

Anti-inflammatory and anti-plaque activity of Ponna yeik (*Ixora coccinea* Linn.) leaves extract used as a mouthwash on chronic gingivitis patients

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Ponna yeik (*Ixora coccinea* Linn.) is locally claimed to be useful in treatment of toothache and oral diseases as a mouthwash in Myanmar. In Myanmar, 80% of school children had gingivitis and 18% of them had periodontal destruction. Bacterial plaque in oral cavity is regarded as the primary local etiological factor in inflammatory disease. Preventing and controlling of periodontal disease would prevent the microbial colonization of plaque on the teeth and gingiva. There are varieties of antiseptic mouthwashes in modern dental practice, but chlorhexidine gluconate is the most effective anti-plaque mouthwash, which is not cheap and easily available. This study with the aim to evaluate the efficacy of Ponna yeik mouthwash, which was easily available at low cost, was conducted at the Institute of Dental Medicine, Yangon. The study design was a randomized controlled clinical trial and chlorhexidine gluconate was used as a positive standard drug. Twenty patients with typical chronic gingivitis who participated in this study were randomly divided into two groups, 10 patients for 0.2% watery extract of Ponna yeik mouthwash and 10 patients for 0.2% chlorhexidine mouthwash two times a day for 4 weeks. The plaque score, bleeding on probing supra-gingival plaque formation, staining effect and severity of gingivitis were examined prior to the clinical trial, as baseline and 4 weeks after trial. Both chlorhexidine and Ponna yeik mouthwashes showed significant effectiveness in plaque score, bleeding on probing and severity of gingivitis when compared to before treatment. Staining effects were observed in patients who used chlorhexidine but not in patients who used Ponna yeik mouthwash. There were no significant differences between two groups in all scores except staining score after 4 weeks of treatment. It was concluded that Ponna yeik mouthwash revealed anti-inflammation and anti-plaque activity without staining.

INTRODUCTION

In the priority ranking of disease based on scoring system (National Health Plan, 1996-2001) [1], oral diseases are placed at 27th rank in Myanmar. In Myanmar, 80% of school children had gingivitis and 18% of them had periodontal destruction [2]. Baseline oral health survey in Myanmar (1990) showed 22.6% of 12 to 13 years old children were free from gum disease and

2.6% of people aged 65 years and above were free from periodontal disease [3]. Among the oral diseases, periodontal disease is the principle disease that affects the morbidity of the dentition. Periodontal disease can be divided into two categories, disease affecting gingival tissue and disease affecting periodontium. Gingivitis, an inflammatory response of the gingiva without destruction of supporting tissue [4], is the common form of periodontal disease

and can be considered as infectious disease. The common form of gingival disease is chronic gingivitis.

Bacterial plaque in oral cavity is regarded as the primary local etiological factor in gingivitis. When uncontrolled, plaque may mature and extend subgingivally leading to periodontitis and eventual loss of teeth [5]. Preventing and controlling of periodontal disease would prevent the microbial colonization of plaque on the teeth and gingiva. Among the varieties of antiseptic mouthwashes in modern dental practice, chlorhexidine gluconate is the most effective antiplaque mouthwash and it is used as standard adjunctive periodontal treatment [6, 7], which is not cheap and easily available in our country. Nowadays, there is a wider area of interest in herbal medicine so that research activities have been done to find out the new, traditional medicine that would be effective and easily available for herbal mouthwash. There are numerous indigenous plants, which are reputed to be effective against the diseases of bacterial origin [8].

Ponna yeik (*Ixora coccinea* Linn.) is locally claimed to be useful in treatment of toothache and oral diseases as a mouthwash in Myanmar. In conditions of toothache, gum swelling in palate is relieved by warm decoction of leaves of Ponna yeik [9]. Ponna yeik is easily available and preliminary study showed the same as chlorhexidine gluconate's anti-bacterial activity on *Staphylococcus aureus*. The present study was, therefore, aimed to find out a new and effective herbal mouthwash in adjunctive periodontal treatment and to compare the effect of *Ixora coccinea* L. (Ponna yeik) mouthwash with 0.2% chlorhexidine solution on chronic gingivitis.

MATERIALS AND METHODS

Collection of medicinal plants

The specimens used in this study were collected from Department of Medical

Research (Lower Myanmar), Yangon. The plants were identified according to the description given in the characterization literature regarding taxonomy [10, 11]. The collection time of the leaves was from November to January after the rainy season and full-grown leaves in nearly uniform size were plucked from the branches.

Preparation of Ponna yeik mouthwash

The fresh leaves were weighed and cleaned with water and then dried at room temperature under the shade. The leaves were grinded into powder form by using electric grinder. Crude watery extracts were prepared by refluxing 50 gm of air-dried leaves powder in a round-bottomed flask on a boiling water bath using 500 ml of distilled water for 6 hours. It was filtered using cheesecloth and the filtrate was evaporated to dryness on a boiling water bath. It was serially diluted to 0.2% solution for mouthwash (solution A).

Preparation of chlorhexidine gluconate mouthwash

Five percent chlorhexidine gluconate stock was serially diluted up to 0.2% solution (solution B). It was used as a positive standard drug.

Experimental design

This study was a randomized controlled clinical trial.

Patients selection

After obtaining the ethical clearance from Institute of Dental Medicine, Yangon, 20 patients within the age of 17 to 22 years from the Institute of Dental Medicine, having typical chronic gingivitis with all anterior teeth and at least one molar tooth present at each quadrant, not taking any antibiotics for the past three months and who received periodontal treatment during the last three months preceding the study were selected after taking written informed consents that they were willing to participate in the trial. Those who had advanced periodontitis or any acute gingival

condition (e.g. acute necrotic ulcerative gingivitis, acute haemorrhagic gingivitis, gingival abscess), enamel hypoplasia or dental fluorosis, taken antibiotics for other reasons during the clinical trial, periodontal problem due to other causes and impacted last molar during the clinical trial were excluded.

Trial procedure

All selected patients were randomly divided into two equal groups and explained about the clinical trial, its objectives and procedure. The degree of plaque accumulation, bleeding on probing and gingival condition of the subjects were examined by only one trained dental surgeon throughout the study. Before starting the study, the examiner was trained by an expert periodontologist with using true pressure sensitive probe, to measure and record the scoring system as expertly and exactly as possible. The proportion agreement was calculated to measure the degree of agreements in both intra-observer and inter-observer variations. The intra-observer calibration was done at 12 anterior teeth of patients with chronic generalized marginal gingivitis of all scoring system used in this study for two consecutive days.

The degree of plaque accumulation, bleeding on probing and gingival condition of the subjects were examined and recorded at baseline and after 4 weeks of treatment. Twenty milliliters of solution were allowed to hold in the mouth for one minute and rinsed with plain water for 3 times before breakfast and before bedtime daily. Every tooth present in the mouth was examined except crowned teeth and abutments of the bridges. The severity of gingivitis was assessed according to noninvasive modification of Loe and Silness index [12], bleeding on probing was assessed according to Sulcus Bleeding Index [13], degree of plaque accumulation was assessed according to Turesky modification of the Quigley-Hein Index 1970 [14] and staining was assessed according to the modification of the Lobene Index 1968 [15].

The scoring units of Sulcus Bleeding Index were used to assess the bleeding on probing. They were as follows:

- 0 = Healthy appearance with no bleeding on probing
- 1 = Healthy appearance with no color or contour changes but bleeding on probing
- 2 = Bleeding on probing and color changes in tissue; but no swelling
- 3 = Bleeding on probing, color changes and slight swelling of the gingival unit
- 4 = Bleeding on probing, obvious swelling, with or without colour changes
- 5 = Spontaneous swelling, bleeding on probing, color changes and significant swelling with or without ulceration

Ethical consideration

Ethical approval for this research proposal was obtained from Institutional Ethical Review Committee of University of Dental Medicine, Yangon. Written and oral information was given to all eligible patients before obtaining their informed consents.

RESULTS

A total of 20 chronic gingivitis patients (10 males and 10 females) participated in this study and their baseline demographic and clinical characteristics are shown in Table 1.

Table 1. Baseline demographic and clinical characteristics of chronic gingivitis patients participating in the study

Total patients evaluated (n=10)	Ponna yeik	Chlorhexidine	p value
Sex (males: females)	1:1	1:1	NS
Age (years) Mean ±SE	19 ± 2.2	19 ± 1.7	NS
Height (cm) Mean ± SE	136.7 ± 3.4	149.1 ± 2.8	NS
Weight (kg) Mean ± SE	96.3 ± 6.9	96.1 ± 6.7	NS
Baseline clinical data (scores) Mean ± SE			
Severity of gingivitis	1.14 ± 0.04	1.2 ± 0.03	NS
Bleeding on probing	0.6 ± 0.052	0.67 ± 0.03	NS
Degree of plaque accumulation	1.58 ± 0.01	1.6 ± 0.008	NS
The staining effect			
Intensity	0.65 ± 0.01	0.65 ± 0.01	NS
Areas	0.6 ± 0.05	0.65 ± 0.01	NS

NS = not significant

It shows that 2 treatment groups were comparable. All patients gave no history of betal chewing and smoking.

Table 2 shows the comparison of patients' response for all scores to Ponna yeik and chlorhexidine gluconate in the two groups tested. After 4 weeks, both Ponna yeik and chlorhexidine gluconate treatments were significantly different from the baseline data scores in all different parameters ($p < 0.01$ to $p < 0.0005$). Both Ponna yeik and chlorhexidine gluconate treatments were significantly different from the baseline data scores in severity of gingivitis ($p < 0.05$ & $p < 0.0005$), but there was no statistically difference between two groups. Comparison of bleeding on probing (Sulcus Bleeding Index) between Ponna yeik and chlorhexidine gluconate mouthwash showed significant reduction after treatment, when compared with the baseline scores in both groups ($p < 0.01$ & $p < 0.005$). But bleeding Index scores after treatment were not significantly different between 2 groups. Comparison of degree of plaque accumulation between Ponna yeik and chlorhexidine gluconate mouthwashes, after 4 weeks of treatment showed no significant difference between two treatments but they were significantly reduced after treatment when compared with before treatment ($p < 0.01$ & $p < 0.005$) (Table 2 & Fig. 1).

Table 2. Comparison of patients' response to different mouthwashes for all scores

	Mean scores \pm SEM					
	Ponna yeik mouthwash			Chlorhexidine mouthwash		
	Before TM	After TM	p value	Before TM	After TM	p value
Severity of gingivitis	1.14 \pm 0.04	0.93 \pm 0.04	$P < 0.05$	1.2 \pm 0.03	0.87 \pm 0.02	$P < 0.0005$
Bleeding on probing	0.6 \pm 0.052	0.34 \pm 0.04	$p < 0.01$	0.67 \pm 0.03	0.36 \pm 0.03	$p < 0.005$
Degree of plaque accumulation	1.58 \pm 0.01	1.5 \pm 0.01	$p < 0.01$	1.6 \pm 0.008	1.5 \pm 0.02	$p < 0.005$
Staining effects Intensity	0.65 \pm 0.01	0.65 \pm 0.01	NS*	0.65 \pm 0.01	0.087 \pm 0.01	$p < 0.005$
Staining effects Areas	0.6 \pm 0.05	0.6 \pm 0.05	NS*	0.65 \pm 0.01	1 \pm 0.01	$p < 0.005$

TM= Treatment, * NS = not significant

Chlorhexidine gluconate treatments showed significant increased staining effect in both area and intensity scores when compared with the baseline data scores ($p < 0.005$). Ponna yeik treatments showed no significant increased staining effect in both area and intensity scores when compared with the baseline data scores (Table 2). Comparison between Ponna yeik and chlorhexidine gluconate treatments at 4 weeks was not significantly different in all different parameters except staining scores ($p < 0.05$) (Fig. 1).

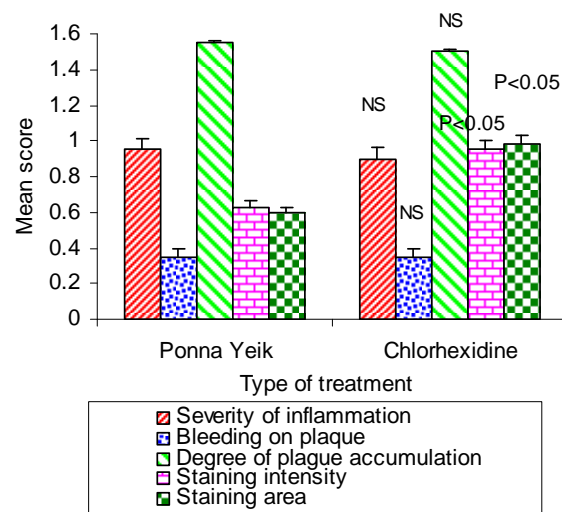


Fig 1. Comparison between effectiveness of Ponna yeik and chlorhexidine mouthwashes after 4 weeks of treatment

DISCUSSION

Since 1960, the role of plaque in the etiology of chronic gingivitis became understood. Periodontal disease can be prevented by either inhibiting the formation of the plaque on the tooth surface or by completely removing the plaque before inflammatory changes occur in the periodontal tissue [16]. There is a direct correlation between the presence and amount of dental plaque on the tooth surface and the presence and severity of periodontal disease. Therefore, plaque control is the most effective method for all inflammation in periodontal tissue. Prevention of plaque formation, removal of plaque by inhibition of calcification of bacteria occurred and elimination of specific pathogenic organism in the plaque are all goals of

chemical therapy by means of anti-plaque mouthwashes.

Current techniques for the assessment of gingival inflammation index system [17], degree of plaque index system [18], bleeding on probing system [17] and probing force investigation were using true pressure sensitive probe and each method had its advantages. One of the significant indices used in epidemiologic research is the Community Index of Periodontal Treatment Needs (CIPTN)(E) which was developed by the World Health Organization [19]. This index assesses the periodontal treatment needs in the community, not simply the level of disease. A study of Lang suggested a weakness in this CIPTN system; it may overestimate the need of treatment. The present method was chosen because it was the most sensitive and having the least error due to use of true pressure sensitive probe [15].

Chlorhexidine gluconate was chosen as a positive control because of its established efficacy and safety [20, 21, 22]. In 1970, Loe & Schiott showed that by rinsing for 1 minute twice a day with 10 ml of 0.2% chlorhexidine gluconate, plaque deposition and gingival inflammation would be completely prevented even in the absence of oral cleaning [23].

In this study, the findings showed that Ponna yeik mouthwash, when used twice a day in conjunction with usual daily oral hygiene measures, significantly reduced gingival inflammation, bleeding on probe and degree of plaque accumulation as good as chlorhexidine gluconate. This finding suggested that the active principle in both rinses (Ponna yeik and chlorhexidine gluconate) effectively control the chronic gingivitis. Therefore, Ponna yeik was evaluated to possess anti-plaque and anti-inflammation activities.

Scientific information on the pharmacologic activity of Ponna yeik for antidiarrhoea, antidysentery, infertility and central nervous system depressant activity had been reported by other researchers [10, 24, 25]. It was also

reported that Ponna yeik (*Ixora coccinea*) root and bark contain glycoside, octadecadienoic acid, mannitol and myristic acid [26]. Anti-plaque and anti-inflammation activities have not yet been reported in the available literature before this study. Therefore, our finding is the first report for the tested activity of Ponna yeik which is cheaper, easily available throughout the country, and easier to make a mouthwash than chlorhexidine which is imported from other countries spending lots of foreign currency.

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