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(RESEARCH ARTICLE)

Aloe vera and *Bixa orellana* in a revitalizing ointment for cattle skin injuries

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Abstract

Phytopharmaceuticals constitute a therapy that, when carried out on a scientific basis, becomes a necessary, safe, effective and, above all, economical alternative in the confrontation of diseases that affect humans and other animals, for the achievement of health. The objective of the research was to evaluate the effectiveness of a revitalizing ointment of *Aloe vera L* and *Bixa orellana* L for skin lesions in cattle. The methods used correspond to the transversal research for case control, a total of 76 animals of the classified by categories, females and males of mestizo breeds, belonging to the Basic Livestock Unit "Liberation of Caibarién", Caibarien, Villa Clara, Cuba were selected during the year 2019 where there were problems with the iron marking, the lesions and the statistical equivalence in both groups were taken into account. From the benefits of the two medicinal plants, an ointment was elaborated to treat skin lesions such as burns, wounds and in general, any lesion that tends to chronicity or infection and therefore it is required to accelerate the healing process. The revitalizing ointment proved to be a good healing agent, since it helps the prompt recovery of the lesions and of the animals. In no case did allergic reactions or irritation occur. Since there are difficulties with the acquisition of healing ointments in veterinary medicine, it constitutes an effective and economical alternative treatment, with a positive impact on livestock and industrial production, as well as on the environmental aspect.

Keywords: *Aloe Vera*; *Bixa Orellana*; Cattle; Skin Lesions; Revitalizing Ointment

1. Introduction

The growing use of medicinal plants among the diversity of Traditional and Natural Medicine (TNM) options is an undeniable social phenomenon. The World Health Organization created the program "WHO Strategy on Traditional Medicine 2002-2005" [1]. This strategy emphasizes the rational use of traditional/complementary medicine and its incorporation into the health system.

Traditional medicine of proven quality, safety and efficacy contributes to ensuring access to health care for all people. It also offers a less harmful and more economically efficient variant of solution, due to savings in industrial chemical drugs and fewer reports of adverse reactions [2].

In Latin America, WHO reports that 71% of the population in Chile and 40% in Colombia use traditional medicine at least once a month [3]. It is now recognized that there is a need to develop a coherent and comprehensive approach to health care and that access to traditional medicine in developing countries is in certain cases, a viable treatment option [4].

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In Cuba, natural medicine modalities that have scientific and traditional validation are used. Although various techniques of traditional and natural medicine were used in the country in selected clinics, since 1996 a program was undertaken with a set of strategic objectives and actions of various kinds, aimed at developing knowledge and procedures, related to TNM [5].

The Cuban population uses medicinal plants to treat their ailments and in rural areas with livestock activities, in addition to the use of plants for that purpose, they use plants to cure the ailments that domestic and barnyard animals present, this is confirmed with studies conducted in the region, which are indicators of the high knowledge and use of plants for such purposes [6].

The use of plants in animal health are popular practices [7]. In Cuba, it is traditional practice to use medicinal plants in some affections of animals and cattle, but reports and evidence of efficacy for veterinary use are scarce. It is necessary to deepen the study of these practices, because alternative solutions to animal health in the region could be found, which would result in undeniable positive effects on livestock production, industrial and environmental aspects, the latter is justified by the effects that certain chemicals (acaricides and insecticides) cause to the environment.

Therefore, it is proposed in the study to evaluate the effectiveness of a revitalizing ointment of *Aloe vera* L and *Bixa orellana* L for skin lesions in cattle.

2. Material and methods

2.1. Description of the study area

A total of 76 animals of the category calves and yearlings, females and males of mestizo breeds, belonging to the self-consumption of the basic livestock unit "Liberation of Caibarien", Villa Clara province, Cuba, where there were problems due to injuries in the animals caused by iron marking during the year 2019, were selected for the study.

The animals were divided into two groups: group I of 50 animals and group II, with 26 control animals, where the lesions and the descriptive and percentage statistical equivalence were taken into account, in both groups.

Based on the review of documents that included the bibliography and scientific articles, where the positive results of the benefits that plants offer in terms of medicinal properties in Cuba and the world are shown, we decided to use *A. vera* and *B. orellana* for the elaboration of the revitalizing ointment and to be able to apply it in the cutaneous lesions in cattle and thus evaluate its effectiveness in the treatment.

2.2. Aloe

A. vera, a plant with about 360 different species, belongs to the family of Asphodelaceae or Liliaceae, with perennial leaves in the form of rosette; its size can reach from a few centimeters to 50 cm [8]. The first references to *A. vera* are found in the Ebers Papyri and there are numerous historical documents from the Egyptians, Greeks, Romans, Algerians, Arabs, Tunisians, Indians and Chinese, among others, that speak of its use for medicinal and cosmetic purposes [9]. Its name comes from the Greek "aloé"; and in Arabic it is called "alloeh", which means: "the shiny bitter substance"; the word vera comes from Latin and means: "truth" [9,10].

- Scientific name: *Aloe vera* (L) = *Aloe barbadensis* Miller Liliaceae.
- Common name: *Aloe*, *Aloe*.
- Useful part: *Aloe* gel.

Description of the plant

A. vera belongs to the herbaceous, shrubby, succulent xerophytic perennial *Aloe* genus [5,11] (Figure 1).

Figure 1 *Aloe vera* L. (leaves)

A. vera L.*,* popularly known as aloe vera, belongs to the Liliaceae family; it is an important plant used in traditional medicine in the cure of various ailments, such as skin diseases, radiation damage, eye disorders, intestinal disorders and antiviral diseases. It is characterized for being one of the greatest cell regenerators that nature has given [5,11,12].

The most used parts of this plant are the leaves (Figure 2), where the fleshy part is extracted, colorless and odorless mucilage, commonly known by the name of crystal [5,11].

Figure 2 Leaves, fleshy part, colorless and odorless mucilage

2.3. Bixa

When the Spanish conquistadors arrived in the New World, they discovered a large number of plant-derived products used by the Mayans and Aztecs. One of them, annatto is a carotenoid-based dye extracted from the seeds of the tropical plant *B. orellana* L. [13]. The etymology of the binomial name corresponds to bixa, Latinization of the Portuguese bija; orellana, dedicated to the Spanish explorer Francisco de Orellana (1490-1546) [13].

Scientific name: *Bixa orellana* L. = *Bixa odorata, B. acuminata, B. americana, B. platycarpa,* belongs to the family Bixaceae [5,14].

Common name: Bija.

Useful part: Seeds [5].

Description of the plant

Present in the Cuban fields, Bija or Achiote is a medicinal plant used mainly in the past, for the elaboration of colorants and as a spice for the elaboration of certain dishes such as "Yellow Rice" or "Cuban soup". The most common names are Achiote, acote, achihuete, saca achote, urcu, annato, Bija [15].

It is described as a shrub up to 9 m tall, with brown bark and young branches commonly scaly. Aovate leaves 8 to 20 cm long and 4 to 15 cm wide, with long petiole (Figure 3).

Figure 3 Bija or Achiote, Shrub. Bark, branches, leaves

Flowers in terminal panicles; corolla with five petals, twisted at the bud, rounded and pinkish; stamens numerous (Figure 4).

Figure 4 Flowers and fruits

Fruits are ovate-globose capsular-shaped, about 4 cm in diameter, densely covered with soft, thin spines. Numerous seeds. Phenology Its flowers between August and September; 60 days after the beginning of flowering ripe fruits are observed, which are possible to locate on the plant until January and February. Native to tropical America. Naturalized in the tropics of the Old World (Figure 5).

Figure 5 Fruits and Seeds

2.4. Preparation of the Revitalizing Ointment

The ointment was elaborated in the specialized Pharmacy classroom of the Faculty of Nursing and Health Technology of the University of Medical Sciences of Villa Clara, taking into account that it should be made in an aseptic environment with all the norms and procedures, using a simple method, the mechanical incorporation of all its ingredients.

Several ways of extracting the colorant from the Bija seeds are known, some very rudimentary and others not so rudimentary that, finally, with the passage of time have been improved. Some of these techniques are described below:

- The seeds, separated from the mature capsules, are placed in enough boiling water so that the dye is easily detached from them; then they are separated, the paste is left to ferment for approximately one week; the water is eliminated and the paste is left alone [13].
- One of the oldest and practically abandoned methods consists of crushing the seeds between cylinders to form a mixture with the dye of the Bija seeds. A sufficient quantity of water is added to the resulting mass and when its sediments, the clear water is removed and it is left boiling for two or three hours. When it is removed from the fire, it is squeezed by means of a press to remove the water [15].

Proper seed selection is necessary, because it has been proven that the fresher the seeds are, the better yield and quality is obtained in the preparations [13,16].

The potential use of *Aloe* products often involves some type of treatment, for example, heating, dehydration or grinding. Unfortunately, due to inadequate processing during gel preparation and stabilization, irreversible modifications are caused in bioactive components such as polysaccharides and antioxidant compounds, affecting their original structure and promoting important changes in biochemical properties, causing many of the products to contain very little or almost no active ingredient [17].

In recent decades, several studies have focused on investigating the main active chemical compounds responsible for the therapeutic effects reported for *A. vera*, and have also been interested in developing an effective method to maintain and preserve these compounds naturally contained in the *A. vera* gel in order to improve the quality of the product [8,18].

The processing of *A. vera* gel described by the authors [8,18] is described below:

- It starts with the harvesting of the *A. vera*; this consists of cutting the leaves by hand from the base of the plant.
- Washing of the leaves received in the plant with water and bactericidal solutions.
- Subsequently, the gel is removed and separated from the bark by filleting. The most used method is the manual separation that consists of making manual cuts to the leaf by filleting the gel with a knife from 2.5 cm above the base and covering the entire upper end and the lateral parts, the gel is liquefied.
- This is done at room temperature (25 °C), so it is recommended to grind for a period of 10 to 20 minutes, since the longer this process takes, the greater the darkening of the gel due to enzymatic browning.
- Through the addition of pectolytic enzymes, stabilization is carried out to preserve the compounds with biological activity, such as polysaccharides, which have a greater presence and importance. This stabilization can be achieved with the addition of these enzymes at 50 ºC for periods of twenty minutes.
- Once the stabilization is done, filtration is carried out. This process also influences the stability of the gel and also separates particles from the product by sedimentation.
- After this, vitamin C or ascorbic acid is added to prevent browning and improve the flavor of the juice. The pH is adjusted to 3.0 or 3.5 by adding citric acid.
- Vacuum drying of the liquid gel is carried out to eliminate oxygen trapped in the form of bubbles and to avoid oxidation of the ascorbic acid, also improving the shelf life of the gel.
- Pasteurization can be carried out according to the HTST process at 85-95 °C, avoiding bad taste and loss of biological activity.
- After this, it is cooled suddenly to 5 °C for ten to fifteen seconds, this stage is fundamental to guarantee the biological activity of the gel.
- Finally, the *Aloe* gel is packaged in glass or plastic.

For the present preparation, the methodology described in the National Form of Phytopharmaceuticals and Apipharmaceuticals is assumed in 2013, and thus extract the active principles in both plants.

By the cooking method, we boil in mineral oil, the previously selected seeds of the Bija, in the following proportion 3 parts of oil for one part of seeds of Bija obtaining 100 ml of the oily extract of the Bija.

With the *A. vera* we peel the leaves and crush (by means of a high-speed commercial crusher) the crystals obtaining the aqueous extract of this, which we boil to concentrate.

Then by mechanical incorporation, we join the components in the following proportions: 90 g of Bija extract in 300 g of Vaseline, from this mixture we take 10 g and join it with the same proportion of *A. vera* extract to obtain an ointment with an oleaginous hydrocarbon base since we use solid Petrolatum or Vaseline as a base.

Steps

- Crush methylparaben and propylparaben.
- Incorporate to the base of the active principles.
- Final mixing until a homogeneous consistency is obtained.

Storage: should be packed in amber colored bottles and in a cool place to avoid contamination and oxidation of its components.

2.4.1. Formulation R /

Bija oil extract 10 g
Aloe vera aqueous extract 10 g
Methylparaben 0.0075 g
Propylparaben 0.0045 g
Petrolatum solid csp 30 g
Legend: csp: Sufficient quantity to prepare

2.4.2. Description of the formula

It is a medicinal preparation, for external use, composed by the association of the active principles of each plant, being these of great utility in the care of the skin and when incorporating the mineral oil, it confers protective, epitheliotropic and antiseptic properties. It is a product that does not contain essences, perfumes or synthetic colorants.

Presentation in amber colored bottles with 30 g.

Once the ointment was obtained and the two groups of animals treated once a day, after cleaning the wound or burn with boiled water and leaving the injured area free of crusts that would prevent penetration and fulfill the effect of the ointment, a thin layer was applied covering the entire surface of the lesion, however, if a more intense effect is required, it can be applied twice a day, repeating the action of cleaning crusts and detritus in each cure.

3. Results and discussion

A. vera, popularly known as *A. vera*, is widely used in skin lesions, mainly for its emollient and softening power. It has been confirmed that these crystals contain vitamins A, B1, B2, B6, C, E and folic acid, in addition to minerals, essential amino acids and polysaccharides that stimulate tissue growth and cell regeneration [5,11].

Chemical composition

Aloe is constituted, fundamentally, by hydroxyanthraquinonic derivatives such as: aloins A and B (barbaloin), 5-hydroxyaloin A, aloe-emodin. Glucosyl chromones (aloerresins A, B, C, isoaloerresin); aloenins A and B (bitter principles) [5,11].

Aloe gel contains, essentially, water and polysaccharides such as pectins, hemicellulose, glucomannans, acemannans and mannose derivatives (mannose 6-phosphate is usually the major constituent within sugars). The presence of amino acids, lipids, sterols (campesterol and sitosterol), triterpenes (lupeol) and enzymes has been identified. Additionally, the presence of lignans, salicylic acid, vitamins (A, C, E, B12, thiamine, niacin and folic acid) and minerals (sodium, calcium, potassium, manganese, copper, magnesium, chloride, zinc and iron) are reported. The fresh gel contains

glutathione peroxidase, superoxide dismutase isoenzymes and the proteolytic enzyme carboxypeptidase. The presence of flavonoids, tannins and coumaric acid has also been determined [5,19].

3.1. Manufacturing procedure

Use *Aloe* leaves from plantations with no less than 1.5 years of sowing, freshly collected. Remove impurities from the leaves with running water and diluted chlorinated water. Remove the outer layers of the leaves including the cells of the pericycle by removing the thorns on the sides, taking care not to tear the green skin so as not to contaminate the gel, on the contrary, remove the part of the gel close to the green skin. Once the gel is obtained, it is pasteurized for 3 minutes at a temperature between 75-80º C. Higher temperatures or longer time could cause changes in the chemical composition, distilled water can be added to each portion to be heated (no more than 1 liter). Filter through a gauze. Dissolve the preservative and add it. If the sodium benzoate is 0.2 or 0.3%, dissolve in hot water, but if the preservative is methylparaben and propylparaben dissolve in alcohol (For 100 L of extract is 180g of methylparaben and 20g of propylparaben). Add 1L of alcohol for 100 L of extract. To complete with distilled water until obtaining the quantity to produce and to filter by gauze and cotton. It is not recommended to work with the whole leaf [5].

Shelf life: 6 months. It is used for the elaboration of other formulations [5].

3.2. Pharmacological effect

3.2.1. Demonstrated biological activity

Analgesic, antibacterial, antiherpetic, anti-inflammatory, antiulcer, fungicidal, emollient and healing. The fleshy part (*Aloe* gel) is the carrier of the components that have healing, anti-inflammatory, skin protective action; it also has bactericidal properties, laxatives and detoxifying agents. Therefore, this plant has a wide range of therapeutic applications [5,19].

A. vera gel showed many physiological and biological activities such as, healing ability of skin burns, skin lesions, acne, psoriasis, anemia, anticancer, antiviral agent, ultraviolet protector, prophylactic effect against accidental nuclear radiations, anti-inflammatory agent, analgesic, antioxidant and can also be used as a natural pesticide [19].

3.2.2. Toxicological effect

Not reported by topical route.

In humans at high doses, orally, if the gel is contaminated with anthraquinones, they can produce an intense emetic, cathartic effect, with bloody diarrhea, intestinal colic, hypothermia, albuminuria, convulsions and collapse. Potassium depletion finally produces a paralysis of the intestinal musculature, which causes a loss of laxative effectiveness and constipation is perpetuated, which forces to gradually increase the dose, causing long-term irreversible damage to the intestinal membrane and musculature, with the appearance of tenesmus, stools with abundant mucus and dark coloration of the intestinal mucosa. Latex can cause a drastic laxative effect. The abuse of laxative anthraquinones can cause the appearance of colon carcinoma, ulcer and intestinal irritation. The alcoholic extract at doses of 100 mg//3 months is toxic in mice [19,20].

Extracts of *B. orellana* are oils or alkaline products obtained by removing the outer layer of annatto seeds by various processes.

3.2.3. Chemical composition

The presence of carotenoids (β-carotene, crocetin, methyl bixin, norbixin, bixin and isobixin (major carotenoids), among other derivatives, has been determined. Acids such as ellagic acid, salicylic acid, maslinic acid, gallic acid; of luteolin-7-glucoside, cyanidin, lignin, lutein, pyrogallol and isoscutelarein (flavonoid) [14,21].

The red color of achiote or annatto is due to several carotenoid compounds, mainly apocarotenes, found in the seed. Bixin is the most sought after and important of these, it is a dark red crystalline substance, soluble in alcohol, oils and fats and insoluble in water [22].

Chemically, it is a carotenoic acid with the empirical formula C25H300, which occurs as a cis-type geometric isomer, but can be converted to its more stable trans form. It is insoluble in water and slightly soluble in chloroform, vegetable oils, ethyl acetate and propylene glycol [15,21].

The seeds also contain vitamin C, iron and proteins.

3.2.4. Recognized medicinal properties

Skin and mucous membranes: Antibacterial

3.2.5. Demonstrated biological activity

Antibacterial, antipyretic, antiseptic, aphrodisiac (female), emollient, fungicidal and healing.

3.2.6. Pharmaceutical forms described

Vegetable drug. Route of administration Topical.

Other attributed properties (Not yet approved) Leaf poultices are emollient, refreshing and anti-inflammatory. The oral decoction is emmenagogue, hepatotropic, antiemetic, diuretic, antivenereal and hemostatic. The seeds are used for asthenia, asthma, diarrhea, dandruff, and its oily maceration for burns [5,11].

Shelf life: 2 years at room temperature [5,11].

3.3. Manufacturing procedure

In a stainless-steel container deposit, the annatto seeds and the liquid petrolatum. Place the resultant of the previous step on the fire. Heat the preparation up to 115ºC, stir constantly with a paddle for 20 minutes. Filter with double gauze [5,11].

3.3.1. Toxicological effects

Toxicity has been demonstrated, in dogs that consumed 60 mg/ of trans-bixin orally. Massive administration of seed causes pancreotoxicity, hepatotoxicity with hyperglycemia and apparent increase of insulin level in dogs; toxicity decreases with riboflavin administration [23].

Taking into account the benefits of the two medicinal plants, it was proposed to create the ointment to treat skin lesions such as burns, wounds and others in general, any healing process that tends to be chronic and therefore requires accelerating the healing process.

Table 1 shows that a total of 76 animals of the category calves and yearlings, females and males of mestizo breeds were taken for this experiment. These were separated into 50 in the first group or treated group and 26 in the control group or untreated animals.

Table 1 Conformation of the Groups for the experiment

Total animals	Group discussed	Control Group
76	50	26

Table 2 shows the lesions obtained and treated by groups I and II, as well as the % representing each lesion.

Table 2 Conformation of the groups by lesions

Lesions	Group I (treated)	%	Group II (control)	%
Iron burns	40	80	19	73.07
Wounds	7	14	5	19.23
Dermatitis in the sun	3	6	2	7.69

Tables 3 and 4 show the groups, as well as the recovery time of the lesions; we can see that in group I treated the recovery is fast and total, not having other reinfections that affect the animal both in weight and life itself. However, the

animals corresponding to the untreated group II had a late recovery and showed the presence of secondary infections that implied losses in body weight of the animals.

Table 3 Animals treated with the revitalizing ointment

Lesions	Number of animals	Recovery in days	Secondary infections	Recovered
Burns	40	5-6	__	total
Wounds	7	4	__	total
Dermatitis in the sun	3	4-5	__	total

Table 4 Untreated animals. Control group

Lesions	Number of animals	Recovery in days	Secondary infections	Recovered
Burns	19	10-15	Miasis Bacterial contamination	total
Wounds	6	8-12	Bacterial contamination	total
Dermatitis in the sun	1	__	__	Not recovered

If we compare the group of treated animals and the control group, we obtain the data shown in Table 5.

Table 5 Comparison between group I and group II

	Animal recovery	Average duration in days	Differences in days
Group I	100%	5-6 days	
Group II		12-14 days	6 days of difference

In group I the healing and recovery of the animal was fast, with an average of 5 to 6 days in field conditions, which represents a success, there were no secondary infections or myiasis processes so the animal is not delayed in its growth and fattening.

In group II, the healing and recovery of the animal was late, full of secondary infections, which were treated with antibiotics and ointments against myiasis, making the recovery of the animals more expensive, as well as affecting their conversion in pounds, which resulted in a delay in the fattening process for which they are destined, making their zootechnical process more expensive.

As shown in table 6, the two groups of animals, both treated and untreated, lost weight during the first six days due to handling, treatment and injuries.

Table 6 Gain or loss between the average weights of group I and group II

	Quantity per category	Initial weight Kg	Average weight at 6 days Kg	Average weight at 12 days Kg
Group I	Calves - 21	1824	1824	1827
	Female/Yearlings -29	4140	4116	4135
Group II	Calves – 11	750	746	744
	Female/Yearlings - 15	1620	1614	1612

Table 7 Results between the different weighings in groups I and group II

	Results At 6 days/kg	**Results At 12 days/kg**	**Differences**
Group 1	-10	+13	+3
	-24	+15	+9
Group 2	-4	-2	-6
	-6	-2	-8

After 6 days, the first group is practically healthy so it does not close any more, but the second group that begins to have difficulties with secondary infestations and myiasis, needs to be treated with antibiotics, as well as with ointments against myiasis.

4. Conclusion

The revitalizing ointment proved to be a good cicatrizing, since it helps the early recovery of the lesions and the animals. In no case there were allergic or irritation reactions. Since there are difficulties with the acquisition of healing ointments in veterinary medicine, it constitutes an effective and economical treatment alternative, with a positive impact on livestock and industrial production, as well as on the environmental aspect.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

References

[1] World Health Organization (WHO). WHO strategy on traditional medicine 2002-2005. WHO/EDM/TRM/2002.1. Geneva. 2002. Available at: https://apps.who.int/iris/handle/10665/67163

[2] López P, García AJ, Alonso, L, Perdomo J, Segredo A. Integration of Cuban natural and traditional medicine in the health system. Cuban Journal of Public Health. 2019; 45(2): e1168.

[3] World Health Organization (WHO). Working Group PSHO/WHO Traditional, complementary and alternative medicines and therapies. Washington DC: WHO. 1999. Available at: https://iris.paho.org/bitstream/handle/10665.2/40368/inditradco_eng.-pdf

[4] Pérez-Nicolás M, Vibrans H, Romero-Manzanares A, Saynes-Vásquez A, Luna-Cavazos M, Flores-Cruz M, Lira-Saade R. Patterns of knowledge and use of medicinal plantsin Santiago Camotlán, Oaxaca, Mexico. Econ Bot. 2017; 71: 209-223.

[5] Ministry of Public Health of Cuba (MINSAP). National Formulary of Phytopharmaceuticals and Apipharmaceuticals. 2nd Version ECIMED (Editorial Medical Sciences). National Direction of Pharmacies, Havana, Cuba. 2013: 162-170.

[6] López P, García AJ, Alonso L, Perdomo J, Segredo A. Integration of Cuban natural and traditional medicine in the health system. Cuban Journal of Public Health. 2019; 45(2): e1168.

[7] Cruz EB, Mendoza YC. Diagnosis on the use of medicinal plants for cattle in 11 communities of the Miraflor Natural Reserve, Moropotente, department of Esteli. Thesis Tec. Sup. Agrop. Esteli, Nic. Dry Tropics Catholic Agricultural University. 2002, pp. 87.

[8] Ramachandra C, Srinivasa P. Processing of Aloe vera leaf gel: A review. American Journal of Agricultural and Biological Sciences. 2008; 3: 502-510.

[9] Boudreau M, Beland F. An evaluation of the biological and toxicological properties of Aloe barbadensis (Miller) Aloe vera. Journal of Environmental Science and Health. 2006; 24: 103-154.

[10] Surjushe A, Vasani R, Saple DG. Aloe vera: a short review. Indian Journal Dermatology. 2008; 53: 163-166.

[11] Roig JT. Medicinal, aromatic or poisonous plants of Cuba. Editorial Scientific Technical Publishing House. Havana; 1991: 267-269. Available at: https://scielo.sld.cu/scieloOrg/php/reference.php?pid=S1028-47962004000100007

[12] Soto-Carbajal D, Hinojosa-Yzarra LA. 2021. Aloe vera and electromagnetic decontamination. Scientific Research Journal. 2021; 1(1): 120-129.

[13] Giuliano G, Rosati C, Bramley P. To dye or not to dye: biochemistry of annatto unveiled. TRENDS in Biotechnology. 2003; 21(12): 513-516.

[14] Duke JA, Bogenschutz-Godwin MJ, Ducellier J, Duke PA. CRC Handbook of Medicinal Spices. By CRC Press LLC. 2003.

[15] Valadez V, López H, Ruiz S, Hernández B, Garcá J. Application of an achiote (Bixa orellana) extract in a formulation of an oily model system. Rev. Research and Development in Food Science and Technology. 2020; 5: 609-613.

[16] Fuentes L, Mateo J, Emiliano Q, Caro I. Changes in quality of nonaged pasta filata Mexican cheese during refrigerated vacuum storage. J Dairy Sci. 2015; 98: 2833–2842.

[17] Kim KH, Lee JG, Kim DG, Kim MK, Park JH, Shin YG. The development of a new method to detect the adulteration of commercial Aloe gel powders. Archives of Pharmacal Research. 1998; 21: 514-520.

[18] Bozzi A, Perrin C, Austin S, Arce VF. Quality and authenticity of commercial Aloe vera gel powders. Food Chemistry. 2007; 103: 22-30.

[19] Cortes I, Rodríguez F, Olalla J, López A, Jiménez A, García L, Camacho B. Biocompatibility Studies of Aloe vera Gel Composite and TiO2 Nanoparticles for Dermal Use. In: E. San Martin Martinez, MA Ramirez Salinas (Eds.). Research advances in Nanosciences, Micro and Nanotechnologies. Barcelona, Spain: Omnia Science. 2020: 133-152.

[20] Soto-Carbajal D, Hinojosa-Yzarra LA. Aloe vera and electromagnetic decontamination. Scientific Research Journal. 2021; 1(1): 120-129.

[21] Tocchini L, Zerlotti A. Extração e Determinação, por CLAE de Bixina e Norbixina em Coloríficos [Extraction and Determination of Bixin and Norbixin in Coloríficos by HPLC]. Cienc Tecnol Aliment. 2001; 21(3): 310-313.

[22] Sandoval CJ, Orozco VJ, Pedrero FD, Colín CMA. Sensory profile development of Oaxaca cheese and relationship with physicochemical parameters. J Dairy Sci. 2016; 99: 7075-7084.

[23] Djerrou Z, Djaalab H, Riachi F, Serakta M, Chettoum A, Maameri Z., et al. Irritantcy potential and sub-acute dermal toxicity study of Pistacia lentiscus fatty oil as a tropical traditional remedy. Afr J Tradit Complement Altern Med. 2013; 10: 480-489.