Therapeutic applications of tincture at 10 % from *Calendula officinalis* in Recurrent Aphthous Stomatitis

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Abstract— *A study about the use of medications (indication-prescription) was realized, prospective in patients with buccal illness, using a tincture at 10 % from Calendula officinalis as therapeutic option, in four different clinics in Havana City, Cuba. 110 patients were chosen randomly, and were collected data from 60 physicians. Results were measured as treatment-response, time of improvement and used dosis with time of improvement. The sample answered to the treatment at 90 %, and from them, 73.7 % in a period between 3-6 days, and 26.3 % in a period of 7-10 days. Calendula officinalis tincture at 10 % was effective in treatment of buccal infections corroborating its antibacterial and anti-inflammatories properties due to the presence of volatile oils, and flavonoids, among others.*

Keywords— Calendula officinalis, Recurrent Aphthous Stomatitis, tincture at 10 %, treatment, dosis.

I. INTRODUCTION

Oral cavity is the entry gate of foods, vitamins, liquids, medications and other that has introducing into the organism. Oral infections are one of the most frequent causes found out in medical practice. Aphtha or buccal ulcers are injuries that appear in oral mucous, when its localization is located to the mouth and not like a consequence of some systemic illness, receiving the name of recurrent aphthous stomatitis (RAS). Probably, an immunological dysfunction jointed to diverse unchain factors facilitate aphtha appearance. That local immunological dysfunction will be related with an increase of lymphocytes T populations (CD4 and CD8) and an increase of α-TNF (Herrera et al., 2015; Cui et al., 2016).

One of most representative periodontal illness by its frequency and symptomatology is recurrent aphthous stomatitis (RAS), which constitute one of stemmatological infections that require immediate attention due to the provoke inconveniences at patients. Affect both sex, but principally female sex in any age, even though is more frequent in teenager associated to risk factors like stress, gastrointestinal disorders, microorganisms (virus, bacteria, fungus), smoke habit, traumatism, psychosomatic and immunity alterations, endocrine factors, nutritional deficiency, allergy, and a hereditary component (González & Montero, 2013; Pérez & Rodríguez, 2006; Solís et al., 2017).

Recurrent aphthous stomatitis (RAS) is one of the most common painful oral mucosal conditions seen among patients. These present as recurrent, multiple, small, round, or ovoid ulcers, with circumscribed margins, having yellow or gray floors and are surrounded by erythematous haloes, present first in childhood or adolescence (Jurge et al., 2006). RAS is characterized by recurrent bouts of solitary or multiple shallow painful ulcers, at intervals of few months to few days in patients who are otherwise well (Scully & Porter, 2008).

RAS has been described under three different clinical variants as classified by Stanley in 1972:

1. Minor RAS is also known as Miculiz's aphthae or mild aphthous ulcers. It is the most common variant, constituting 80% of RAS. Ulcers vary from 8 to 10 mm in size.
2. Major RAS is also known as periadenitis mucosa necrotic recurrent or Sutton's disease. It affects about 10–15% of patients. Ulcers exceed 1 cm in diameter.

3. Herpetiform ulceration is characterized by recurrent crops of multiple ulcers; may be up to 100 in number. These are small in size, measure 2–3 mm in diameter.

Treatments are pharmacological principally, and consist in diminishing symptoms and avoid secondary infections. Using topic anesthetics (lidocaine 10 %), collusive (sodium perborate, chlorhexidine 0,2 %) and phytotherapy (calendula, chamomile, *Plantago mayor*, aloe), propollis, honey, homeopathy, laser therapy, acupuncture, digit puncture, etc. Analgesics and anti-inflammatory agents, topic antivirals and steroids, vitamins, systemic antifungals, or antibiotics are commonly used (Zhu et al., 2010; Nolan et al., 2006).

Various predisposing factors are associated with RAS, among them, Genetic predisposition, Trauma, Tobacco, Drugs, Hematinic deficiency, Gluten sensitive enteropathy/celiac disease, Inflammatory bowel disease, Sodium lauryl sulfate-containing toothpaste, Hormonal changes, and Stress (Gallo et al., 2009).

Several microorganisms have been implicated in the pathogenesis of RAS. Several contrary findings have been reported in the various studies published. Among them, are oral streptococci (*Streptococcus mitis*); *Helicobacter pylori*; Viruses (human cytomegalovirus DNA (HCMV); Epstein-barr virus (EBV)) (Sun et al., 1998). Tumor necrosis factor alpha (TNF-α) is a pro-inflammatory cytokine and is one of the most important cytokine implied in the development of new aphthous ulcers in patients (Jacobson et al., 1998; Zabel et al., 1993).

Calendula officinalis L. (Asteraceae), commonly known as caléndula, copetuda, botón de oro, corona del rey, flor de difuntos, rosa de muertos, maravilla, marigold, etc., was the plant used to elaborate the tincture at 10%. Being the flowers its useful organ. Their name derived from Latin word *calendae*, which mean calendar (Enríquez et al., 2010; Hernández et al., 2011).

Native from Egypt, cultivate in Europe in XII century, widespread later to the rest of the World. In Mediterranean region, it grows easily during the Summer in British Islands, and enjoy with great reputation like ornamental plant farming in courtyards, gardens and gavels. Cultivated in Europe since XVII century, specifically in England and ten countries more, among them: Germany, Spain, France, Hungary, Poland, Romania, Switzerland and Russia. Was introduced in Kuwait, Japan, Mexico, Combia, Costa Rica and USA. The date of introduction in Cuba remains unknown, although is very recognized as wreath padded and to give atmosphere to avenues and parks (Lastra & Piquet, 1999; MINSAP, 2002).

Floral stubborn or ligulae flowers (Figure 1) are broadly used for its anti-inflammatory, spasmodic, sedative, sudorific, vulnerary and bactericide properties against *Staphylococcus aureus* and *Staphylococcus faecalis*. In inner applications is recommended to stimulate the hepatic activity and because of biliary secretion, treatment of gastric ulcerations. In external application, decoctions, tinctures and ointments are employed in mask, varicose ulcers, and cutaneous eruptions. Infusions are informed as ant ulcerous, and to the treatment of skin affections (García, 2017).

The aim of this research was to evaluate the affectivity of treatments with a tincture of calendula at 10 % in diagnostic patients with RAS in stemmatological clinics in Centro Habana Municipality in Havana, Cuba.

FIGURE 1: Flowers of *Calendula officinalis*.

II. MATERIALS AND METHODS

2.1 Context

The research was developed in 4 stemmatological clinics from Centro Habana Municipality in 2017, with a total of 45 armchair attended by 65 stemmatologists. The annual average incidence in Centro Habana Municipality is around 200 patients (Dept. Registros Médicos, 2018).

2.2 Classification and Methodology

A retrospective, longitudinal and descriptive study of utilization form of tincture of calendula at 10 % in RAS was designed. With the objective of known the information that the stemmatologists about the tincture an interview was realized to all personal (56) that consent their participation on the research, considering criteria of some authors (ULP, 2016; Larios, 2016).

2.3 Universe, Sample and Criteria

Universe was constituted by 180 patients with diagnosed RAS. Having into account the selection criteria, 110 patients were selected (sample). The criteria selection was: Clinic History, both sex, older than 18 years, and RAS diagnose. Clinic History with not all information or illegible writing was discarded.

Three different objectives were included into the questionnaire to the specialists: 1. Knowledge about medicinal properties of *Calendula officinalis*, 2. Knowledge about medical indications of tincture at 10 % f *C. officinalis*, and 3. Knowledge about the efficacy of this therapy in stemmatological affections. Answers were valorized according to the following criteria: Adequate knowledge; Middle adequate knowledge and Inadequate knowledge.

2.4 Socio demographic and clinic characterization of patients with RAS

For socio demographic and clinic characterization of patients with RAS, the variables used were:

Age: 19-29 years old; 30-39; 40-49; 50-59; 60-69; 70-79; 80 and more than that.

Gender: Male or Female.

Toxic habits: smoker; nonsmoker; ex-smoker; without references.

Alcohol: drinker; nondrinker; ex-drinker; without references.

Kind of RAS: according the classification given by Scully and Porter, 2008: Minors, Majors, Herpetiform.

Frequency of RAS: Casual RAS; Acute RAS; Recurrent RAS; Without references.

2.5 Wound evolution in time with the treatment of the tincture

Each patient was orientated verbal and in writing to purchase the tincture in the pharmacy, way of application (topically), Do not ingest any food until 1 hour after the application, go to consultation every 48 hours until improvement or change of treatment.

Days of treatment with the tincture: between 3-6 days; between 7-10 days.

Administration frequency: 3 times per day.

Dosage: 5 drops/100 mL of water; 10 drops/100 mL of water.

Treatment's response: cured/ satisfactory: all symptoms disappear after 10 days; not cured/ unsatisfied: symptoms not disappear after 10 days.

Effectiveness index of treatments:

Was calculated by $\text{Effectiveness} = \frac{\text{Cured patients}}{\text{Total patients}} \times 100$

Effective: effectiveness equal or higher than 90%

Half effective: effectiveness between 80-89%

No effective: effectiveness less than 79%

2.6 Ethical considerations

Research was done taking into account the Helsinki's Declaration, 2013. Protocol was submitting under consideration and approval of Master's Academic Committee and requesting the approval of the Clinic and Policlinic Administration. Investigating made a compromise of confidentiality about patient's identity, just like the data used to realize this study. All specialists involved in the research were asked about their consent to participate in the investigation.

2.7 Analysis and processing of the results

Data base was making in Microsoft Excel including all variables and categories. Calculation of percentage and absolute figures were expressed in tables and imagine facilitating the analysis and discussion. Statistical processing was done using the SPSS. 22 Window's package.

2.8 Research's limitation

Because of the research was a retrospective investigation being able to find slanting of information.

III. RESULTS AND DISCUSSIONS

3.1 Identification of information degree of specialists about the therapy with calendula tincture at 10%.

Table 1 shows the number of armchairs and sex of specialists interviewed by each clinic under study. Clinic "Enrique Sainz Casado" own the biggest amount of armchairs due to its exclusivity for stemmatological attention. As it can see, female gender is predominating among the stemmatologists. 56 specialists gave their consent to participate in the research, representing 86.2 % of the whole in Centro Habana Municipality, being a thoroughly representative value to validate their answers.

TABLE 1
ARMCHAIR NUMBER AND SPECIALIST'S COMPOSITION ACCORDING TO SEX INTERVIEWED IN CENTRO HABANA MUNICIPALITY

Place of stemmatological attention	Number of armchairs	Specialists		
		Females	Males	Total
Policlinic "Luis Galván Soca"	11	13	1	14
Policlinic "Joaquín Albarrán"	9	6	4	10
Policlinic "NGuyen Van Troi"	11	10	0	10
Clinic "Enrique Sainz Casado"	14	20	2	22
Total	45	49	7	56

56 specialists answered properly question 1 whose objective was to identify the knowledge about the medicinal properties of *C. officinalis*. Correct answer is that recognize the anticoagulant, anti-inflammatory, antiseptic, antibacterial and antiemetic activities of the plant. Taking into account their answers, we consider the consciousness adequate.

Question 2 had six possibilities to replay about indications of tincture at 10% of calendula in stomatology. Only four of them where properly answered, as is shown in Figure 2, qualified as Middle Adequate Knowledge.

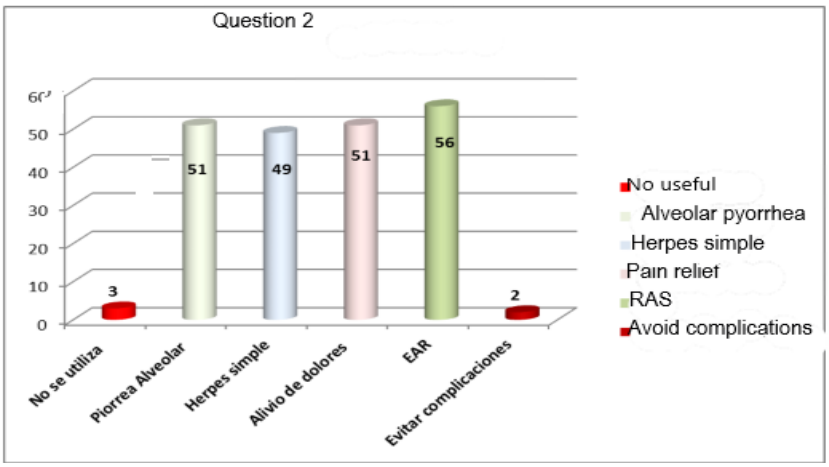

FIGURE 2: Results after question 2.

Figure 3 show the answers given by the specialists at question 3, related with the criteria about the effectiveness of treatment using that therapy in stemmatological affections. It is evident that 11 specialists' unknown or consider unsatisfied the effectiveness of this therapy, classifying as unsuitable knowledge.

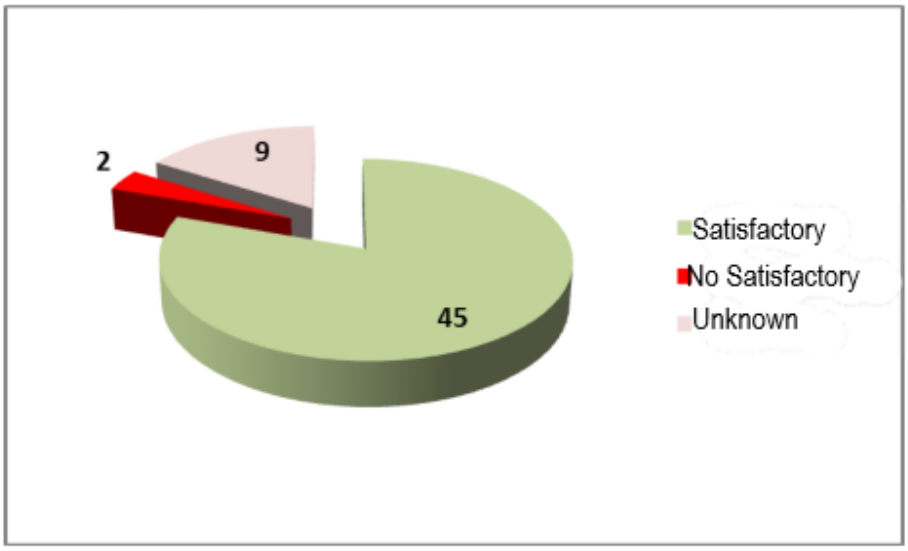

FIGURE 3: Obtained results in question 3.

Table 2 show the global results about the valuation of knowledge degree of specialists. The specialists hold knowledge about the medical properties of *C. officinalis*, however, 11 answered negatively about the effectiveness of the treatment using this therapy in stemmatological affections, indicating the ignorance of the term effectiveness, regarding the design an investigation to determine the cause of this result. Because of the fact that they do not trust in the effectiveness of the treatment in some stemmatological pathologies, confine its prescription.

TABLE 2
VALUATION OF KNOWLEDGE DEGREE OF THE SPECIALISTS ABOUT THE USE OF TINCTURE AT 10% OF *C. OFFICINALIS*

Question No.	Specialists with correct answer	Qualification
1	56	Adequate knowledge
2	52	Middle adequate knowledge
3	45	Inadequate knowledge
Final qualification		Middle adequate knowledge

Three decades ago, WHO be carried out a calling to the Governments and International Community with the end to incorporate in Health National Systems the use of traditional therapies and alternative medications. Cuba, has trace general rules of acting in view of the immediate and mediate future. One of them, demand maximum attention to improve the natural and traditional medicine.

3.2 Sociodemographic and clinic characterization of patients with RAS

Table 3 relates the group of ages and sex of the 110 patients with RAS selected in the research because they fulfill the established selection criteria. Female sex with 60 patients and ages between 30-50 years old in both sex prevailed. Consulted literature confirm the overrule of female sex and middle ages with RAS, associated to active labor life and the increment of stress by this fact, appearing gastrointestinal problems, immunological and nutritional deficiencies, among others, coinciding with our results (Morales & Ventura, 2000).

TABLE 3
SAMPLE COMPOSITION WITH RAS, AGES AND SEX TREATED WITH TINCTURE AT 10%

Age	Female	%	Male	%	Total	%
19-29	1	1.6	2	5	3	2.6
30-39	17	28.3	11	22.9	28	25.3
40-49	15	25	14	23.2	29	26.3
50-59	9	15	11	22.9	20	18.1
60-69	7	11.7	4	9	11	9.9
70-79	7	11.7	5	11	12	10.9
80 and more	4	6.7	3	6	7	6.9
Total	60	54.5	50	45.5	110	100

Recent studies establish a similar situation in Morocco, where the 60% of patients are females (Rodríguez & Raissouni, 2018). In Cuba, there are references of realized studies in Las Tunas Province and Moron Municipality with 65.5 % and 65.8 %, respectively (Díaz et al., 2018; Báez et al., 2015).

Recent epidemiologic studies indicate that the prevalence of RAS fluctuate between 2-50 % in general population, with a total estimated between 5-25 %. Average age is 19-20 years old, and its presence has not relation with geographic location or race. There is a prudent prevalence in female sex, although many authors point out that there are not significant differences between one group and any other (Pacho & Piñol, 2005).

Diverse research report that in patients with RAS exist an important deficit of certain nutritional elements (Casiglia, 2002). Inside of lacking elements we can find the deficit of folic acid, vitamin C, vitamins B12 and B1, minerals like iron, zinc, and calcium, and states of hypoproteinemia (Haisraeli-Shalish et al., 1996).

Some report indicate that some patients have certain hyper sensibility to certain foods, such as celiac illness, whom have an intolerance to gluten and other alimentary allergy (Sedghizadeh et al., 2002). Nevertheless, the results are still controversial in Turkey, because at the end of the research the levels of vitamin B12 were low significantly in patients with RAS comparing with control normal fellows. They concluded that the deficit of vit B12 is an important factor in genesis of RAS (Ogura et al., 2001).

Some investigations mention smoke habit as an unchaining factor in RAS. Figure 4 represent the composition of the sample taking into account the presence or not of smoke habit.

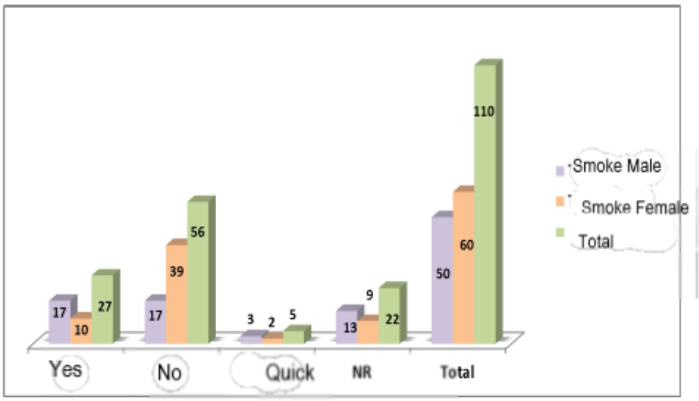

FIGURE 4: Patients with RAS with Smoke habits.

24.5 % of patients (27) smoke actually, coinciding with the result of Risk National Factor Survey that said that 27.3 % older than 15 years are smokers. So that, 31.1 % are males and 16.4 % are females, coinciding with obtained result, where exist a prevalence of masculine gender (17) and seven patients were smokers, being smoke habit, even it has stop, a defined risk factor to appearance the RAS (Edgar et al., 2017).

Only one male patient is a drinker, while a female patient leaves to drink or is considered ex-drinker. This result has not coincidence with National Survey of risk factors in transferable diseases (Pacho and Piñol, 2005), where was reported the prevalence of 41.7 %, from them, 71.9 % were males, while the groups of ages between 25-34 and 35-44 years' old have the

higher presence. Be a drinker is not a risk factor to unchain the illness but, malnutrition and immunologic deficit constitute a risk by themselves.

Table 4 shows the kind of RAS considering the size of ulcer and its relationship with tobacco habit according to Scully and Porter.

TABLE 4
PATIENTS COMPOSITION WITH RAS ATTEND TO KIND OF RAS AND TOBACCO HABIT

Kind of aphtha	Smoker	No Smoker	Ex-Smoker	Without reference	Total
Minor	11	43	1	13	68
Major	9	6	1	1	27
Herpetiform	7	7	3	8	35
Total	27	56	5	22	110

Obviously, minor aphtha is predominant, associated to no-smoker patients. In the case of herpetiform, appear at the same time in smoker patients and no-smoker patients, and on three of the five ex-smoker patients. Authors like Tamayo Ortíz and Scully & Porter, publicized similar results with the apparition of minor aphtha.

Tobacco is not an unchain factor, but it can complicate the established periodontal pathology. Investigation indicates that smokers from both sex have a high prevalence of gingivitis and chronical destructive periodontitis, among others. Table 5 summarized the appearance frequency of aphtha according to the sex.

TABLE 5
PATIENTS WITH RAS TAKING INTO ACCOUNT SEX AND APPEARANCE FREQUENCY.

Frequency	Female	Male	Total
Occasional aphtha	39	21	60
Acute aphtha	11	18	29
Recurrent aphtha	9	9	18
Without reference	1	2	3
Total	60	50	110

Occasional aphtha is the most represented where lesions are isolated, intervals of appearance fluctuate between months and years, but lesions heal up without obstacle. In the case of acute aphtha episode can persist during weeks, appearance the lesions in different zones of the mouth, replacing aphtha in way of healing or cured, commonly in children or adults with acute gastrointestinal dysfunctions that remit when improve the gastrointestinal dysfunction. There are 18 patients with recurrent aphthae, where affections be possible to continue months or years, there is a lesion present always, and more difficult to get well (Morales & Ventura, 2000).

3.3 Evaluation of effectiveness of treatment of RAS with tincture of calendula at 10 %

Days of treatments with the tincture of calendula at 10 % are represented in Figure 5. From whole patients, 86 of them using the tincture between 3-6 days, and 24 patients between 7-10 days. Among them, 18 patients classify as recurrent aphtha, which is known to be the most arduous to respond to any therapy (Jiménez & Rivera, 2017).

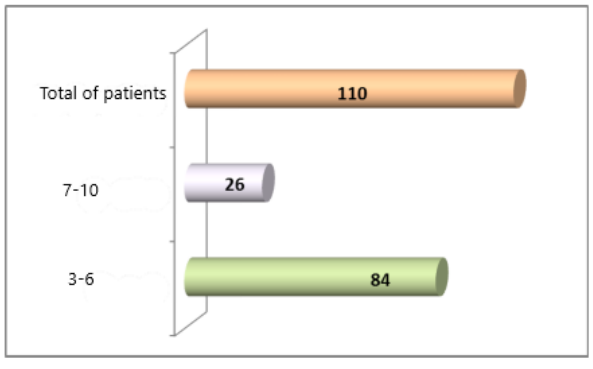

FIGURE 5. Response in time to the treatment of RAS with tincture at 10 %.

Obviously, exist a prevalence of patients with positive answer to the treatment between 3-6 days. In the sample under study had a prevalence of patients with occasional aphtha, reason why it could be influenced on high effectiveness of the treatment with calendula at 10 % deal with small and a little complicate lesions.

Table 6 summarizes the distribution of patients according to dosage and frequency of administration of tincture from calendula at 10 %.

TABLE 6
RESPONSE TO THE TREATMENT OF RAS WITH TINCTURE OF CALENDULA AT 10 %.

Dosage and frequency of administration	Patients		Days of treatments			
	Amount	%	3-6	%	7-10	%
5 drops/100 mL 3 times per day	49	44.5	45	53.6	7	26.9
10 drops/100 mL 3 times per day	61	55.5	39	46.4	19	73.1
Total	110	100.0	84	100.0	26	100.0

Clinical history reported that whole patients were administrated three times per day, being 55.5 % the patients using 10 drops/100 mL of water, and 73.1% with a treatment for a time between 7-10 days. Those results are in correspondence with those realized at the University of Nayarit using calendula in the scaring in oral cavity in post extraction of third molar, in patients treated with mother tincture of *C. officinalis* (Hernández et al., 2009).

Similar results were demonstrated by Sagué et al., 2010, in stemmatological clinic "José Luis Tassende" in Santiago de Cuba, where was demonstrated the efficacy of the tincture at 20% of *C. officinalis* in stomatitis subprotesis grade I, as much as palate like superior alveolar edge, through a therapeutic clinic assay phase II randomized and monocentric.

The research done by Fang et al., 2013, at the Odontology University of Cartagena, Colombia, pose that oral rinsing with calendula, allow the opportune healing of tissues from post-surgery gingival mucosa; medication with this plant, contemplates a little proportion of antimicrobial capacity and a high inductive ability on tissue healing.

Ojeda et al., 2007; Lima et al., 2011 and Tamayo et al., 2019, reporting similar results related with the days of treatments, only with a little difference in the case of Ojeda, related with the fact that the most used posology was 5 drops/100 mL of water. The treated sample under study (110 patients) answered in a 90 % in a period of 3-6 days (73.7 %), and 7-10 days (26.3 %), respectively.

Figure 6 summarized the answer at the treatment with tested patients, where only 11 patients not healing because the symptoms not dissolve at 10 days, and 4 of them, was realized a change in the treatment.

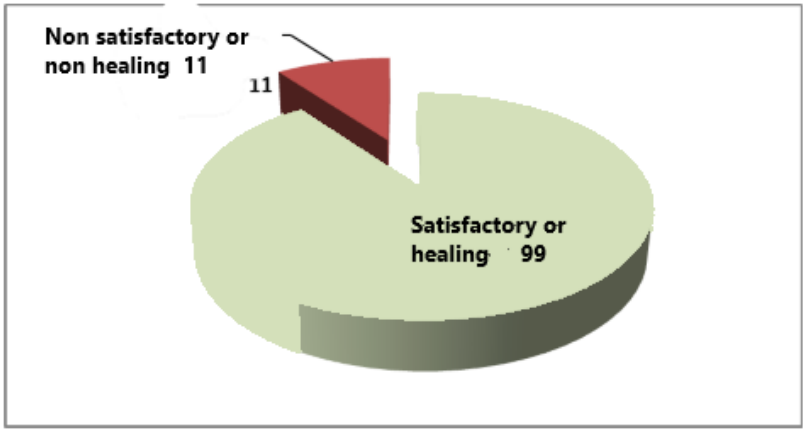

FIGURE 6: Treatment response with tincture at 10 % of *C. officinalis*.

Similar results were obtained by Roveroni et al., 2009 in patients with RAS under topical treatment with tincture at 10 % against exfoliative cheilitis. Treatment of chronic gingivitis in Higher Institute of Medical Sciences in Villa Clara Province, Cuba, obtained satisfactory results in spite of it is another pathology, but, calendula has healing properties in optimal treatment (Veitía et al., 1998).

Result of the estimate of effectiveness index to the 90 % of applied treatment had a high coincidence with several authors in Centro Habana Municipality, as is shown in Figure 7. It was different if the study had been designed towards fulfillment of therapy.

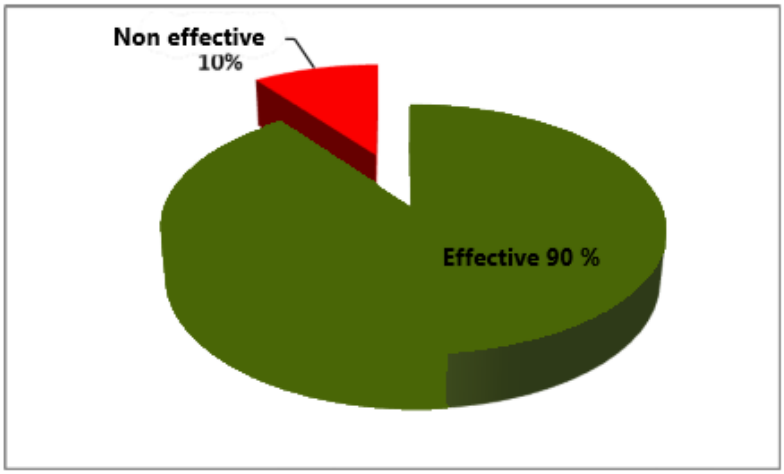

FIGURE 7. Effectiveness index of treatment with tincture at 10 % of *C. officinalis*.

Aphthous lesions are, generally, benign and present auto imitated evolution. By this reason, it is not available a specific treatment that cure them and are treat in palliative manner. In our country, there is a great experience in natural and traditional medicine, where rules have been created to the use of phyto medication and bee medication, recommended to use in treatment of RAS, like aloe, calendula, etc., all of them with anti inflammatory and cicatrizing activities.

Sometimes, oral erosions disguise the first signals of a heterogeneous group of diseases such as Behçet illness, Crohn illness, celiquism, or hematological alterations like agranulocytosis. There are denominate aphthous lesions and present an accompanying symptomatology, regarding to consult a physician immediately.

IV. CONCLUSIONS

Information degree of interviewed stemmatologist about the use of calendula's tincture at 10 % was qualified as middle adequate knowledge. Female sex predominated in patients with RAS as well as ages between 30-59 years' old. Predominant RAS was of minor type (54.5 %), followed by herpetiform (22.7 %). Treatment of lesions in time with tincture of calendula at 10 % result between 3-6 days, fundamentally with a posology of 5 drops/100 mL of water, with the 90 % of patients cured before the 10 days of initialized the therapy. Effectiveness of therapy with tincture at 10 % of calendula was evaluated in a 90 % of whole cases. Authors recommend designing effectiveness or efficacy tests in a clinic and aleatory assay, to demonstrate the definitive efficacy of tincture of *C. officinalis* in this one and other stemmatological affections.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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