa, cancerosa, salva-vidas,” this plant is originally from Brazil, its leaf is used. The “espinheira-santa” is a sub-shrub, its size can vary from 2 to 5 m in height. The stem is
woody and has pointed and jagged leaves 4 to 12 cm long. The primary chemical constituents reported for *Maytenus* spp. include terpenes (maytin, tringenone, isotenginone II, congorosins A and B, maitenoic acid), triterpenes (friedelanol and friedelin), essential oils (frienedenol), tannins, especially galics (epicatechin, epigallocatechin, and gallate of epigallocatechin), glycol monogalactosyldiacylglycerol, digalactosyldiacylglycerol, trigalactosyldiacylglycerol, tetragalactosyldiacylglycerol and sulphoquinovosyldiacylglycerol) and, lastly, alkaloids (maitein, maytinprine and maytol).\(^6\)\(^8\)\(^9\) While Chiapetti et al\(^1\)\(^2\) established methods of analyzing the different plant extracts of *Maytenus ilicifolia* by the HPLC technique.

*Maytenus ilicifolia* can be used for gastritis, dyspepsia, and adjunctive treatment of peptic ulcers. It acts as a regulator of stomach functions and promotes the protection of the gastric mucosa.\(^10\)\(^11\)

This paper aims to develop an effervescent medicinal powder containing a standardized extract of *Maytenus ilicifolia*.

### Methodology

#### Materials

In these experiments we use the following equipment: volumetric flask, analytical balance (explorer Ohaus), water bath, beaker, crucible, condenser, spatula, spectrophotometer, greenhouse, amber container, funnel, refrigerator, mortar, and pestle, warming blanket, muffle, filter paper, pH meter (Ohaus), rotavapor (Fisatom), test tube.

#### Methods

##### Plant Quality Control

The plant drug consists of dry leaves containing at least 2% of total tannins. Total tannins consist of at least 5% tanning fraction and at least 4% non-tanning fraction, as described in the Farmacopéia Brasileira. The tannins found in *Maytenus ilicifolia* were quantified by the gravimetric method through acetate precipitation of copper, method developed by Caldeira.\(^16\) The *Maytenus ilicifolia* samples used in the tests were from pharmacies in the city of São Paulo, Brazil.

The determination of organoleptic characteristics, such as appearance, color, and odor, were visually evaluated.\(^17\) Briefly, the plant samples were processed according to the sensory characteristics evaluation, according to the monographs in the Farmacopéia Brasileira.\(^18\)

Moisture/Volatile materials tests were performed as follows:

- A dry, empty crucible was weighed, added 2 g of the dried plant, brought to the greenhouse at 105°C, weighed every hour until it was kept constant. A trial was done in triplicate.
- In order to determine the % total ashes, after keeping the constant weight of the moisture/volatile materials test, it was taken to muffle for two hours after it was taken out and weighed. A trial was done in triplicate.

To determine the extractive content, 4 g of the plant was weighed and placed in a volumetric flask and made up to 100 mL of water, using the heating blanket and the condenser was kept at temperature for one hour, filtered to obtain the plant extract, then 10 mL was taken. The solution is placed in the oven at 105°C and weighed every hour to constant weight. A trial was done in triplicate.

##### Method of Preparing Extracts

The method used was maceration. 200 g of the dried plant was weighed. This was placed in an amber glass vial and made up to 2000 g with 77°GL cereal alcohol. This was kept in the dark environment and was shaken daily for 2 minutes for better extraction, and this was held for 7 days. Then the extract was filtered on filter paper. The obtained extract in rotavapor, to make a fractional distillation, thus removing the alcohol, leaving only the concentrated extract of the plant.\(^20\)

Sampling was performed according to the Farmacopéia Brasileira.\(^21\) All samples were evaluated in duplicate. Generic tannin extraction: we weighed 1g of the sample in a 100 mL beaker and added 25 mL of distilled water, boiled for 2 minutes; the supernatant was filtered using quantitative filter paper (Quality brand, weight 80 g/m², thickness 205 µm and gray 0.5%) to keep the powder at the bottom of the initial container; this process was repeated twice. At the end of the procedure, the extract was transferred to a 100 mL volumetric flask and made up to 100 mL (1:100) with distilled water.\(^22\)

The organoleptic characteristics, such as appearance, color and odor were visually evaluated, to perform the quality control of the extract.\(^17\)

The determine pH as follows: the electrode was removed from the KCl solution, washed with distilled water jets, and then dried with filter paper. Calibration pH, the electrode was washed with distilled water and dried with filter paper.\(^23\) Triplicate pH measurement.

##### Method of Preparing Effervescent Powder

To prepare the effervescent powder, tartaric acid was added to 100 mL (1:100) with distilled water. The determine pH as follows: the electrode was removed from the KCl solution, washed with distilled water jets, and then dried with filter paper. Calibration pH, the electrode was washed with distilled water and dried with filter paper.\(^23\) Triplicate pH measurement.
weighed, placed in a porcelain mortar and ground to reduce the particle size of the powder. The citric acid was weighed and put to the same mortar, ground, and homogenized. The sodium bicarbonate was weighed and added to the same mortar, ground and homogenized. The prepared powder was used to add varying percentages of the Maytenus ilicifolia extract, making the mixture with a dough consistency. This formed mass was passed through a sieve 60 to form granules and then placed in the oven to dry 45°C for 24 hours. This was removed from the greenhouse and re-sieved in the same mesh, obtaining an effervescent powder. This effervescent powder was used as a base for the addition of organoleptic modifying agents such as flavoring and sweetening agents.

Stability Assessment Method

Cycle Testing
The stability of the Maytenus ilicifolia extract powder was evaluated per sample, which was stored 24 hours at room temperature (25°C), 24 hours in the oven (45°C), and 24 hours in the freezer (-5°C), requiring a repetition of this procedure for 42 days. In the end, the organoleptic characteristics (appearance, color, taste, odor), effervescence time, pH, density were evaluated. Every 2 weeks a sample was taken from each temperature, and 24 hours later, it was evaluated.

Accelerated Stability Test
The stability of Maytenus ilicifolia extract containing powder was evaluated under different temperature conditions for a period of 42 days. The prepared powder was packed in 16 amber vials. The samples were divided into four groups of four samples each, and each group was subjected to one of the following temperature conditions: room temperature (25°C), oven (45°C), freezer (-5°C) and refrigerator (+4°C). On the first day, the organoleptic characteristics, effervescence time, pH, and density were analyzed. Every two weeks a sample was taken from each temperature and 24 hours later it was evaluated.

Determination of Bulk Density

A clean, dry, and previously calibrated metal pycnometer was used for density determination. Calibration consisted of determining the mass of the empty pycnometer and the weight of its contents with water already distilled at 20°C. The sample was placed on the pycnometer, and the excess substance was removed and weighed. The weight of the sample was obtained by the difference in the mass of the full and empty pycnometer. The ratio of net mass to water mass, both at 20°C, is the relative density.

Results

Plant Quality Control
The plant was identified as Maytenus ilicifolia, according to the report analyzed from the supplier. The result of organoleptic characteristics was aspect ground, color greenish brown, and odor plant characteristic.

In the moisture/volatile materials test, the sample presented a value of 6.18%, having as maximum reference value 8% shown in the Farmacopéia brasileira, so there was no deviation in the result.

In the total ashes test, the sample presented the value of 6.18%, having as maximum reference value 15% presented in the Farmacopéia portuguesa, so there was no deviation in the result.

The value of the extractive content in the sample was 26%, with a minimum reference value 15% presented in the British pharmacopeia, so there was no deviation in the result.

Extract Quality Control
The result of organoleptic characteristics was aspect liquid, color greenish brown, flavor extremely bitter, and odor plant characteristic. The pH value was 5.29.

The final formulations and the degree of usual proportions in the formula for effervescent sodium phosphate are shown in Table 1.

The formulation was developed to contain 1 g of standardized dry extract of Maytenus ilicifolia equivalent to 35 mg of total tannins expressed as Maytenus tannins distributed in a 50g sample of effervescent powder.

Discussion

According to some studies, M. ilicifolia present action against peptic ulcer and gastritis. Coulaud-Cunha et al report that the action of M. ilicifolia on the peptic ulcer and gastritis involves more than one mechanism of action, not
Table 7. Raw Material Quantity

| Material                                      | Quantity |
|-----------------------------------------------|----------|
| Dibasic sodium phosphate dry and powdered     | 18%      |
| Tartaric acid, dry powder                     | 23%      |
| Sodium bicarbonate                            | 42%      |
| Citric acid, monohydrate                      | 15%      |
| Standardized dry extract M. ilicifolia Mart. ex Reissek | 2%       |

yet conclusively elucidated, that both tannins, especially epigallocatechin, and essential oils, especially fridenol, are responsible by the gastroprotective effects.

Carlini and Frochtingarten,28 in studies with M. ilicifolia stuffy, report that the longer the treatment, the higher the gastroprotection without changes in pH. Such observation of M. ilicifolia in frogs proved that this has an inhibitory effect on histamine H₂ mediators in parietal cells. According to Gilman et al,32 when stimulated, cause the activation of adenylyl cyclase, initiating a series of complex morphological and biochemical alterations, which leads to increased gastric secretion, functioning as an H₂ antagonist, besides inhibiting the effect of gastrin. It has also been shown that both epigallocatechin (tannin) and fridenol (essential oil) are responsible for part of the protective effect of the gastric mucosa.29,33

The evidence of the synergistic effect between the components of M. ilicifolia was corroborated in studies by Queiroga et al,34 which demonstrated that tannins, when used separately in indomethacin-induced ulcer models, have no activity.

Carlini and Frochtingarten35 related the action of M. ilicifolia with its richness in tannins and those used. Several gallic tannins, including epigallocatechin, have been shown to inhibit the potassium-dependent membrane from offering a new product ATPase of gastric mucosa cells responsible for the secretion of hydrochloric acid in the stomach. This mechanism is processed by competitive inhibition. Later studies, such as Murakami et al36 and Annuk et al37 proved that there is a non-competitive inhibition, suggesting two distinct places of action. Still, according to Murakami et al,35 epigallocatechin-3-gallate proved to be the most active compound. According to Annuk et al,38 there is another mechanism of action related against H. pylori, frequently involved in clinical studies. Studies showed that gallic tannins of different medicinal plants had bacteriostatic action against H. pylori in vitro. Such action was mainly due to the change in membrane permeability, leading to electrolyte and water losses. It was also demonstrated that they acted to the bacteria's adhesion to the gastric mucosa, preventing its pathogenic action.

Clinical studies have shown M. ilicifolia antimicrobial activity, antiviral potential, antifungal, antiprotozoal, and anti-inflammatory activity.37,38

According to Ferreira et al,39 in a work carried out with aqueous extract of M. ilicifolia leaves it was demonstrated the inhibition of histamine stimulated gastric secretion in frog gastric mucosa. After this experiment, it was concluded that the freeze-dried aqueous extract reduced the basal acid secretion in the frog's isolated gastric mucosa by the antagonistic effect of histamine H₂ receptors, as well as cimetidine and ranitidine.

According to studies carried out in ethanolic extract and freeze-dried aqueous extract of M. ilicifolia, the chemical components mauritianin, trifoline, hyperine, epicatechin, canferol, and galactiol were isolated. From these, it was proved by high-performance liquid chromatography that only the compounds mauritianin and kaempferol have activities on the volume and pH of gastric secretion of rats, being glycosides of great importance on the gastroprotective effect.40

Isolation of friedelan-3β-ol and friedelin triterpenes from M. ilicifolia leaves confirmed that these two substances were not able to decrease indomethacin-induced gastric ulcers in rats.41

Several clinical studies have been performed with M. ilicifolia in the treatment of peptic ulcer and dyspepsia28,42,43,44 and corroborated the actions described in animal models.

“Espinheira-santa” (M. ilicifolia) is a phytotherapeutic of relevant therapeutic action, especially anti-ulcerogenic, given its pharmacological efficacy and safety. It is worth remembering that, due to lack of studies, it is not recommended for children and should not be used by pregnant women, since studies in mice (female and pregnant) indicated a significant decrease in the number of embryos, besides having estrogenic activity, which may interfere in the uterine receptivity of the embryo. Among its pharmacological activities, anti-ulcerogenic activity stands out, which can be compared to the action of ranitidine and cimetidine.45

Tabach et al demonstrated in important preclinical46 and clinical47 studies the toxicological safety and therapeutic efficacy in humans following the use of standardized M. ilicifolia extract.

H₂ receptor antagonists like ranitidine, cimetidine, reversibly, and competitively inhibit histamine binding while proton pump inhibitors like omeprazole and pantoprazole by inhibiting gastric acid secretion. Generally, these drugs are well tolerated; however, occasional adverse effects may occur.

As noted throughout the paper, there are quite effective drugs for the treatment of gastric disorders, but there are always some exceptions to their use. The use of these drugs is widespread and used in chronic treatment, and these need to be monitored for any adverse effects that can only be observed with the abuse or long-term use of the drug.

The new product is a powder containing M. ilicifolia extract. Over time, various formulations were tested to meet market expectations.
In this work, we demonstrate for the first time that an effervescent, stable, and effective formulation can be developed using a standardized extract of *Maytenus ilicifolia* and may serve as a prototype for other herbal formulations (Figure 2).

With the information discussed above, it was possible to conclude that the product had good stability, keeping its organoleptic characteristics stable, with no change in pH, a slight increase in effervescence time, suffering only a change in density, but did not interfere with product quality.

Further quality control testing is also required, as we do not take the time to develop these tests in this paper. Study of the most suitable packaging for this product so that it can enter the market without stability problems.

**Competing Interests**

None.

**References**

1. Sgambato D, Miranda A, Romano L, Romano M. Gut microbiota and gastric disease. Minerva Gastroenterol Dietol. 2017;63(4):345-354. doi:10.23736/s1121-421x.17.02380-7

2. Burukoglu D, Baycu C, Taplamacioglu F, Sahin E, Bektur E. Effects of nonsteroidal anti-inflammatory meloxicam on stomach, kidney and liver of rats. Toxicol Ind Health. 2016;32(6):980-986. doi:10.1177/0748233714538484

3. Uchida M, Kobayashi O, Shimizu K. Gastric emptying after artificial ulceration in rats: differences due to the site of the ulcer and the effects of prokinetic drugs. J Smooth Muscle Res. 2017;53(0):48-56. doi:10.1540/jsmr.53.48

4. Calam J, Baron JH. ABC of the upper gastrointestinal tract: pathophysiology of duodenal and gastric ulcer and gastric cancer. BMJ. 2001;323(7319):980-982. doi:10.1136/bmj.323.7319.980

5. Herrington JL, Jr, Sawyer JL. Gastric ulcer. Curr Probiol Surg. 1987;24(12):759-865. doi:10.1016/0011-3840(87)90018-9

6. Tari A, Hamada M, Kamiyasu T, et al. Effect of enprostil on omeprazole-induced hypergastrinemia and inhibition of gastric acid secretion in peptic ulcer patients. Dig Sci. 1997;42(1741-1746. doi:10.23736/s1121-421x.17.02380-7

7. Hermansson M, Stal1 von Holstein C, Zilling T. Peptic ulcer perforation before and after the introduction of H2-receptor blockers and proton pump inhibitors. Scand J Gastroenterol. 1997;32(6):523-529. doi:10.3109/00365529709025093

8. Fonseca APND, Silva GDF, Carvalho JJ, Salazar GDCM, et al. Estudo fitoquímico do decocto das folhas de Maytenus truncata Reissek e avaliação das atividades antinociceptiva, antiedematogênica e antiulcerogênica de extratos do decocto. Quim. Nova. 2007;30(4):842-847. doi:10.1590/S0100-40422007000400016

9. Mendes BG, Machado MJ, Falkenberg M. Triagem de glicolipídios em plantas medicinais. Rev Bras Farmacogn. 2006;16(4):568-575. doi:10.1590/S0102-695X2006000400022

10. Ohlsaki A, Imai Y, Naruse M, Ayabe S, Komiyama K, Takashima J. Four new triterpenoids from Maytenus ilicifolia. J Nat Prod. 2004;67(3):469-471. doi:10.1039/np030379d

11. Lameira OA, Pinto JEBP. Plantas medicinais: do cultivo, manipulação e uso à recomendação popular. Belém: Embrapa; 2008:19-26.

12. Lorenzi H, Matos FJA. Plantas medicinais no Brasil nativas e exóticas. 2nd ed. Nova Odessa: Plantarum; 2008:544.

13. Chiapetti TP, Malavasi UC, Braga GC, Malavasi MM. Effects of the extraction method and chromatographic separation solvent in the antioxidant activity of different species of “espíneira-santa”. J Food Sci Technol. 2019;56(11):5056-5062. doi:10.1007/s13197-019-03978-1

14. Brasil. Agência Nacional de Vigilância Sanitária. Farmacopeia Brasileira. Formulário Farmacéutico Nacional. http://portal.anvisa.gov.br/documents/338312/259456/Suplemento+FFFB.pdf/478d1f83-7a0d-48aa-9815-37d6e62b999a. Accessed 11 Dec 2019.

15. Santos-Oliveira R, Coulaud-Cunha S, Colaço W. Revisão da Maytenus ilicifolia Mart. ex Reissek, Celastraceae. Contribuição ao estudo das propriedades farmacológicas [Review of Maytenus ilicifolia Mart. ex Reissek, Celastraceae. Contribution to the studies of pharmacological properties], Rev Bras Farmacogn. 2009;19(2b):650-659. doi:10.1590/S0102-695X2009000400025
16. Caldeira MMV, Schumacher MV, dos Santos EM, Viejas J, Pereira JC. Quantificação de tanino em três povoaamentos de Acacia mearnsii de Wild. Colombo: Boletim de Pesquisa Florestal; 1999:81-88.

17. Gomes MJV, Reis AMM. Ciências farmacêuticas: uma abordagem em farmácia hospitalar. São Paulo: Atheneu; 2003.

18. Farmacopéia Brasileira. 4th ed. Parte II – fascículo 4 São Paulo: Atheneu; 2003.

19. Costa AF. Farmacognosia. 5th ed. Lisboa: Fundação Gulberkion; 1994.

20. Ansel HC, Popovich NG, Allen LV Jr. Farmacotécnica: formas farmacêuticas & sistemas de liberação de fármacos. São Paulo: Premier; 2000.

21. Farmacopéia Brasileira. 5th ed. Rio de Janeiro: Ed. Fiocruz; 2010.

22. Mouco GB, Bernardino MJ, Cornélio ML. Controle de qualidade de ervas medicinais. Biotecnologia Cienc Desenvolv. 2003;31:68-73.

23. Farmacopéia Brasileira. 4th ed. São Paulo: Atheneu; 1988.

24. Rieger MA. Teste de estabilidade para microemulsão. Cosmetics &Toiletries. 1996;8(5):47-53.

25. Farmacopéia Portuguesa. 7th ed. Lisboa: Farmacopéia; 2002:641.

26. Farmacopéia Brasileira. 6th ed. Vol. II – Monografia, Agência Nacional de Vigilância Sanitária, Brasília; 2019

27. British Pharmacopoeia Volume I & II. London: The Stationery Office; 2009

28. Carlini EA, Frochtingarten ML. Toxicologia clínica (Fase II) da espinheira-santa (Maytenus ilicifolia). Brasília: Distrito Federal; 1988:67-73.

29. Ming LC, Castro DM, Delachiave ME. Plantas medicinais aromáticas e condimentares. Botucatu: Universidade Estadual Paulista; 1998.

30. Coulad-Cunha S, Oliveira RS, Waissmann W. Venda livre de Sorocea bomplandi Bailon como Espinheira Santa no município de Rio de Janeiro. Congresso Ibero-Americano de Plantas Medicinais, Angra dos Reis. 2004.

31. Carvalho ACB, Ballino EE, Maciel A, Perfeito JPS. Situação do registro de medicamentos fitoterápicos no Brasil. Rev Bras Farmacogn. 2008;18(2):314-319. doi:10.1590/S0102-695X2008000200028

32. Gilman AG, Hardman JG, Limbird LE. Goodman & Gilman as bases farmacológica da terapêutica. 9th ed. Río de Janeiro: McGraw-Hill; 1996.

33. Pereira AMS, Rodrigues DC, Cerdeira RM, França SC. Isolamento de metabólitos de maytenus associadas à ação anti-ulcera gástrica. Curtiba: Simpósio de Plantas Medicinais do Brasil; 1993.

34. Queiroga CL, Silva GF, Dias PC, Possenti A, de Carvalho JE. Evaluation of the antitumorigenic activity of friedelan-3beta-ol and friedelin isolated from Maytenus ilicifolia (Celastraceae). J Ethnopharmacol. 2000;72(3):465-468. doi:10.1016/s0378-8741(00)00237-3

35. Murakami S, Muramatsu M, Otomo S. Gastric H+, Ki+ and Acetylcholine inhibition by catechins. J Pharm Pharmacol. 1992;44(11):926-928. doi:10.1111/j.1365-2130.1992.tb03238.x

36. Annuk H, Hirmo S, Turi E, Mikelsaar M, Arak E, Wadstrom T. Effect on cell surface hydrophobicity and susceptibility of Helicobacter pylori to medicinal plant extracts. FEMS Microbiol Lett. 1999;172(1):41-45. doi:10.1111/j.1574-6968.1999.tb13447.x

37. dos Santos MC, Lopes CV, Borges AM, Heck RM, Leite MCL. Regate histórico de um grupo rural de estudos das plantas medicinais: educação em saúde. v. 39 . Cadernos de Educação & Saúde. 2011:285-299. doi:10.15210/cacud.v39i3.1537

38. Wonfor R, Natoli M, Parveen I, Beckman M, Nash R, Nash D. Anti-inflammatory properties of an extract of M. ilicifolia in the human intestinal epithelial Caco-2 cell line. J Ethnopharmacol. 2017;209:283-287. doi:10.1016/j.jep.2017.08.006

39. Ferreira PM, de Oliveira CN, de Oliveira AB, Alzamora F, Vieira MA. A lyophilized aqueous extract of Maytenus ilicifolia leaves inhibits histamine-mediated acid secretion in isolated frog gastric mucosa. Planta. 2004;219(2):319-324. doi:10.1007/s00025-004-1222-9

40. Leite JVP, Braga FC, Romussi G, et al. Constituents from Maytenus ilicifolia leaves and bioguided fractionation for gastroprotective activity. J Braz Chem Soc. 2010;21(2):248-254. doi:10.1590/S0103-50532010000200009

41. Queiroga CL, Barbieri GA, Galieta MX, et al. Avaliação do potencial anticâncer de fração ativa antitumorogênea de Maytenus ilicifolia (Celastraceae). In: Águas de Lindoia: Reunião Anual da Sociedade Brasileira de Química; 2007.

42. da Silva LM, Boeing T, Somensi LB, et al. Evidence of gastric ulcer healing activity of Maytenus robusta Reissek: In vitro and in vivo studies. J Ethnopharmacol. 2015;175:75-85. doi:10.1016/j.jep.2015.09.006

43. Costa MA, Andrade CLZ, Vieira RF, Sampaio FC. Plantas e saúde: guia introdutório à fitoterapia. Brasilia: Secretaria de Saúde do Distrito Federal; 1992:63-65.

44. Geoceze S, Vilela MP, Chaves BDR, Ferrari AP, Alinea EA. Tratamento de pacientes portadores de dispesia alta ou de última peptic com preparações de Espinheira-santa (Maytenus ilicifolia). Publicação CEME, PPPM no. 2, p.75-87. 1988.

45. Carlini E A, Frochtingarten ML. Em Toxicologia clínica (Fase II) da espinheira-santa (Maytenus ilicifolia). CEME/FAP, 2007:67-73.

46. Tabach R, Duarte-Almeida JM, Carlini EA. Pharmacological and toxicological study of Maytenus ilicifolia leaf extract. Part I - preclinical studies. Phytother Res. 2017;31(6):915-920. doi:10.1002/ptr.5818

47. Tabach R, Duarte-Almeida JM, Carlini EA. Pharmacological and Toxicological Study of Maytenus ilicifolia Leaf Extract Part II- Clinical Study (Phase I). Phytother Res. 2017;31(6):921-926. doi:10.1002/ptr.5816