Title: IMPROVED EXTRACTS OF PSDIUM GUAVA L., METHODS FOR ITS OBTAINING AND USE FOR THE TREATMENT OF GASTROINTESTINAL DISORDERS

Abstract: The invention is related to the obtaining of phytomedicaments that contain standardized extracts coming from the guava plant (Psidium guajava L.), particularly from its leaves (Psidium guajavae folium), useful for the treatment of diverse gastrointestinal diseases related to dysfunctions of the nervous system associated to gastrointestinal function. The anti-microbial, anti-motility, anti-spasmyelic, anti-inflammatory, anti-oxidant and anti-secretory properties of the clinically evaluated phytomedicaments of the invention, as well as its null toxicity, allow its usage in the clinic for the treatment of disorders of the gastrointestinal function.
Improved extracts of *Psidium guajava* L., methods for its obtaining and use for the treatment of gastrointestinal disorders.

Field of the Invention.

The present invention relates to the obtaining of plant extracts for medical use, more specifically to the obtaining of standardized guava (*Psidium guajava* L.) extracts and particularly to the standardized extracts of the leaves of guava (*Psidii guajavae folium*), to produce phytomedicaments useful in the treatment of gastrointestinal disorders.

Background of the invention.

In developing countries, acute diarrhea represents the primary cause of death in small children. In all the world more than five millions of deaths annually occurred among smaller children to a year of age during the decade of the eighties. Although oral rehydration is the most recommended therapy for almost every cases of diarrhea, it basically treats the problem of dehydration, so that in some cases it becomes necessary to administer spasmyloytic drugs that could be used in conjunction with this therapy. Nevertheless, this kind of drugs presents important non wanted side effects when used for the treatment of acute diarrhea.

The search of new medical products with anti-diarrheic properties is a topic of great importance in contemporary pharmacology. In spite of the spread use that for decades has had compounds such as atropine, kaolin or pectin, careful evaluation of their effects on the intestinal function has shown that its efficacy is very relative in the treatment of diarrhea. With the discovery of the opioids and its synthetic derivatives like the diphenoxylate and loperamide, a new chapter was opened in the therapeutics of this disease. Nevertheless, given that these products can sometimes have toxic effects (for example on the central nervous system of children with different degrees of development in their haematoencephalic barrier), the search of other medicines to combat the diarrhea is still an important task, considering it as a requisite the specific anti-diarrheic properties of the new products.

To achieve the former and to avoid the adverse side effects, one of the options would be to obtain the derived products from plant species that has been used in traditional medicine to cure or treat acute diarrhea.
According to Mexican traditional medicine, the oral administration of infusions obtained from the *Psidium guajava* *folium* to individuals that suffer from diarrhea, decreases or eliminates the discomfort and abdominal pain, and significantly reduces the frequency of intestinal discharges. The medical use of the water infusions prepared with the leaves of the tree to relieve gastrointestinal disorders, is a common practice in Mexico, dating back to the Aztec medicine. The therapeutic properties of the leaves from the tree are described with detail in diverse historical sources that deal with Mexican herbs for about the last 500 years. The plant is always referred to the treatment of dysentery, relieve of colic, abdominal distension and the cure of diarrhea. Mexican traditional medicine recommends the use of infusions of the guava leaves for its oral administration, three times a day, as an effective resource for the treatment of acute diarrhea, colitis, flatulence, and gastric pain. In accordance with the available ethnomedical information, adverse effects from the use of this folk remedy have not been observed and traditional healers consider it efficient and safe.

The "guayabo" or guava tree (*Psidium guajava* *L.*, from the *Myrtaceae* family) has a Mexican origin, and its leaves have been used for medical purposes since remote times among the so-called traditional or folk medicine. Its presence is constant in all historical sources dealing with indigenous herbology. Among the ancient Mexicans these plant received the nahuatl denomination of *xalxócotl* which refers to the fruit that is "of a hard and acid shell (*xócotl*) and sandy (*xalí") given its abundant content of small seeds that look like sand. The name *guayaba* has a Caribbean origin with unknown etymology, but that has prevailed after the discovery of America to refer itself to the fruit of the tree that is held in high esteem given its pleasant sweet-sour smell and taste.

The plant is distributed across the whole tropical fringe of the world having a medical use in places far apart of its origin such as China, India, Philippines and Central Africa. In Mexico as in other Latin American countries, the principal medical use of its leaves is that of a traditional remedy to fight diarrhea. They are commonly used like a tea or infusion that when orally administered alleviate intestinal cramps and reduces the episodes of acute diarrhea.

The first scientific studies of this plant were undertaken in Mexico and other countries during the XX century. In Mexico, extended ethnobotanical studies were performed
between 1985 and 1989, years in which a national ethnobotanical survey established that the leaf of the guayabo was, among traditional medicine, the principal resource used for the relieve of disorders that involved the presence of diarrhea. This information indicates that the preparation of an infusion made from 1 or 2 grams of dried or fresh guava leaves boiled during 5 to 8 minutes in 250 to 300 ml of water. The infusion obtained that way is then strained, sweetened with sugar or honey, and administered orally 3 to 4 times a day for about two or three days. In accordance with traditional medicine, this remedy is very efficient in reducing the diarrheic episodes, and having also a good acceptance by the patients given its absence of toxicity and non-wanted side effects\textsuperscript{7,12}. In the scientific field, the observed curative effects of the \textit{Psidium guajava L.} extracts have been studied in products obtained from the plant, some of which will be mentioned next:

\textbf{a) Anti microbial properties.}

The first formal scientific publication of the properties from the guava leaves extracts was realized by Colliere, who observed that the aqueous extract inhibited the growth of the \textit{Staphylococcus aureus} and other pathogen microorganisms cultivated \textit{in vitro}\textsuperscript{13}. Later, El Khadem and Mohamed\textsuperscript{14} made the same observation with a methanolic extract of the leaf from which they identified and isolate like possible responsible of this property, two glycosylated derivative from quercetin flavonoid, been these denominated: avicularin (quercetin-3-O-gentobioside) and guajavarin (quercetin-3-O-\alpha-L-arabinoside). Posterior studies verified that the flavonoids present in the leaves are capable to inhibit the \textit{in vitro} growth of \textit{Escherichia coli}, \textit{Pseudomonas aeruginosa} and \textit{Candida albicans}, among other microorganisms\textsuperscript{15,16}.

It is known that the anti microbial properties from the guava leaves extracts are owing to the antibiotic effects given by the combination of some of these flavonoids (synergetic effect) in comparison with that of each of these individually. It is known that in the leaf of the guayabo two groups of flavonoids are found:

\textbf{a) Quercetin derivatives} (guajavarin, isoquercitrin, hyperin, quercitrin, avicularin), and

\textbf{b) Morin derivatives}, (morin-3-O-\alpha-L-lixopyranoside and morin-3-O-\alpha-L-arabopyranoside).
The combination of quercetin, quercitrin and morin possesses a potent inhibitory activity upon the growth of the cultured microorganisms mentioned above, and also upon the development of \textit{Salmonella enteritidis} and \textit{Bacillus cereus}, in this last case the effect is enhanced by means of the addition of another flavonoid, the rutin\textsuperscript{17,18}. The extracts of the \textit{Psidium guajavae folium} that contain a mixture of those flavonoids are effective against pathogen intestinal flora responsible for the most part of the common gastrointestinal disturbances of infectious origin\textsuperscript{19,20}.

\textit{b) Anti motility intestinal effect.}

The explanation of the effect that the extracts of the guava leaves has upon intestinal motility is the result of research started twenty years ago. In Malaysia where the guava leaves have the same popular use as an anti-diarrheic resource as in Mexico, Lutterodt\textsuperscript{21} described that the methanolic extract obtained of them inhibited gastrointestinal secretion of acetylcholine in the isolated intestine of the guinea pig, mechanism which he proposed to explain its anti-diarrheic effect. It was postulated that the quercetin, being the principal detected flavonoid in the extract, could be responsible for that effect. Nevertheless, Fanning and coworkers\textsuperscript{22} had already reported that quercetin, obtained from other plants, inhibited the induced contractions in the isolated ileum of the guinea pig. Macander\textsuperscript{23} and Capasso\textsuperscript{24} concluded also that quercetin and some other flavonoids inhibited \textit{in vitro} the muscular contractions of the ileum from the guinea pig.

In previous studies performed by the applicants\textsuperscript{25} regarding the methanolic and aqueous compounds of the extracts from \textit{Psidium guajavae folium} using an experimental model of intra luminal perfusion of the guinea pig ileum \textit{in vitro}, it was demonstrated that the portion rich in flavonoids was responsible for the anti-spasmodic effect and inhibitory function of intestinal peristalsis. In another study, Meli\textsuperscript{26} described that when quercetin is administered intra-peritoneally (50mg/Kg) to laboratory mice, inhibited the intestinal transit in a way similar to than produced by verapamil, a medicine that blocks the Ca\textsuperscript{2+} channels.

Previous studies by the applicants showed that both in the methanolic extract from the leaves and in the infusion of popular use, exist five glycosylated flavonoids derivative from quercetin (quercetin-3-O-\textalpha-L-arabinoside, quercetin-3-O-\textbeta-glucoside, quercetin-3-
O-ß-D-galactoside, quercetin-3-O-ß-L-rhamnoside and quercetin-3-O-gentobioside) that became hydrolyzed in the digestive tract and liberated the aglycone denominated quercetin, which is the anti-motility and anti-spasmodic active principle of the extract. The action mechanism of quercetin was also described, showing that it has an action as a Ca\(^{2+}\) antagonist inhibiting the ion incorporation into the intestinal smooth muscle, which traduces itself in a temporal decrease of peristaltic movements. This mechanism also explains the intestinal spasmolytic action of this substance that blocks the flux of Ca\(^{2+}\) ions into intestinal muscle fibbers contracted previously by diverse agents.

Galvez reported the same results when studying the effect of quercetin over intestinal contraction using different experimental models, finding that the substance acts as a selective Ca\(^{2+}\) antagonist. Later, Hammad and Abadia defined that the spasmyolitic power of the flavonoids of the type of the pentahydroxil flavons (which is the case of quercetin), depends on the presence of hydroxyl groups in the C-3 and C-5 positions of the molecule and the absence of them in position C-2': This, in the case of the guava leaves products makes quercetin the spasmyolitic principle with more activity, followed by another flavonoid also present in its leaves, the morin, that besides being a powerful anti-microbial shows also certain spasmyolitic intestinal activity.

Lutterodi described that the aqueous extract from *Psidii guajavae folium* (orally administered) reduced the acute diarrheic syndrome produced by Microlax™ in laboratory rats, and that besides the anti-motility effect associated with quercetin there existed an intestinal anti-secretor action which explained the water re-absorption that is produced in animals treated with guava leaves extracts.

Soon after, Martin reported that quercetin had also an effect over gastric secretion thus reducing gastritis and the formation of gastric-duodenal ulcers in experimental animals. Ocete described the anti-inflammatory action attributed to the morin flavonoid, studied in induced acute colitis in rats.

c) *Anti-oxidant effect.*

Quercetin has become one of the natural origin flavonoid with high pharmacological potential in pathological processes related to the oxidative stress of the cells. The antioxidant activity of quercetin has been widely spread and is related to the presence
in its molecule of hydroxyl groups in the A and B rings, with a double bond in the 2-3 position and hydroxyl in the position 3. Studies with diverse in vitro models indicate that this compound posses antioxidant and free radicals capturing properties, inhibits the lipid per-oxidation and the oxidation of the mitochondrial membrane. It has been reported in animal studies that quercetin in high concentrations (250 mg/Kg of weight) when orally administered, enter the blood stream where it converts itself into quercetin-3-O-beta-D-glucuronide, a compound that acts as an antioxidant of plasmatic low density lipoprotein.

\textit{d) Toxicity.}

Preclinical toxicological studies have been performed with the \textit{Psidii guajavee folium} extracts, determining the median lethal dose (LD50) in Swiss rats (OFI) and the alternative toxicology (acute toxic types) in Wistar rats. Also, a genotoxical evaluation of the extracts in an in vitro system for an induction essay of short term somatic segregation in the fungus \textit{Aspergillus nidulans} and an in vivo essay in the induction test of micronucleus in the bone marrow of mice, have been performed. Toxicological results do not show any deaths among neither of the two experimental models in the range of the employed dose, that reached as far as 2000 mg/Kg of body weight. Histological results show no damage attributable to the toxicity of the plant material tested. In the \textit{Aspergillus} study results demonstrates absence of genotoxical effects of the extracts, which was also observed in the micronucleus induction system of the mice bone marrow. The mentioned studies concluded that guava leaves extracts lack of toxic effects and are safe for its medical use.

Given the former and based on published biomedical literature that supports the anti-diarrheic properties of the guava, some herbal products have been developed and commercialized for their oral administration in the treatment of gastrointestinal disorders.

In this sense, Robinson described the obtaining of acetone extracts from the \textit{guayabo} fruits for the treatment of cancer in animals and humans. The extracts were obtained by blending the fruit with sugar, boiling of the mixture and posterior adding of acetone, leaving it rest for 12 hours, then filtering and heating the mixture under vacuum to concentrate it and eliminate the acetone. The obtained extract is orally administered in
volumes of 30 ml three times a day, administering a mean of 1,000 to 3,000 ml of extract depending on the type of cancerous affection of the patient under treatment. Even tough the document shows data of the effectiveness of the extract in animals, it does not include clinical studies made on patients to evaluate its therapeutic effectiveness and toxicity.

Kiyoshima describes the obtaining of the *Psidii guajavae folium* extracts for the treatment of viral infections such as influenza. The extract is obtained with a hydrophilic organic solvent such as alcohol in a process of autoclave extraction, characterizing that extract by the presence of a fraction of molecular weight of 12,000. The extract is then frequently administered as a solid dose of 15 mg. Although the extract has the effect of delaying the virus infection, the document does not show the standardized and determinate quantities of the active constituents enclosed in it.

Ishihara describes the obtaining of ethanolic extracts from the *Psidii guajava folium* as an inhibitor in the production of peroxylipids and as an antioxidant agent. Nevertheless, the process of elaboration submits the raw material to extraction stages of high pressure (1.5 to 5 atmospheres) and to heating. The described extract has not therapeutic application by itself, given that it is used in combination with grape seeds and pine cone extracts to observe its effect. There is also no mention of determined quantities of the active principles of the obtained extract.

More simple extracts have been obtained from the *Psidii guajavae folium* to use as beverages or teas, but no therapeutic application has been reported for that extracts.

However, in spite of the efforts previously dedicated for the obtaining of phytomedicaments derivative from the *Psidium guajava L.*, these products are not considered official drugs given that they had not been clinically evaluated in patients and do not contain standardized quantities of their active constituents.

Given the former it is important to develop drugs with a multiplicity of convenient effects for the treatment of acute diarrheic disease that can be used in combination of clinical evaluation and re-hydrating therapy that do not generate unwanted toxicity or side effects for the patient and can be administered in a controlled and safe manner.
Objectives of the invention.

It is one of the objectives of the present invention to provide standardized extracts from the *Psidium guajava* L. for the treatment of gastrointestinal disorders.

It is other of the objectives of the present invention to provide standardized extracts from the *Psidii guajavae folium* for the treatment of gastrointestinal disorders.

Another objective of the present invention is to provide standardized extracts from the *Psidium guajava* L. that do not present toxicity for the patient and allow relieving of the diverse symptoms involved in gastrointestinal disorders.

Another objective of the present invention is to provide standardized extracts from the *Psidium guajava* L. by means of the normalization of its flavonoid concentration for the treatment of gastrointestinal disorders.

It is another objective of the present invention to provide standardized extracts from the *Psidium guajava* L. for the obtaining of phytomedicaments that can be administered in a controlled manner in diverse therapeutic regimes.

Another objective of the present invention is to provide phytomedicaments for the treatment of gastrointestinal disorders containing standardized extracts from the *Psidium guajava* L. as a therapeutic agent.

It is another objective of the present invention to provide phytomedicaments containing standardized extracts from the *Psidium guajava* L. in several pharmaceutical forms for its controlled administration in patients.

Another objective of the present invention is to provide low cost and efficient methods for the obtaining of standardized *Psidium guajava* L. extracts that conserve their therapeutic activity.

Another objective of the present invention is to provide efficient methods that allow standardizing the concentration of the active constituents of the *Psidium guajava* L. extracts by means of normalizing its flavonoid concentration.
Another objective of the present invention is to provide an effective method for treatment for the irritable intestine syndrome using standardized *Psidium guajava* L. extracts as a therapeutic agent.

Another objective of the present invention is to provide an effective treatment method to control and relieve the symptoms of the irritable bowel syndrome, the acute diarrheic syndrome, gastritis and nervous colitis using the standardized extracts of the *Psidium guajava* L. as a therapeutic agent.

**Brief description of the figures.**

**Figure 1.** Shows the chemical structure of the flavonoids derivative from quercetin.

**Figure 2.** Shows a graphic where it can be observed the quercetin concentration in the leaves of the *Psidium guajava* L. plants found in several parts of Mexico and in different seasons of the year. It can be observed the plants from the states of Michoacán (1), Querétaro (2), of México (3) and Aguascalientes (4), during winter (A), spring (B), summer (C) and autumn (D). The bars represented with an asterisk show the resulting highest averages from a two-way ANOVA statistical analysis.

**Figure 3.** Shows a graphic illustrating the differences between groups of patients with acute diarrheic syndrome under clinical essay in regard to the intensity of abdominal pain or cramps using the phytomedicament of the invention. It can be observed the group treated with the phytomedicament at the beginning of the study (Baseline), at the first day (Day 1), second day (Day 2) and third day (Day 3), compared with the control group treated with placebo (C).

**Detailed description of the invention.**

The anti-microbial, intestinal motility inhibitor, spasmolytic, anti-inflammatory and anti-secretor properties of the flavonoids present in the *Psidii guajavae folium* give foundation for the use of this vegetal drug for the development of phytomedicaments for the treatment of disturbances of intestinal hyper-motility. Acute states of diarrhea, cramps and intestinal blowing are frequent among urban society of every country, and even considered as "auto limiting syndromes" attributed to the intake of bad quality food, the stress of modern lifestyle and the excess of eating and drinking, representing
a health problem for which they are not sufficient safe medicaments without collateral effects.

In accordance with the established International regulations for the development of phytomedicaments given by the World Health Organization\textsuperscript{49}, in between the numerous requisites that should accomplish a medicament of this type, the following stand out:

- The scientific foundation of the traditional use of the plant with the chemical and pharmacological studies that support their medicinal properties,
- The control and standardization of the vegetal drug, derivative from the medicinal plant for its commercialization, and
- The chemical standardization of the active constituents in the proposed medicament that permits its dosage for the clinical essays.

In the present application the applicants present a phytomedicament based on standardized extracts obtained from the \textit{Psidii guajavae folium} (guava leaves) that include studies of the toxicological and standardizing type established for the phytomedicaments, based on clinical studies in patients. The phytomedicament of the present invention is a safe medicament, given that it does not present toxicity, and can be administered to patients with diverse intestinal disorders, like for example the irritable bowel syndrome, acute diarrheic syndrome and nervous colitis and gastritis.

The phytomedicament of the invention is very useful for the treatment of the most common dysfunctions of the gastrointestinal tract, which includes diarrhea. The combined effects that produce their flavonoids is an advantageous characteristic with respect to other medicines, given that solely with the application of this phytomedicament, gastrointestinal disorders that produce diarrhea, cramps and abdominal blowing can be treated, which require a symptomatological management and without the need to apply several medicaments for the treatment of each of one of these clinical symptoms.

The phytomedicament described in this document represents a product of natural origin designed as an aid in the treatment of the most common "auto-limiting" gastrointestinal affections, which provide important benefits given its complete therapeutic effect without the unwanted side effects in the patients. Furthermore, its controlled
administration and dosage and its efficient therapeutic effect in patients with intestinal affections, allows its usage in conventional medical treatments.

Before the present invention, there did not existed any *Psidium guajava* L. extracts, particularly from the *Psidii guajavae folium*, that contained standardized and determined quantities of the active principles responsible for the therapeutic effect of the extract. This characteristic of the extract of the invention permits its handling for the frequent and controlled obtaining of pharmaceutical compositions that contain it, allowing this to control the quantity of the active constituents found in the given compositions. In consequence it becomes possible to obtain phytomedicaments with known and predetermined doses than can be administered to the patient by several means in adequate volumes. Moreover, with the administration of these phytomedicaments it is certain that the patient receives constant and controlled quantities of the phytomedicament for different treatment regimes, according with the gastrointestinal affection treated. In addition to the former, this phytomedicament allows the physician to design controlled treatments for the patients that suffer gastrointestinal disorders, given that it is possible to administer controlled doses of the medicament and to continue an adequate follow up of the treatment.

Also, the standardized extract of the invention allows its easy combination with multiple helpful compounds for the production of diverse conventional pharmaceutical forms, powder or liquid depending on the administering needs and therapeutic regime. The extract maintains its pharmacological properties, independently of the process upon which is submitted for the obtaining of convenient pharmaceutical forms, which permits an ample versatility in its application.

The extract contains pre-established quantities of its active principles (glycoside flavonoids) standardized as equivalents of quercetin, using for that purpose the quantitative determination of the quercetin molecule in the extract, for example by means of a high performance liquid chromatography (HPLC). Given that the observed therapeutic effect of the *Psidium guajava* L. extracts results from the presence of diverse flavonoid glycosides\(^\text{27}\) that have in common the quercetin molecule (see figure 1), the extract of the invention possesses precise quantities of this glycoside flavonoids that in combination exert the therapeutic action, measured as the total concentration of...
quercetin in the extract. For the purpose of the invention, the extract described here possesses a concentration of 4 to 6 mg of active principles, measured as the total concentration of quercetin for each gram of the obtained extract, measured principally as dried extract. The active principles in the extract of the invention correspond to the glycoflavonoids normally found in the Psidii guajave folium (see figure 1), such as guaijavarin (Quercetin-3-O-α-L-arabinoside), isoquercitrin (Quercetin-3-O-β-D-glucoside), hyperin (Quercetin-3-O-β-D-galactoside), quercitrin (Quercetin-3-O-β-L-rhamnoside) and avicularin (Quercetin-3-O-gentobioside), and morin derivatives, morin-3-O-α-L-lyxopyranoside and morin-3-O-α-L-arabopyranoside.

The molecule of quercetin has been used as an ingredient for the obtaining multiple antioxidant compositions\(^5^0,5^1\), nutritional\(^5^3\) or food supplements\(^5^2,7^4\), and for the obtaining of derivatives with a determined therapeutic activity\(^5^4\). Multiple therapeutic applications of quercetin and its derivatives have been reported for the treatment of osteoporosis\(^5^5\), allergies\(^5^4\), neuronal damage and diseases related with the 5HT1A receptor (Alzheimer, for example)\(^5^6\) and rheumatoid arthritis\(^5^7\).

In spite that the molecule of quercetin as such possesses a greater anti-spasmodic activity in vitro compared to the flavonoids contained in the extracts\(^2^7\), the low absorption that presents this compound represents an important limiting factor for its exclusive administration in addition to its low solubility in aqueous solutions under physiological conditions. In this sense quercetin is only absorbed by 1% of the orally administered dose, moreover for its effective cellular capture it is required the use of water miscible dissolvents such as the dimethylsulfoxide\(^5^4\).

In the case of the extract of the invention, the flavonoid glycosides contained in it get hydrolyzed in the digestive tract freeing thereby its aglycone, the quercetin molecule that is the active biological constituent\(^2^7\). In this way, the flavonoids contained in the extract (quercetin-3-O-β-glucoside, for example) facilitate the absorption of quercetin by the wall of the small intestine\(^5^8\), permitting the consecutive pharmacological action of the extract. The high concentrations of the diverse flavonoid glycosides derivative from quercetin which are contained in the extract (principally quercetin-3-O-β-D-glucoside and quercetin-3-O-β-D-galactoside, see table 1), allow the active ingredients to reach
its action site (intestinal tract) exerting its localized pharmacological activity after its administration.

As can be observed in table 1, the proportion of each of one of the flavonoid glycosides present in the extract of the invention conserves themselves with respect to the detected proportions in the raw material from which the extract was obtained. This shows that with the methods for obtaining of the extracts described in the present application, extracts without significant variations in the proportions of its active ingredients from the chosen vegetal material of the plant can be obtained. In this way the originally observed therapeutic effects of the products derivative from the Psidii guajavae folium are conserved in the extract described here. With this it is feasible to obtain multiple pharmaceutical compositions in different presentations from the concentrated extract of the invention.

A pharmaceutical composition that uses the extract of the invention as a therapeutic agent can be prepared in accordance with whatever conventional methods and procedures are used. In the preparation of the composition, the active ingredient is blended or diluted with a carrier, or preferentially included into the carrier, which could be in form of capsules, sachets or any other container. When the carrier serves as a dissolvent, this can be a solid, semisolid or liquid material that acts as a vehicle, an excipient or as a medium for the active ingredient. Thereby the compositions can be manufactured in the form of tablets, pills, powder, sachet, elixir, suspension, emulsion, solution, syrup, aerosol, soft and hard gelatin capsule, sterile injectable solution, sterile packed powder and the like.

The pharmaceutical examples of convenient carriers, excipients and dissolvent are lactose, dextrose, saccharose, sorbitol, manitol, starches, acacia gum, alginates, gelatin, calcium phosphate, calcium silicate, cellulose, methyl-cellulose, microcrystalline cellulose, polyvinylpyrrolidone, water, hydroxy-methyl-benzoate, hydroxy-propyl-benzoate, talcum powder, magnesium estearate and mineral oil. The composition can also include anti-agglutinate agents, filler materials, flavoring agents, lubricant agents, humectant agents, emulsifying agents, preservatives and the like. The pharmaceutical compositions of the invention can also be formulated to provide rapid,
sustained or retarded liberation of the active ingredients after its administration to a mammal employing whatever procedures or methods well known in the art.

The pharmaceutical composition of the present invention contains a quantity of 1 to 5 mg of glycosylated flavonoids measured as the total concentration of quercetin, for each 400 to 500 mg of composition, but preferentially 1 to 4 mg of the mentioned flavonoids. With respect to the several pharmaceutical forms that can be obtained using the extract, the concentration of the active principles can vary within the mentioned ranges depending upon the pharmaceutical forms in question.

As for the extract of the invention, the pharmaceutical compositions that contain it are stable to their storage under environment temperature for a period of at least of 4 months, conserving its concentration of active principles and its therapeutic effect. This allows eliminating the elevated risks of contamination that present the aqueous extracts from *Psidium guajava* L. or *Psidii guajavae folium* commonly obtained in the traditional way.

The composition of the invention can be orally administered. A dose of the extract equivalent to 0.01 - 0.05 mg of flavonoid glycosides for each Kg of body weight is adequate to be administered in human patients; however a dose of 0.01-0.02 mg of flavonoid glycosides for each Kg of body weight is preferred. Those given doses can be administered on a single time or daily in separate doses until the complete disappearance of the clinical symptoms. The exact quantity of the composition administered to the patient can vary according to its age, sex, body weight, severity of the illness and formulation to be administered.

In spite that several types of extracts and therapy applications have been reported for different parts of the *Psidium guajava* L., none of them constitute extracts with controlled concentrations of the known active principles (standardized extracts), making it not possible to administrate them in a constant and controlled manner to the patients. Furthermore, this impedes in the clinic to determine with certainty the effective therapeutical quantity of the extracts to be applied in the patients with respect to its active principles; for this reason is not possible to design effective therapeutic treatments using said extracts and make a suitable follow up of the treatment. In
another sense, the extracts obtained are given to the patients in considerable volumes and in a very frequent manner (several times a day), by which it becomes not possible to maintain a general and trustworthy administration regime to all kind of patients. The frequent administration of the mentioned extracts, imply the administration of a low concentration of the active principles contained in them; for this reason, with the administration of said extracts, is complicated the maintenance of the suitable therapeutic levels of flavonoids in the patients receiving the treatment.

The extract of the invention is useful in the relieving of symptoms associated with diverse gastrointestinal disorders related to dysfunctions of the enteric nervous system linked to gastrointestinal function, like for example the irritable intestine, irritable bowel and acute diarrheic syndromes, including nervous colitis and gastritis.

In the case of the irritable bowel syndrome (IBS), the clinical manifestations of this disorder are owed to the dysfunctional state of the intestinal nervous system (enteric) produced by diverse factors, been observed:

- An intense intestinal colic, resulting from the sustained contraction of the flat muscle fibers of the intestine,
- Diarrheic episodes consequently associated with states of temporal dehydration owed to the hyperactivity of intestinal peristaltic movements and the lost of liquids given their poor absorption and active secretion, and
- Intestinal blowing linked to moderate infections produced by the own intestinal flora (endogenous) constituted generally by *E. coli*, *Staphylococcus aureus* and other microorganisms.

Given that the extract of the invention lacks of toxicity, has a localized effect over intestinal tissue and its therapeutic action combine four beneficial and complementary effects, phytomedicaments containing the extract can be used as a convenient therapeutic agent for the gastrointestinal diseases mentioned above. For example, in the case of the acute diarrheic syndrome, the extracts results very helpful because:

- It possesses a spasmolytic effect thereby eliminating the intestinal colic,
- It has an anti-secretor effect, which permits the re-absorption of liquids thereby avoiding dehydration,
- It has an antioxidant action, thus eliminates the inflammation of the intestinal tissue, and
- It has an anti-microbial action over pathogen and opportunistic intestinal flora.

In consequence, with the administration of the standardized extract of the invention the described dysfunctions can be treated in a convenient manner, be administered in low volumes and without the necessity to resort to multiple medicines to treat each one of the symptoms to achieve the desired therapeutic effect. The effect of neuroenteric regulation that provides the phytomedicament of the present invention allows the efficient and complete treatment of the diseases mentioned above.

The efficiency of the phytomedicament of the present invention as a therapeutic agent was evaluated among human patients with acute diarrheic syndrome. The oral administration of 400 to 500 mg of the phytomedicament of the invention every 8 hours during three days (containing a total concentration of flavonoids of 1mg/500mg of the phytomedicament, estimated as total quercetin) to the patients under treatment, resulted in a significant difference with respect to the control group since the first day of treatment, in the number of diarrheic episodes and intensity of the colic and abdominal pain (see figure 3). The clinical evolution of the group treated with the phytomedicament of the invention was more efficacious and rapid, well tolerated by all the patients and with the absence of collateral effects of any type59.

In a study developed by Wei60 regarding the clinical usefulness of the Psidii guajavae folium extracts in the treatment of rotavirus enteritis in children, a fastest recovery in the treated patients (87%) was observed in comparison with the control group (58%) and also a negative conversion of the antigen of the human rotavirus (HRV). Nevertheless, the used extracts were not standardized, thereby is not possible to determine the most adequate dosage regime to continue with the treatment.

In marked difference with the former, the clinical studies realized with the standardized extract of the invention, permits to determine with certainty the adequate doses to be administered to patients with gastrointestinal disorders, allowing this to design adequate therapeutic regimes. Moreover, the patients treated in the mentioned study were only children with specific viral intestinal disorders, whereas in the clinical study
performed with the extract of the invention, patients of different ages with acute diarrheic syndrome were included. The results obtained by the clinical study with the extract of the invention indicate that the extract can be used as a phytomedicament for the treatment of intestinal diseases under controlled and precise dosage regimes.

In the case of small children, the symptoms of the acute diarrheic syndrome are not considered during the treatment of the disease, owed to the impossibility that have these patients to describe them. In this case, the medical procedures and principal indications that are performed for its treatment are focalized to the avoidance of dehydration. In the case of the adult patients, the treatment strategy is the opposite in the medical practice; in this case, the increase in the diarrheic episodes becomes a secondary symptom to treat, given that abdominal inflammation and colic are the principal affection to eradicate in the shortest time possible, for which analgesics, spasmyotics and anti-inflammatory are used.

The phytomedicament of the invention can be used in an efficient and safe manner to relieve the symptoms associated with acute diarrheic syndrome such as colic and abdominal pain.

Before the present invention, a comparable phytomedicament did not exist that provided the therapeutic effects mentioned above in just one product. For example, irritable bowel syndrome is conventionally treated with the administering of several medicines which action is only partial, administering an anti-diarrheic, an anti-inflammatory, Ca⁺ antagonists, etc.

The extract of the invention has in common with the product usually used for the relieve of diarrhea (Loperamide, for example, and others), an effect as a calcium ions antagonist, but differs from the former in that it lacks of toxicity and possesses an anti-inflammatory, anti-microbial and anti-secretor effect, properties that in combination provides its therapeutic safety and efficiency.

In spite of the use of aqueous standardized extracts from other plants (Indigofera arrecta) for the relieve of peptide ulcers in doses of 2.5 to 5 mg/Kg of body weight, that show inhibitory effects of the intestinal passage and decrease of volume and acidity of
the basal gastric secretions, these extracts do not display the cluster of effects provided by the *P. guajava* L. extracts of the invention.

In reference to the method for the obtaining of the extract of the invention, this permits the elaboration of hydro-alcoholic extracts of *Psidii guajavae folium* helpful as an anti-diarrheic resource, given the verification of the anti-microbial, spasmytic, anti-motility, anti-secretor, and anti-inflammatory properties of the flavonoids contained in it, in addition to the results provided by the clinical tests undertaken in patients with acute diarrheic syndrome treated with the extract of the invention.

In general way, the extract of the present invention is prepared from dry and milled selected leaves of *Psidium guajava* L. with a minimum concentration of flavonoids (estimated as total quercetin) in the vegetal material no less than 5 to 8 mg per gram of vegetal material. The material obtained is put into contact with ethanol for a period of 24 to 48 hrs., under a temperature of 30 to 50°C; the extract obtained is then filtered, then concentrated by means of distillation of the solvent, blended with convenient excipients, and spray dried. This extract is standardized in its content of glycosylated flavonoids derivative from quercetin, quantified as equivalents of the quercetin flavonoid in a concentration of 4 to 6 mg per gram of total extract, through the addition of convenient quantities of excipients to finally obtain a powder through spray drying. The powder is then mixed with acceptable pharmaceutical excipients with the objective of getting the most convenient pharmaceutical forms containing determined concentrations of the active principles, whether by volume or weight.

In one of the preferred modalities of the invention, the mixture of powder with the excipient is compressed for the obtaining of tablets with a weight of 400 to 500 mg and a concentration of 1 to 4 mg of glycoside flavonoids per tablet. The obtained tablets are orally administered for the treatment of gastrointestinal disorders.

The process of the invention permits the obtaining of *Psidium guajava* L. extracts with such adequate concentrations of flavonoids, that it is afterwards possible to standardize the concentration of these in the extract, with the purpose of administering effective doses with the adequate therapeutic outcome in the patients under treatment.
In the same way as for the present invention, several general procedures for the obtaining of the extracts of different plants have been reported. Examples of these are those that involve the diffusion of the bioactive substances to extract from the plant, in phases of liquid/steam extraction under controlled conditions of pressure and temperature\textsuperscript{62}, or else using supercritical fluid extraction through fluorocarboned solvents and their posterior separation by chromatography\textsuperscript{63}.

Sohn\textsuperscript{64} describes the obtaining of a quercetin derivative (quercetin-3-O-β-D-glucuronid) from the \textit{Rumex aquaticus} plant and its application for the treatment of gastritis and refluxing esophagitis. For its obtaining, crude extracts from the leaves of the plant are initially obtained through the mixing of the dry leaves with alcohols of 1 to 5 carbons under a temperature of 40 to 60°C for a period of 1 to 24 hrs, then centrifuged, filtered and concentrated through reduced pressure. The extract obtained is then refrigerated until its utilization. Afterwards the crude extract is mixed with a non-polar solvent for lipid removal and subjected to chromatography with alcohols of 1 to 5 carbons to obtain the quercetin derivative through a process of crystallization. By means of the mentioned process, 2.1 g of the compound for each 4 Kg of dried leaves can be obtained, and in a general manner a description of the feasibility of administering 0.1 to 500 mg/Kg of weight \textit{per} day of the compound is presented, without the exact determination of the adequate doses to be administered in human patients. The mentioned document only shows the effect of the compound isolated and characterized solely in rats and it is only demonstrated the pharmacological effects in the extracted tissues of the sacrificed animals. Moreover by means of the method described, complete extracts from the plant can not be obtained.

Feng\textsuperscript{65} describes the obtaining of the \textit{Loranthus} plant extract that possesses quercetin and avicularine, to obtain compositions with antihistaminic effects for the treatment of allergies and hypersensitive reactions. The process for the obtaining of the extract involves the mixing of different parts of the plants of a determined particle size with ethanol to the 95%, heated to 80°C, and filtering of the resulting solution which is then treated with heat through steam until the obtaining of a creamy paste that is finally dried by means of conventional methods. The obtained powder is then reconstituted with water and the resulting solution purified through chromatography using specific resins until the obtaining of a creamy paste that is dried to powder. Nonetheless in the
mentioned document no therapeutic effects on human patients are shown, neither the obtaining of the therapeutic compositions to be applied as such in the therapy of the mentioned diseases. Moreover, the method of obtaining is complicated and with regular yields (150g of extract for each 5 Kg of plant).

Flavonoid enriched extracts from Typhae pollen have been obtained through the blending of the biological material with water and posterior extraction of impurities with a low polarity dissolvent (ether, alkanes, esters) for the final adding of a polar dissolvent (butanol, isopropanol or chloroform). The extract possesses quercetin, kaempferol and isorhamnetin, and some derivatives of these molecules; it is mentioned that the extract is helpful for the treatment of diseases that induce poor blood circulation. Nevertheless, the extracts obtained do not contain determinate quantities of the active principles and the methods of obtaining do not permit the getting of homogenous extracts.

Stander reports the obtaining of Barosma betuliona extracts for the treatment of inflammatory or hypertensive conditions. The extract possesses quercetin, rutin diphenols and diosmin, obtained from fractions got through distillation from the oil of the plant. In spite of the demonstrated effectiveness, the volumes administered to the patients are high (250 ml per dose).

Aqueous extracts rich in flavonoids including quercetin from the Brickellia californica plant have been obtained, through the combination of the leaves and stalks which are blended with water, and then heated until boiling is achieved. The obtained extract is rich in luteolin and administered as such to patients with diabetes, showing in these a decrease in the sugar blood levels several weeks later; nevertheless no determined concentrations of the active principles are shown, and the effects are delayed owed to the low doses administered of the extract.

Aqueous and ethanolic extracts from the Achoyrocline satureoides ("Marcela") have been reported to be useful in the treatment of neurodegenerative diseases given the presence of compounds similar to quercetin. The extracts are obtained from the macerated inflorescence of the plant by means of the addition of ethanol, the filtering of the dissolvent and posterior evaporation of it under reduced pressure, for the final
extraction of the remnant with petroleum ether. The solution is then extracted with ethyl acetate and n-butanol. Although the antioxidant effects of the extracts were tested and verified in animal models, no clinical tests in human patients are shown which allow discerning the adequate quantities of the extract to be used, and besides the process for the obtaining of the extract is complicated.

Extracts from *Fructus crataegus* with representative quantities of bioflavonoids including quercetin, have shown they pharmacological activity in the treatment of cardiovascular diseases related to high levels of plasmatic lipids\(^{70}\). The extracts are obtained through the adding of water or ethanol to the dried fruits under temperatures between 25 and 75°C for a maximum period of 10 hrs; afterwards the mixture is filtered and concentrated for the obtaining of the extracts. However, the process described in the document does not show a previous analysis of the flavonoid concentration in the raw material for the obtaining of the extracts. Neither are tests in human patients nor the adequate doses to provide a treatment against the disease is shown.

There has been reported the obtaining of flavonoid enriched compositions such as quercetin and isoquercitrine through the enzymatic conversion of rutin solutions using naringinase, with therapeutic effects for the treatment of disorders that involve pathological enzymatic activities\(^{71}\), like oxidative stress, and increased levels of cholesterol and triglycerides among others. However, the therapeutic activity of the obtained extracts for the treatment of intestinal diseases are not reported, neither the description of the obtaining of pharmaceutical compositions with standardized doses of active principles.

Combinations of several plant extracts that contain minimal quantities of quercetin for its application in the therapy of renal affections, incontinence and cancer, have also been used\(^{72}\).

Hammamelis extracts that contain quercetin as part of the flavonoids present, have been obtained using extraction with alcohols of 1 to 4 carbons, water, propylene glycol and acetone in different proportions to be used in cosmetic applications\(^{73}\).
Derivative polyphenolic extracts from apple that contain quercetin have been obtained for its application as antioxidants and later adding to food. The hydroalcoholic extracts are obtained under a temperature between 20 to 100°C and an alcohol content of 0 to 90%. However, no results in human patients have been showed and the use of the extract is limited to its incorporation in food supplements.

Even though the extracts of different plants containing quercetin (including Psidium or Psidium guajava L.) or the active principles with proved pharmacological activity could be obtained with the methodologies described previously, the obtaining procedures are very complicated and no extracts with determined concentrations of the active principles of interest are obtained to manufacture phytomedicaments that can be applied in a straightforward manner in the clinic.

The flavonoid concentration obtained in the extract with the method of the invention, is so adequate that its concentration can be adjusted by manipulating the proportions of weight and volume, both during the obtaining of the concentrated extracts as in the pharmaceutical compositions obtained.

As a way to illustrate the present invention the next examples are shown, without enclosing the scope of it by these:

**Example 1. Selection and obtaining of the vegetal material.**

*Psidium guajava L.* leaves were recollected and inspected by means of the criteria and methodology described by Rivera-Arce with the objective of getting leaves from the plant with determined morphological characters that could be identified in a precise manner. Leaves selected in agreement with the procedure described by Rivera-Arce, presented the typical morphological characters that identify leaves of the *Psidium guajava L.*, as well as the following typical anatomical characters:

- Poligonal and isodiametric cells of rectilinear contour in the adaxial epidermis.
- Abaxial epidermis constituted by multiform cells with wavy contour,
- Anisocytic and anamocytic stomata in the abaxial epidermis,
- Long, curve, simple, unicellular trichomes or hairs,
- Massive primary vein of straight stroke,
- Isolateral mesophyll with six layers of palisade cells,
• Hypodermis developed nearby the adaxial epidermis constituted by two to three layers of cells,
• Schizo-lysigenous cavities.

On the other hand, a determination of the active principles measured as the concentration of quercetin in the dry leaves of the *Psidium guajava* L. plants localized in several parts of Mexico and in different seasons of the year was carried out. As can be observed in figure 2, the plants localized in the States of Mexico and Aguascalientes presented a minimum concentration of 8 mg of quercetin for each gram of dried leaves; and the season of the year in where maximum concentrations were found were winter and spring.

As was previously described, the vegetal material (leaves) of the *Psidium guajava* L. that reunited the typical anatomical and morphological features of the plant and a minimum content of 8 gm of active principles per gram of dried leaves was selected. The quantification of the active principles was achieved by means of the method mentioned in the example 6.

**Example 2. Obtaining of the extract.**

The selected dried leaves from the example 1 (6 Kg) were milled and sieved through a 40 mesh. The obtained material was then extracted in a stainless steel reactor with a mixture of ethanol-water (80/20 vol/vol), under a temperature of 50°C for 24 hours, to afterwards recover the solvent through filtration. The filtrate was concentrated by distillation and the concentrate recovered; the distilled dissolvent was used for a second extraction under the same conditions. The obtained concentrates of the previous extraction were blended and the total extract obtained was weighted, carrying out the analysis of active principles by means of the method mentioned in the example 7.

The concentrated extracts containing a concentration of active principles no less than 10 mg/g of concentrated extract were selected for the obtaining of the dried pulverized extract.
Example 3. Preparation of the dried pulverized extract.
The concentrated extract obtained in the example 2 was diluted with a sufficient volume of ethanol-water (80/20 vol/vol) for mixing with maltodextrin through mechanical agitation and heating, and afterward submission to spray drying. The quantity of excipient aggregated to the mixture was in a sufficient quantity so that the final powder contained 4 to 6 mg of active principles per gram of final pulverized. The final concentration of active principles in the powder was effected by means of the method mentioned in the example 8. The active principles contained in the obtained extract correspond to glycoflavonoids (guaijavarin, isoquercitrin, hyperin, quercitrina and avicularina).

Example 4. Manufacture of tablets with carboxymethylcellulose.
The pulverized extract obtained in the example 3 was mixed with carboxymethylcellulose (20% weight/weight) and magnesium stearate (0.2% weight/weight), submitting the obtained mixture to compression for the obtaining of tablets with an approximate weight of 350 to 400 mg with a minimal concentration of 1 mg of active principles per tablet. The final concentration of the active principles in the tablets was determined by means of the method mentioned in example 8.

Example 5. Manufacture of tablets with maltose.
The dried pulverized extract obtained in example 3 was blended with appropriate quantities of maltose and magnesium stearate. Then the obtained mixture was submitted to compression for the obtaining of tablets with an approximate weight of 350 to 400 mg with a minimal concentration of extracts of 2 mg of active principles per tablet. The final concentration of active principles in the tablets was determined by means of the method mentioned in the example 8.

Example 6. Quantification of the active constituents contained in the leaf.
A quantity of 10 g of _P. guajava_ leaves was pulverized in a mortar. In an analytical balance 1 g of the obtained powder was weighted and placed in a Whatman #1 paper cartridge for submitting it to an exhaustive extraction with methanol in a Soxhlet equipment until the dissolvent of the reflux appeared without coloration. The methanol was evaporated and the remainder re-suspended in 20 ml of HCl 0.5N in MeOH, and the mixture heated by reflux for 30 min. The mixture was adjusted to 25 ml with
methanol, and then 1 ml of this mixture transferred to a graduated flask of 10 ml token to volume with methanol. Of the resulting mixture an aliquot was token and then filtered by a membrane of 0.45 μm. A volume of 10 μl of the resulting solution was utilized for its chromatographic analysis as described in the example 10.

Example 7. Quantification of the active constituents contained in the concentrated extract.

0.5 g of the concentrated extract were weighted and dissolved in 20 ml of HCl 0.5N in MeOH, and the mixture heated by reflux for 30 min. The mixture was then adjusted to 25 ml with methanol, and then 1 ml of this mixture transferred to a graduated flask of 10 ml token to volume with methanol. Of the resulting mixture an aliquot was token and then filtered by a membrane of 0.45 μm. A volume of 10 μl of the resulting solution was utilized for its chromatographic analysis as described in the example 10.

Example 8. Quantification of the active constituents principles contained in the dried pulverized.

1 g of the pulverized was weighted and suspended in 20ml of HCl 0.5N in MeOH, and the mixture heated by reflux for 30 min. The mixture was filtered through Whatman #1 paper and then the paper filter washed with methanol. The filtrate was adjusted to 25 ml with methanol, and then 1 ml transferred to a graduated flask of 10 ml and token to volume with methanol. Of the resulting mixture an aliquot was taken and filtered through a membrane of 0.45 μm. A volume of 10 μl of the resulting solution was utilized for its chromatographic analysis as described in the example 10.

Example 9. Quantification in the tablets.

Ten tablets were milled in a mortar. Then 1 g of the obtained powder was weighted and re-suspended in 20 ml of HCl 0.5N in MeOH, and the mixture heated by reflux for 30 min. The mixture was filtered through Whatman #1 paper and the paper filter washed with methanol. The filtrate was adjusted to 25 ml with methanol, and then 1 ml transferred to a graduated flask of 10 ml and token to volume with methanol. Of the resulting mixture an aliquot was taken and filtered through a membrane of 0.45 μm. A volume of 10 μl of the resulting solution was utilized for its chromatographic analysis as described in the example 10.
Example 10. Chromatographic analysis.

The concentration of active principles in the leaf, concentrated extract, dried pulverized and tablets was quantified as equivalent concentration of quercetin, given that the active principles are a combination of flavonoids derivative from this compound.

Approximately an equivalent of 0.025 g of standard quercetin was weighted, dissolved in methanol and adjusted to 50 ml (conc. 0.0005 g/ml). Seriated dilutions with methanol were obtained in concentrations of 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml which were injected three times into a liquid chromatograph for its analysis. The obtained area averages were calculated and with that value the lineal regression curve was traced. The elution of the samples under analysis was achieved by lineal gradient with H₃PO₄ 10mm in water and acetonitrile, across a C-18 Kromasil column (5 μm particle diameter) (MetaChem Technologies, Inc, Torrance CA, USA), under a temperature of the column of 35°C. For the detection, an UV light detector set to a wavelength of 254nm was used.

Example 11. Identification of the active principles contained in the extracts.

The active principles of the obtained extract from the example 2 and from the raw material used for its obtaining according to example 1, was determined through high pressure chromatography whereby the molecules and its quantity were identified according to the elution of each one of it. The obtained fractions were analyzed for the subsequent separation of the individual compounds, using elution and banding techniques over Whatman paper⁷⁶. Each of the products was purified by means of Sephadex LH-20 columns⁷⁷.

The structures of the purified compounds were determined by standard methods using acid hydrolysis with HCl to 100°C for 60 min, enzymatic hydrolysis with β-glucosidase and β-galactosidase using buffer acetates (pH 5), oxidation with hydrogen peroxide, UV spectroscopy, ¹H-NMR, ¹³C-NMR, mass spectroscopy and by means of its comparison with control samples. The obtained aglycone got by hydrolysis of the isolated compounds was identified through co-chromatography with control samples using chromatography in paper, UV spectrum, ¹H-NMR y ¹³C-NMR. The sugars freed by hydrolysis were identified by chromatography in paper using standard methods⁷⁸,⁷⁹. The results of this determination are shown in table 1.
### Table 1

Flavonoid content in the extract of the invention and its concentration in the *Psidium guajava* L. leaf.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Extract</th>
<th>Leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>$10^{-4}$ (mol/g)</td>
<td></td>
</tr>
<tr>
<td>Isoquercitrin</td>
<td>1</td>
<td>32.57</td>
</tr>
<tr>
<td>Hyperin</td>
<td>0.85</td>
<td>27.70</td>
</tr>
<tr>
<td>Guaijavarin</td>
<td>0.65</td>
<td>21.17</td>
</tr>
<tr>
<td>Quercitrin</td>
<td>0.33</td>
<td>10.75</td>
</tr>
<tr>
<td>Avicularin</td>
<td>0.16</td>
<td>5.21</td>
</tr>
<tr>
<td>Quercetin</td>
<td>0.08</td>
<td>2.60</td>
</tr>
</tbody>
</table>


A controlled, random and double blind clinical study was performed in a group of patients with acute diarrheic disease. Patients of both sexes, between 20 and 59 years with acute diarrheic disease defined by a clinical history characterized by at least three evacuations in the last 24 hrs, decreased in consistency with respect to the normal pattern of evacuations, were included. The patients of the experimental group (n=50) received 400 mg of the phytomedicament of the invention in capsules presentation that were administered orally, one dose every 8 hours during 3 days. The control group (n=50) received placebo in the same type of pharmaceutical forms and the same treatment scheme. Oral hydration therapy was applied to all patients in both groups in accordance with conventional procedures recommended by WHO for the management of acute diarrheic disease (WHO, 1990). The variables to verify were: consistency and number of evacuations a day, presence of mucus in feces; degree of abdominal pain (in a range of 0 to 10 units); number of spasms in 24 hours; presence of fever and vomit episodes.

The diarrheic episodes, that represented the motives for medical consultation, were moderated (the median of evacuations in the last 24 hrs was 6, and almost all liquid); more than 80% of all patients presented abdominal pain of moderate to severe intensity, and additional symptoms reported frequently were abdominal distention and vomiting (see table 2).
Table 2
Clinical conditions of the patients

<table>
<thead>
<tr>
<th></th>
<th>Group under treatment n=50</th>
<th></th>
<th>Control group n=50</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Days with diarrhea</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Interval</td>
<td>1-4</td>
<td></td>
<td>1-4</td>
<td></td>
</tr>
<tr>
<td><strong>No. evacuations in the previous 24 hours</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>6</td>
<td></td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Interval</td>
<td>3-14</td>
<td></td>
<td>3-15</td>
<td></td>
</tr>
<tr>
<td><strong>Consistency</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liquid</td>
<td>31</td>
<td>62</td>
<td>27</td>
<td>54</td>
</tr>
<tr>
<td>Semi-liquid</td>
<td>19</td>
<td>38</td>
<td>23</td>
<td>46</td>
</tr>
<tr>
<td><strong>Abdominal pain</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>44</td>
<td>88</td>
<td>42</td>
<td>84</td>
</tr>
<tr>
<td><strong>Intensity of the abdominal pain (scale 0-10)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderated (3-5)</td>
<td>20</td>
<td>40</td>
<td>13</td>
<td>26</td>
</tr>
<tr>
<td>Severe (6-10)</td>
<td>21</td>
<td>48</td>
<td>29</td>
<td>58</td>
</tr>
<tr>
<td>Median</td>
<td>6</td>
<td></td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td>Interval</td>
<td>3-10</td>
<td></td>
<td>3-10</td>
<td></td>
</tr>
<tr>
<td><strong>Additional symptoms on the first day</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal distention</td>
<td>40</td>
<td>80</td>
<td>32</td>
<td>64</td>
</tr>
<tr>
<td>Mucus in the evacuation</td>
<td>2</td>
<td>4</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Fever</td>
<td>7</td>
<td>14</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Vomit</td>
<td>21</td>
<td>42</td>
<td>25</td>
<td>50</td>
</tr>
</tbody>
</table>

The evolution of symptoms in the patients once the treatment was established (Table 3), was analyzed for each day of follow up. The group that received the treatment with the phytomedicament of the invention showed significant differences with respect to the control group in the number of diarrheic episodes and abdominal pain from the first day of treatment (see table 3 and figure 3). The clinical evolution of the group treated with the phytomedicament was better and faster in comparison with the control group (see figure 3, column C), the used product was well tolerated and did not produce any side effects.
Table 3
Evolution during the treatment

<table>
<thead>
<tr>
<th></th>
<th>Group with treatment</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Patients</td>
<td>Median</td>
</tr>
<tr>
<td><strong>Number of evacuations</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>50</td>
<td>5</td>
</tr>
<tr>
<td>Day 2</td>
<td>40</td>
<td>3</td>
</tr>
<tr>
<td>Day 3</td>
<td>32</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total of evacuations</strong></td>
<td>50</td>
<td>8</td>
</tr>
<tr>
<td><strong>Consistency (liquid or semi-liquid)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>45</td>
<td>4</td>
</tr>
<tr>
<td>Day 2</td>
<td>29</td>
<td>3</td>
</tr>
<tr>
<td>Day 3</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td><strong>Abdominal pain (number of episodes)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>30</td>
<td>3</td>
</tr>
<tr>
<td>Day 2**</td>
<td>10**</td>
<td>1</td>
</tr>
<tr>
<td>Day 3**</td>
<td>3**</td>
<td>2</td>
</tr>
</tbody>
</table>

** p = 0.02

These results show that the phytomedicament of the invention provides fast and effective relief of the intestinal colic, a characteristic symptom of the acute diarrheic syndrome. The results obtained from the clinical study using the phytomedicament of the invention, allows inferring by means of the analysis of all and each of the variables that with the use of one dose (400 mg every 8 hours during 3 days) the obtained effects resulted very satisfactory.

References.


Claims.

1. A standardized extract of *Psidium guajava* L., characterized because has a concentration of flavonoid glycosides of 4 to 6 mg per gram of extract, measured as total concentration of quercetin.

2. The extract of the claim 1, characterized because the flavonoid glycosides are selected from the group that comprise glycosides derivative from quercetin and glycosides derivative from morin.

3. The extract of the claim 2, characterized because the glycosides derivative from quercetin are selected from the group that comprises quercetin-3-O-α-L-arabinoside, quercetin-3-O-β-glucoside, quercetin-3-O-β-D-galactoside, quercetin-3-O-β-L-rhamnoside and quercetin-3-O-gentobioside.

4. The extract of the claim 2, characterized because the glycosides derivative from morin are selected from the group that comprises morin-3-O-alpha-L-lixopyranoside and morin-3-O-alpha-L-arabopyranoside.

5. The extract of the claims 1 to 4, characterized because the extract is in form of a powder.

6. The extract of the claims 1 to 5, characterized because the extract additionally contains a pharmaceutically acceptable excipient.

7. The extract of the claim 6, characterized because the excipient is maltodextrin.

8. The extract of the claims 1 to 4, characterized because the extract is in a form of liquid.

9. The extract of the claims 1 to 8, characterized because it is stable for at least 4 months under room temperature.

10. A pharmaceutical composition for the treatment of gastrointestinal diseases in mammals, characterized because it includes a therapeutically effective quantity of the extract of the claims 1 to 9 in a pharmaceutically acceptable vehicle.

11. The pharmaceutical composition of the claim 10, characterized because is in a pharmaceutical presentation selected from the group that comprise tablets, capsules or pills.

12. The pharmaceutical composition of the claim 11, characterized because is in a form of tablet.

13. The pharmaceutical composition of the claim 11, characterized because it is found in a form of capsule.
14. The pharmaceutical composition of the claim 11, characterized because is in a form of pill.

15. The pharmaceutical composition of the claims 10 to 14, characterized because it contains a concentration of 1 to 4 mg of flavonoid glycosides measured as total concentration of quercetin for each 400 to 500 mg of the composition.

16. A method for the obtaining of the extract of the claims 1 to 9, characterized because it comprises the steps of:
   a) Selecting the *Psidium guajava* L. leaves, dried previously with a minimum content of 8 mg of flavonoid glycosides, measured as total concentration of quercetin per gram of leaf,
   b) Milling the selected leaves in step a) and sieving the powder through a 40 mesh,
   c) Extracting the powder of the step b), by addition of a mixture of alcohol of 1 to 4 carbons and water, in a proportion of 50:50 to 80:20 vol/vol and incubation of the mixture under a temperature of 30 to 50°C for a period of 24 to 48 hrs.
   d) Filtrating the obtained mixture, concentrate the filtrate by distillation and recovering the dissolvent for the consecutive extraction of the powder in accordance to the step c),
   e) Mixing the concentrated extracts and selecting those with a minimum content of 10 mg of flavonoid glycosides, measured as total concentration of quercetin for each gram of concentrated extract, and
   f) Adding a sufficient quantity of a pharmaceutically acceptable excipient to the concentrated extract of the step e) until the obtaining of a concentration of flavonoid glycosides of 4 to 6 mg per gram of the extract, measured as total concentration of quercetin.

17. The method of the claim 16, characterized because in the step c) the powder is extracted with a mixture of ethanol-water in a proportion of 80:20 vol/vol under a temperature of 50°C for a period of 48 hrs.

18. The method of the claim 17, characterized because the concentration of flavonoid glycosides is determined through a high performance liquid chromatography.

19. The method of the claim 18, characterized because the pharmaceutically acceptable excipient is maltodextrin.

20. The method of the claims 16 to 19, characterized because additionally the mixture obtained in the step f) is submitted under dehydration,
21. The method of the claim 20, characterized because the dehydration is carried out through spray drying.

22. The use of the extract of the claims 1 to 9, for the manufacturing of a medicament for the treatment of gastrointestinal diseases in mammals.

23. The use of the claim 22, characterized because it is administered a dose of the extract equivalent of 0.01 to 0.05 mg of flavonoid glycosides per Kg of corporal weight.

24. The use of the claim 23, characterized because it is administered a dose of the extract equivalent of 0.01 to 0.02 mg of flavonoid glycosides for each Kg of body weight.

25. The use of the claims 22 to 24, characterized because the extract is orally administered.

26. The use of the claim 25, characterized because the extract is administered in only one dose.

27. The use of the claim 26, characterized because the extract is administered in several doses and one to three times a day for 3 to 5 days.

28. The use of the claims 21 to 25, characterized because the extract is administered until the complete disappearance of the clinical symptoms.

29. The use of an extract of *Psidium guajava* L. for the manufacturing of a medicament for the treatment of gastrointestinal diseases in mammals caused by dysfunctions of the nervous system associated to gastrointestinal function.

30. The use of the claim 29, characterized because the gastrointestinal diseases are selected from the group that comprises the irritable intestine syndrome, irritable bowel syndrome, acute diarrheic syndrome, nervous gastritis and colitis.

31. The use of the claim 30, characterized because the gastrointestinal disease is irritable intestine syndrome.

32. The use of the claim 30, characterized because the gastrointestinal disease is acute diarrheic syndrome.

33. The use of the claim 29 to 32, characterized because it is administered a dose of the extract equivalent to 0.01 to 0.05 mg of flavonoid glycosides *per* gram of body weight.

34. The use of the claims 33, characterized because it is administered a dose of the extract equivalent to 0.01 to 0.02 mg of flavonoid glycosides *per* Kg of body weight.
35. The use of the claims 29 to 34, characterized because the extract is orally administered.

36. The use of the claim 35, characterized because the extract is administered in only one dose.

37. The use of the claim 36, characterized because the extract is administered in several doses and one to three times a day for 3 to 5 days.

38. The use of the claims 29 to 35, characterized because the extract is administered until the complete disappearance of the clinical symptoms.

39. A method for the standardization of the active constituents in *Psidium guajava* L. extracts, characterized because it comprises the determination of the flavonoid glycosides contained in the extract, measured as total concentration of quercetin.

40. The method of the claim 39, characterized because it comprises the steps of:
   a) Determining the concentration of flavonoid glycosides, measured as total concentration of quercetin, contained in the vegetal material to be used for the obtaining of the extract and,
   b) Determining the concentration of flavonoid glycosides, measured as total concentration of quercetin, contained in the extract.

41. The method of the claim 40, characterized because the minimal concentration of flavonoid glycosides, measured as total concentration of quercetin, contained in the vegetal material is of 8 mg per gram of it.

42. The method of the claim 41, characterized because the vegetal material are leaves.

43. The method of the claim 40, characterized because the minimal concentration of flavonoid glycosides, measured as total concentration of quercetin, contained in the obtained extract is of 10 mg per gram of it.
# INTERNATIONAL SEARCH REPORT

**International application No.**

PC: /1B2005/002172

## A. CLASSIFICATION OF SUBJECT MATTER

A61K31/7048  A61K36/00  A61P1/12

According to International Patent Classification (IPC) or to both national classification and IPC.

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols):

A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched:

Electronic data base consulted during the international search (name of data base and, where practical, search terms used):

EPO-Internal, BIOSIS, EMBASE, WPI Data, PAJ

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
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Further documents are listed in the continuation of Box C. See patent family annex.

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**Date of the actual completion of the international search:**

9 March 2006

**Date of mailing of the international search report:**

24/03/2006

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