

Ferdinand Z. Guintu, MD
Antonio H. Chua, MD

Department of Otorhinolaryngology
Head and Neck Surgery
Jose R. Reyes Memorial Medical Center

Correspondence: Dr. Ferdinand Z. Guintu
Department of Otorhinolaryngology
Head and Neck Surgery
Jose R. Reyes Memorial Medical Center
San Lazaro Compound, Rizal Avenue
Sta. Cruz, Manila 1003
Philippines
Phone: (632) 743 6921;
(632) 711 9491 local 320;
(632) 922 8978070
Email: ferdinandguintu@yahoo.com
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Effectivity of Guava Leaves (*Psidium guajava*) as Mouthwash for Patients with Aphthous Ulcers

ABSTRACT

Objective: To determine whether *Psidium guajava* leaves mouthwash is effective in the management of patients with aphthous ulcers.

Methods:

Design: Randomized prospective open label clinical study

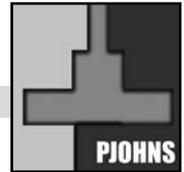
Setting: Tertiary Government Training Hospital

Subjects: Thirty two patients diagnosed with aphthous ulcers were randomly divided into two groups, a treatment group using prepared guava leaves mouthwash, and a control group using isotonic sodium chloride solution mouthwash given thrice a day for seven days. Patients were evaluated using a 10-point Visual Analog Scale. The sizes of the aphthous ulcers were measured using a caliper, and compared on Day 1 and Day 7 for both treatment and control groups. Results were subjected to statistical analysis using T-test, Mann-Whitney U test and Fisher Exact test.

Results: Comparison of VAS scores of guava treatment and NSS control groups showed that there were no differences in pain experienced on Days 1 and 2. However, the VAS scores from Day 3 to 7 had p values ranging from 0.02 - 0.0001 which showed significant differences in resolution of pain. There was statistically significant marked improvement of pain symptoms as early as three days post-treatment among patients who were administered guava leaves mouthwash. Complete resolution of aphthous ulcers in 75% of the study group was observed on Day 7. Mean ulcer size post-treatment with guava gargle was 0.25mm compared to 0.75mm for NSS gargle. The mean size difference at day 7 was 1.44mm for the guava treatment group and 0.88mm for the NSS control group. There was a statistically significant faster resolution of ulcer size on day 7 in 16/16 or 100 % of patients in the treatment group compared with only 10/16 or 62.5% of patients in the control group. Patients who were administered guava leaves mouthwash generally fared better than those administered isotonic sodium chloride solution.

Conclusion: Guava leaves mouthwash was effective for aphthous ulcers in terms of reduction of symptoms of pain and faster reduction of ulcer size. Further clinical trials comparing this mouthwash against other treatment options are recommended.

Keywords: *guava leaves (Psidium guajava)*, *aphthous ulcers*, *mouthwash*



Herbal medicine is one of the main modalities in traditional as well as complementary and alternative medicine; and is increasingly acknowledged due to the extensive use of herbal remedies among the general population in developed and developing countries worldwide.¹

"Bayabas" or guava (*Psidium guajava*) is a plant of the family *Myrtaceae*.¹ In the backyards of most Filipino homes in the rural countryside, this plant is commonly seen and grown because of its many uses as fruit and as traditional remedy to treat various ailments. Research studies have shown that almost all of the parts of this plant have medicinal qualities, making it one of the most popular therapeutic plants in the Philippines and one of the approved medicinal plants of Department of Health.²

Guava (*Psidium guajava*) is a small tree that can grow up to 3 meters tall. The fruit, bark and leaves are used as herbal medicine. A decoction of its leaves is recognized for its effectiveness in curing several ailments, including the treatment of chronic diarrhea and gastroenteritis. The most common use of the leaves is for cleaning and disinfecting wounds by rinsing the afflicted area with a decoction of the leaves. It can also be used as a wash for uterine and vaginal problems, and is good for ulcers. Leaf decoction may also be used as mouthwash.² *Psidium guajava* has also been used for the management of various diseases like toothache, sore throat, and inflamed gums.³

Aphthous ulcers are the most common oral mucosal disorder.⁴ These are acute and extremely painful mouth ulcers usually involving non-keratinized oral mucosal sites. Aphthous ulcers are usually round with a slightly raised margin and erythematous halo⁴ and may be classified into minor, major and herpetiform.⁵ Approximately 80 percent of patients have minor aphthous ulcers.⁵ These are 2 to 8 mm in diameter affecting nonkeratinized mucosa such as the labial and buccal mucosa and the floor of mouth or the ventral surface of the tongue. Much less common are major aphthous ulcers, larger than minor ulcers, often 1 cm or more in diameter.⁵ A third and even less common variety is termed "herpetiform ulceration" and comprises ulcers that are initially multiple and pinpoint.⁵ These may interfere with eating, drinking and swallowing. Several causes have been implicated: local trauma, viral infection, systemic causes, poor oral hygiene, stress and others. Although patients have spontaneous healing within 10-14 days, no specific treatment for aphthous ulcers is yet available.^{4,6} The goals of aphthous ulcer treatment have been to control pain and promote healing.⁷ Mouthwashes may also help.⁸

This study was designed to determine whether *Psidium guajava* leaves mouthwash is effective in the management of patients with aphthous ulcers. Specifically, we sought to determine whether *Psidium*

guajava mouthwash as compared to isotonic saline mouthwash, decreases the symptoms of pain in patients with aphthous ulcers; and to assess whether *Psidium guajava* mouthwash, as compared to isotonic saline mouthwash, will hasten aphthous ulcer resolution.

METHODS

Study design: Randomized Prospective open label clinical study

Setting: Tertiary government training hospital

Study population: Patients aged 10 years old and above, diagnosed with minor aphthous ulcers in our hospital outpatient department from July to December 2011, who were able to understand and give written informed consent (or assent) and report adverse events were considered.

Excluded were those with major and herpetiform aphthous ulcers; those with an oral cavity mass with superimposed ulcer where malignancy could not be totally ruled out; those with co-morbid conditions, uncontrolled metabolic conditions or psychiatric conditions; and pregnant patients.

Thirty-two patients meeting the inclusion and exclusion criteria and who gave informed consent (or assent, where consent was obtained from the parent or legal guardian in those < 18 years of age) were included in the study, fulfilling the pre-determined sample size.

Patient Procedure:

Subjects were divided into a treatment group and control group using a randomization table. Treatment group patients used the *Psidium guajava* leaves mouthwash. They were each given prepared guava mouthwash solution to gargle and instructed to gargle 250 ml of the solution for 3 minutes using any watch or clock thrice a day for one week. Control group patients used isotonic sodium chloride solution (NSS) mouthwash. They were each given prepared NSS mouthwash and instructed to gargle 250 ml for 3 minutes using any watch or clock thrice a day for one week. Patients were asked on Day 7 if there was any adverse reaction to the gargle.

Treatment and control groups self-accomplished aphthous ulcer pain scoring daily using a 10-point Visual Analog Scale (VAS) where 0 indicated no symptoms and 10 indicated worst symptoms for the entire treatment phase, beginning with a baseline in clinic on Day 1. Blank VAS scales (*Figure 1*) were given to each participant and collected after 7 days.

Aphthous ulcer sizes for both treatment and control groups were also measured and recorded using one caliper (Aesculap, Tuttlingen, Germany) pre-treatment (day 1) and post-treatment (day 7) by a blinded outpatient resident on duty.

Day 1: Aphthous Ulcer Pain Scoring (Visual analog pain scale)

Date: _____
 Patient no.: _____

1. Circle the number that shows how painful is your aphthous ulcer right now
 (Bilugan ang numero na nagpapakita ng gaano kasakit ang iyong singaw ngayon)

No symptom 0 1 2 3 4 5 6 7 8 9 10 very

Day 2: Aphthous Ulcer Pain Scoring (Visual analog pain scale)

Date: _____
 Patient no.: _____

1. Circle the number that shows how painful is your aphthous ulcer right now
 (Bilugan ang numero na nagpapakita ng gaano kasakit ang iyong singaw ngayon)

No symptom 0 1 2 3 4 5 6 7 8 9 10 very

Day 3: Aphthous Ulcer Pain Scoring (Visual analog pain scale)

Date: _____
 Patient no.: _____

1. Circle the number that shows how painful is your aphthous ulcer right now
 (Bilugan ang numero na nagpapakita ng gaano kasakit ang iyong singaw ngayon)

No symptom 0 1 2 3 4 5 6 7 8 9 10 very

Figure 1. Sample blank visual analog scale

Preparation of Materials

Fresh guava leaves were gathered from Pampanga province and verified by a local biologist. The guava leaves were washed with water, and batches of 200 gm of guava leaves were boiled in 750 ml of water for 8 to 10 minutes. The boiled leaves were removed and the solution was cooled and transferred into sealed 1 liter clean clear plastic containers and placed in the refrigerator. Commercially-available Isotonic Saline Solution (Euro-med, Cavite) were transferred into sealed clean clear plastic containers for the control group.

Data management and analysis

Outcome measures were based on comparison of the two groups after 7 days. Primary outcome measures were reduction in the median VAS pain score and reduction in the median ulcer size. Summary measures (mean and percentage) were also determined for the demographic distribution of study participants and other outcomes.

The null hypotheses tested were as follows:

1. There is no significant difference in VAS scores for pain between patients using guava mouthwash and patients using isotonic sodium chloride solution mouthwash.

2. There is no significant difference in size of the aphthous ulcer between patients using guava mouthwash and patients using isotonic sodium chloride solution mouthwash.

All data were encoded and tallied in SPSS version 10 for windows (IBM, New York, USA). Descriptive statistics were generated for all variables. For nominal data, frequencies and percentages were computed. For numerical data, mean ± SD were generated. Analysis of the different variables was done using the T-test, Mann Whitney U test and Fisher Exact test.

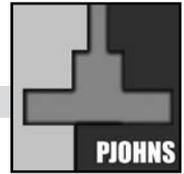
RESULTS

Of a total of 37 patients considered, three patients with uncontrolled diabetes mellitus and two patients with pulmonary tuberculosis were excluded. A total of 32 patients were included in this study. There were 26 females and six males, with ages ranging from 17 to 69 years. These patients were randomly allocated into two groups of 16 each: the guava leaves mouthwash treatment group (N=16) and isotonic sodium chloride solution control group (N=16). All participants completed the study. No adverse effects were recorded although one participant in the treatment group complained of nausea attributed to laryngopharyngeal reflux. There was no significant difference between the two groups in terms of demographic characteristics of age and sex with all p values >0.05 (Table 1).

Table 1. Comparison of demographic characteristics between the two groups

	NSS (n=16)	Guava (n=16)	P value
<u>Age in years</u>			
Mean ± SD	44.06 ± 15.24	39.40 ± 12.30	0.34 (NS)
<u>Sex</u>			
Female	15 (93.8%)	11 (68.8%)	0.17 (NS)
Male	1 (6.2%)	5 (31.2%)	

There was no significant difference in VAS scores of both groups on Days 1 and 2 (p = 0.78 and 0.32 respectively). However, there was a significant difference in VAS scores of both groups from Day 3 until Day 7 (all p values <0.05). The VAS scores of those given guava mouthwash



were significantly lower than those given NSS (Table 2). Comparison of the differences in VAS scores at different intervals between the two groups showed that there was a significant difference from Days 3 to 7 (all p values <0.05). The decrease in VAS scores of those given guava was significantly greater than those given NSS (Table 3). The mean VAS scores

at different intervals between the two groups are presented graphically in (Figure 2). There was a statistically significant, marked improvement of pain symptoms as early as three days post-treatment in the guava leaves mouthwash group as compared to the isotonic sodium chloride solution mouthwash group.

Table 2. Comparison of VAS Scores at different intervals between the two groups

	NSS (n=16)	Guava (n=16)	P value
Day 1 Mean ± SD	5.62 ± 1.36	5.50 ± 1.26	0.78 (NS)
Day 2 Mean ± SD	5.44 ± 1.41	4.88 ± 1.74	0.32 (NS)
Day 3 Mean ± SD	4.75 ± 1.00	3.62 ± 1.58	0.02 (S)
Day 4 Mean ± SD	3.94 ± 1.34	2.31 ± 1.74	0.005 (S)
Day 5 Mean ± SD	3.18 ± 1.38	1.31 ± 1.30	0.0004 (S)
Day 6 Mean ± SD	3.12 ± 1.36 (3)	0.38 ± 0.72 (0)	<0.0001 (S)
Day 7 Mean ± SD	2.68 ± 1.14 (3)	0.31 ± 0.60 (0)	<0.0001 (S)

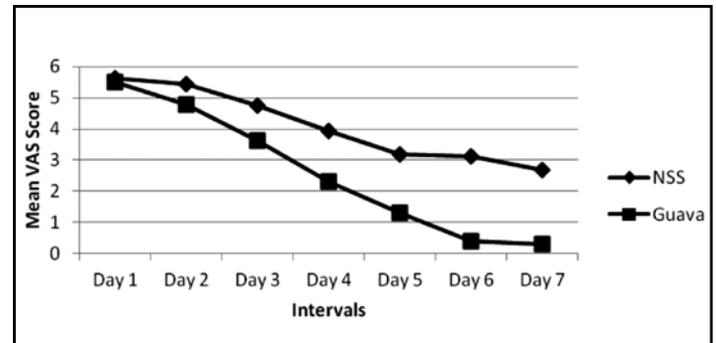


Figure 2. Mean VAS scores at different intervals between the two groups

Table 3. Comparison of the differences (decrease) in VAS scores at different intervals between the two groups

Difference	NSS (n=16)	Guava (n=16)	P value
Day 1 vs Day 2 Mean ± SD	0.18 ± 0.54 (0)	0.62 ± 1.02 (0)	0.17 (NS)
Day 1 vs Day 3 Mean ± SD	0.88 ± 0.96	1.88 ± 1.36	0.02 (S)
Day 1 vs Day 4 Mean ± SD	1.68 ± 1.44	3.18 ± 1.94	0.01 (S)
Day 1 vs Day 5 Mean ± SD	2.44 ± 1.26	4.18 ± 1.68	0.002 (S)
Day 1 vs Day 6 Mean ± SD	1.68 ± 1.44	3.18 ± 1.94	0.01 (S)
Day 1 vs Day 7 Mean ± SD	2.50 ± 1.03	5.12 ± 1.31	<0.00001 (S)
Day 1 vs Day 7 Mean ± SD	2.94 ± 1.18	5.18 ± 1.16	<0.00001 (S)

The comparison of ulcer sizes between the two groups showed no significant difference on day 1 (p = 0.80). On the other hand, there was a significant difference noted in size on day 7 (p = 0.004). The mean size of ulcers in those given guava mouthwash was significantly smaller than those given NSS with a mean of 0.25mm and 0.75mm respectively (Table 4). There was a significant size difference between the two groups (p = 0.03). The decrease in size of those given guava mouthwash was significantly greater than those given NSS with a mean decrease in size of 1.44mm and 0.88mm respectively (Table 5). The distribution of subjects according to ulcer size at Days 1 and 7 is presented graphically in (Figure 3). There was a statistically significant,

Table 4. Comparison of ulcer size at Days 1 and 7 between the two groups

	NSS (n=16)	Guava (n=16)	P value
Day 1			
1mm	9 (56.2%)	6 (37.5%)	
2mm	4 (25.0%)	9 (56.2%)	
3mm	3 (18.8%)	1 (6.3%)	
Mean ± SD	1.62 ± 0.80	1.69 ± 0.60	0.80 (NS)
Day 7			
0mm	4 (25.0%)	12 (75.0%)	
1mm	12 (75.0%)	4 (25.0%)	
Mean ± SD	0.75 ± 0.45	0.25 ± 0.45	0.004 (S)

Table 5. Comparison of ulcer size difference between the two groups

	NSS (n=16)	Guava (n=16)	P value
<u>Size Difference</u>			
<u>0mm</u>	6 (37.5%)	0	
<u>1mm</u>	6 (37.5%)	9 (56.2%)	
<u>2mm</u>	4 (25.0%)	7 (43.8%)	
<u>Mean ± SD</u>	<u>0.88 ± 0.80</u>	<u>1.44 ± 0.51</u>	0.03 (S)

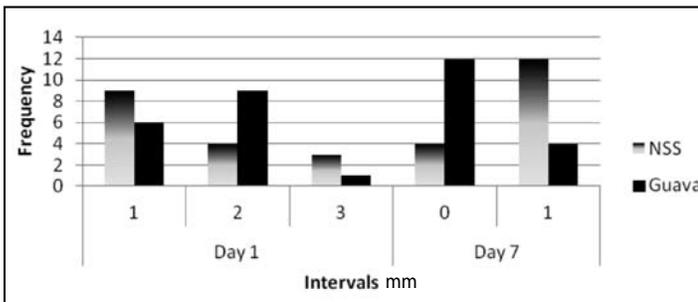


Figure 3. Distribution of subjects according to size at day 1 and day 7 between the 2 groups

markedly faster resolution of aphthous ulcer size in the guava leaves mouthwash group as compared to the isotonic sodium chloride solution mouthwash group.

The results obtained suggest that guava leaves mouthwash is effective as an alternative treatment for aphthous ulcers.

DISCUSSION

P. guajava has been known to have antimicrobial,⁹ anti-inflammatory,¹⁰ antitumor,¹¹ antiallergic,¹² antihyperglycemic,^{10,13} and antimutagenic¹⁴ activities. It has been used to treat wounds,¹⁵ cough³ and dental diseases.¹⁶ Flavonoids extracted from guava leaves including morin-3-O-lyxoside, morin-3-O-arabinside, quercetin and quercetin-3-O-arabinside were reported to have strong antibacterial action.⁹ An in vitro study of aqueous extract of *Psidium guajava* reduced the cell-surface hydrophobicity of *Strep. sanguinis*, *Strep. mitis* and *Actinomyces sp.* responsible in development of dental plaques.¹⁶ *Psidium guajava* also has been used for the management of various diseases like toothache, sore throat, inflamed gums, and a number of other conditions.³ Growth of *Staphylococcus aureus* and beta-streptococcus group A, as determined by the disc diffusion method, was inhibited by extract of dry guava leaves.³ The anti-inflammatory and analgesic properties of the plant's leaf extract were determined by using experimental animals. Diclofenac

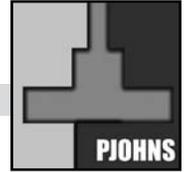
and morphine were used as standard reference for comparison.¹⁰ The numerous tannins, polyphenolic compounds, flavonoids, ellagic acid, triterpenoids, guajaverin, quercetin and other chemical compounds present in the plant account for the observed anti-inflammatory and analgesic effects of the plant's leaf extract.¹⁰ Much of the traditional uses of *P. guajava* have been validated by scientific research. Toxicity studies in mice and other animal models as well as controlled human studies show both leaf and fruit are safe without any side effects.¹⁷

The effectivity of guava leaves in pain alleviation may largely be due to the numerous flavonoids, tannins, polyphenolic compounds, ellagic acid, triterpenoids, guajaverin, quercetin and other chemical compounds present in the plant.¹⁰ Flavonoids inhibit biosynthesis of prostaglandins, which are involved in various immunologic responses and are the end products of the cyclooxygenase and lipoxygenase pathways.¹⁸ Protein kinases are another class of regulatory enzymes affected by flavonoids. Inhibition of these enzymes provides the mechanism by which flavonoids inhibit inflammatory processes.¹⁹

Significant decrease in the size of aphthous ulcers may be attributed to the presence of flavonoids extracted from guava leaves including morin-3-O-lyxoside, morin-3-O-arabinside, quercetin and quercetin-3-O-arabinside which were reported to have strong antibacterial and antiviral action.⁹ Since it is hypothesized that one of the causes of aphthous ulcer is hypersensitivity and inflammation, the resolution of size may be due to the effects of flavonoids.

A significant amount of work has been done on the pharmacological and biological activity and possible application of chemical compounds. The taste and scent of guava are a limiting factor in proper standardization of clinical trials as patients could easily recognize its taste and scent. Hence, VAS was combined with measurement of aphthous ulcer size to more objectively document its therapeutic potential.

Our study showed that guava leaves mouthwash, compared to isotonic saline solution, was effective for aphthous ulcers in terms of reduction of symptoms of pain and faster reduction of ulcer size. Further clinical trials comparing this mouthwash against other treatment options are recommended.



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