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## Preparation and Evaluation of Herbal Tea and Toothpaste of Mulberry Leaves (*Morus alba* L.).

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### ABSTRACT

The objective of this research was to prepare herbal tea and toothpaste of mulberry leaves extract and evaluate anti-dental plaque activities of their preparations. The herbal material of mulberry leaves was successfully prepared by collecting, wet sorting, washing, cutting, dry sorting, packaging and storing the leaves. Further, the dried mulberry leaves were characterized for macroscopic and microscopic character as well as physicochemical character. The dried mulberry leaves were treated as chopped, grain powder and fine powder to make herbal tea. While the dried mulberry leaves were extracted with ethanol to make viscous extract. The mulberry leaves extract were prepared into toothpaste. The mulberry toothpaste were evaluated and compared their physicochemical properties and antidental plaque to the existing toothpaste. The herbal material of mulberry leaves meet the macroscopic and microscopic properties of *Materia Medica* Indonesia. The physicochemical properties of dried mulberry leaves were as follow: water content  $9.36 \pm 0.34$  %, total ash content  $11.04 \pm 0.91$  %, acid insoluble ash content  $3.77 \pm 0.10$  %, water soluble extract  $16.25 \pm 0.21$  % and ethanol soluble extract  $12.45 \pm 0.48$  %. The herbal tea of mulberry leaves meet the green tea standard. The extract of mulberry leaves at concentration of 4 % in toothpaste basis has the physicochemical properties as follow: form soft, smell herbal like, homogeneous, pH  $7.80 \pm 0.18$ , spreading power  $2.84 \pm 0.18$  cm<sup>2</sup>/g, froth height  $1.02 \pm 0.77$  cm. The antimicrobial activity of this toothpaste was  $21.88 \pm 1.21$  mm of inhibition power toward dental plaque. The three kind of mulberry tea meet the requirement of green tea. The mulberry leaves extract toothpaste can be successfully prepared by mixing 1, 2, and 4 % of mulberry extract with basis of toothpaste. Formula 3 containing 4 % of mulberry extract has a similar inhibition power to Betle Leaves Extract<sup>®</sup> and Pepsodent<sup>®</sup> toothpaste toward dental plaque.

**Keywords:** Herbal material, herbal tea, mulberry leaves extract, toothpaste, dental plaque

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## INTRODUCTION

The literature review of mulberry (*Morus alba* L.) reveals the wide range of important pharmacological activities including antidiabetic, antimicrobial, antimutagenic, antioxidant, anticancer, anxiolytic, anthelmintic, antistress, immunomodulatory, hypocholesterolemic, nephroprotective, hepatoprotective and various effects like adaptogenic effect, effect on hyperlipidemia, inhibition of melanin biosynthesis, used in psychiatric disorder and in gut and airways disorders. This plant showed also antiatherogenic activity and anti-HIV activity [1-3].

Recently in Indonesia, mulberry-leaf tea has gained more popularity as a health beverage and is seeking for industrial manufacture. In the process of tea manufacturing, three main steps are needed namely, blanching, whitening and drying [4]. Drying processes may effect the quality of mulberry tea. In addition, particle size may effect the quality of mulberry tea [5]. The effect of particle size were investigated for optimizing the most suitable quality of mulberry tea.

Traditionally, mulberry is used for treatment of dental pain by chewing to avoid further teeth damage. The purified compound of mulberry, 1-deoxynojirimycin, has an important role as a therapeutic agent by controlling the overgrowth and biofilm formation of *Streptococcus mutans* [6]. The extract of mulberry leaves has been tested for anthelmintic and antimicrobial activities [7]. Therefore, the present study was undertaken to investigate the preparation and evaluation of mulberry tea and toothpaste of mulberry leaves extract.

## EXPERIMENTAL

### Materials

Mulberry leaves were collected from Medicinal Plant Park Andalas University on January 2014. The plant was determined by Herbarium of Andalas University and specimen of this plant was saved in the Herbarium. Other chemicals were purchased from local supplier. Betle Leaves Extract<sup>®</sup> toothpaste and Pepsodent<sup>®</sup> toothpaste were purchased from the market. All chemicals used were of analytical grade.

### Preparation of herbal material and herbal tea of mulberry leaves

Herbal material of mulberry leaves were prepared by collecting, wet sorting, washing, cutting, dry sorting, packaging and storing the leaves. Evaluation of mulberry leaves were performed according to *Materia Medica Indonesia* [8]. Herbal tea of mulberry leaves were prepared from herbal material by three treatments, namely by chopping and grinding the dried leaves and sieving by sieve number 8, 20 and 60. The three kinds of herbal tea were weighed 2 g respectively, and packed with special filter paper for tea bag.

### Evaluation of mulberry leaves tea

Evaluations of mulberry leaves tea were undertaken according to the Indonesia National Standard [9]. These were including condition of brewed water, water content, content of water extract, content of total ash, content of soluble ash in water, content of acid insoluble ash and alkalinity of soluble ash.

### Preparation of mulberry leaves extract

Mulberry leaves powder (200 g) were input into dark bottle, added 2 liter of ethanol 95 %, soaked during the first 6 hours while occasionally stirring, then allowed to stand for 18 hours. The liquid was separated by filtering, and the process was repeated 2 times with type and amount of the same solvent. All liquid collected were evaporated in a rotary evaporator to obtain thick extract [10].

### Preparation of mulberry leaves extract toothpaste

Formula of toothpaste were prepared according to Table 1.

**Table 1: Toothpaste formula of mulberry leaves extract**

Ingredient	Formula 0	Formula 1	Formula 2	Formula 3
Mulberry leaves extract	0 %	1 %	2 %	4 %
Calcium carbonate	37 %	37 %	37 %	37 %
Glycerine	27 %	27 %	27 %	27 %
Sodium carboxy methyl cellulose	0.5 %	0.5 %	0.5 %	0.5 %
Nipagin	0.2 %	0.2 %	0.2 %	0.2 %
Menthol	0.3 %	0.3 %	0.3 %	0.3 %
Sodium lauryl sulphate	2 %	2 %	2 %	2 %
Sodium metabisulphite	1 %	1 %	1 %	1 %
Colloidal silica dioxide	3.5 %	3.5 %	3.5 %	3.5 %
Distilled water	to 100 %	to 100 %	to 100 %	to 100 %

**Preparation of mulberry leaves extract were undertaken as follow:**

Sodium carboxy methyl cellulose was dispersed in a part of glycerine and stirred up until wetted perfectly (mass 1). Sodium metabisulphite was diluted with distilled water by stirring up (mass 2). Mass 1 and 2 were mixed and ground homogeneous. Nipagin was ground and added colloidal silica dioxide while ground slowly, and then added calcium carbonate little by little while ground slowly until homogeneous (mass 3). Mass 3 was added little by little into a mixture of mass 1 and mass 2 to obtain mass 4. Menthol was dissolved in 96 % ethanol and then added sodium laurylsulphate and another part of glycerine (mass 5). Mass 5 was added to the previous mixture (mass 4) to obtain mass 6. In a separate mortar, mulberry leaves extract was ground and added mass 6 little by little while ground homogeneously.

**Evaluations of mulberry extract toothpaste**

Evaluation of mulberry extract toothpaste was undertaken according to the Indonesia National Standard [11]. These were including organoleptic inspection, homogeneity, pH, spreading power and frothing power.

**Microbiological test of mulberry leaves extract toothpaste**

Plaques were taken from a dental clinic in Padang. The plaques were sown on nutrient agar which has been solidified in a Petri disk, incubated for 18-24 hours at 37 °C . The growth of bacterial colonies were selected and moved on slant agar medium [12].

The bacterial colonies were suspended in physiological saline and homogenized. This suspension was measured its transmittance by using spectrophotometer at wavelength of 580 nm to obtain 25 % transmittance [13-14]

A total 1 mL of bacterial suspension was added to petri dish, then added 15 mL of nutrient agar and homogenized by rotating the Petri dish, and then allowed to solidify. The solidified media was perforated by using the sterilized pipette and put into the hole about 50 mg of mulberry leaves extract toothpaste. In other holes, betel leaves toothpaste and Pepsodent® toothpaste were entered as comparison. All of them were incubated for 24 hours at 37 °C and observed the growth of microbes as well as measured the minimal inhibitory concentration.

**RESULTS AND DISCUSSION**

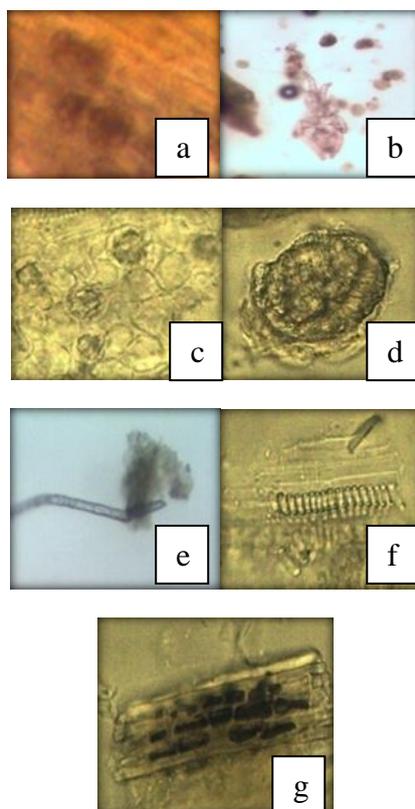
**Preparation of herbal material and herbal tea of mulberry leaves**

Results of macroscopic evaluation were showed in Fig 1. It has a single folium, stalk, 1-4 cm in length, green-yellowish in color. The leaf sheet was oval or heart-shaped, sharp and pointed, margin of serration, 2.5 – 20 cm in length, and 1.5 – 12 cm in width. Upper surface of leaf was coarse and scraggly, dark green to green brownish in color. The bone of leaf was be fin and rather protrusion, lower surface was coarse and scraggly, color was more light than upper surface. The two surface of leaf were more hairy. The character of mulberry leaf was agree with *Materia Medica Indonesia* [8].



**Figure 1: Herbal material of mulberry leaves**

Microscopic evaluation of dried mulberry leaf was showed in Fig 2. The microscopic character of dried mulberry leaf meet the requirement of *Materia Medika Indonesia* [8].



**Figure 2: Upper epidermis (a), lower epidermis (b), lithocyst cell (c), palisade tissue (d), cover hair (e), mesophyll (f) and parenchyma (g) of mulberry leaves**

Physicochemical evaluation of mulberry leaves were showed in Table 2. Values of physicochemical parameter meet the requirement of *Materia Medika Indonesia* [8]. Therefore, the dried leaves of mulberry can be used as herbal tea.

**Table 2: Physicochemical characteristic of mulberry leaves**

Parameter	Value (%)
Water content	9.36 ± 0.34
Total ash content	11.04 ± 0.91
Acid insoluble ash content	3.77 ± 0.10
Water soluble extract	16.25 ± 0.21
Ethanol soluble extract	12.45 ± 0.48

Result of preparation of mulberry tea in three level of sizes, namely chopped, grain powder and fine powder, were revealed in Fig 3. Evaluation of mulberry tea was showed in Table 3. Values of parameter for mulberry herbal tea meet the requirement of the National Standardization Agency of Indonesia [9]. The three kinds of mulberry tea do not differ its quality, but the best herbal tea is the chopped leaves. This kind of herbal tea does not experience the mechanical process.

**Table 3: Characteristic of mulberry tea**

Parameter	Chopped	Grain powder	Fine powder
Condition of brewing tea:	Green yellowish to red brownish	Green jellowish to red brownish	Green yellowish to red brownish
• Color	Fair	Fair	Fair
• Odor	Spesific	Spesific	Spesific
• Taste			
Water content (%)	9.52 ± 0.54	9.74 ± 0.08	10.08 ± 0.23
Water extract (%)	50.43 ± 3.38	41.37 ± 1.35	41.37 ± 2.57
Total ash content (%)	11.17 ± 0.24	11.19 ± 0.51	11.06 ± 0.36
Water soluble ash (%)	11.01 ± 0.51	10.55 ± 0.27	10.88 ± 0.13
Acid insolube ash (%)	0.36 ± 0.04	0.40 ± 0.01	0.37 ± 0.05
Alkalinity (%)	1.31 ± 0.02	1.32 ± 0.01	1.08 ± 0.08



**Figure 3: Herbal tea of mulberry leaves (A = chopped, K = grain powder, H = fine powder)**

**Table 4: Physicochemical evaluation of mulberry extract toothpaste, Betle Leaves Extract® toothpaste and Pepsodent® toothpaste**

Formula	Organoleptic character	Homogeneity	pH	Spreading Power (cm <sup>2</sup> /g)	Froth height (cm)
F0	Semi-solid Mint White Sweet-spiced	Homogeneous	8.92±0.14	3.63±0.29	1.25±0.24
F1	Semi-solid Mint Pale green Sweet-spiced	Homogeneous	8.65±0.34	3.25±0.23	0.90±0.20
F2	Rather soft Herb like Green Sweet-spiced	Homogeneous	8.49±0.20	2.62±0.15	0.79±0.14
F3	Soft Herbal like Dark green Sweet-spiced	Homogeneous	7.80±0.38	2.84±0.18	1.02±0.77
Betle Leaves Extract® toothpaste	Soft Betle like Green Sweet-spiced	Homogeneous	7.19±0.08	4.48±0.30	1.31±0.18
Pepsodent® toothpaste	Soft Mint White Sweet-hot	Homogeneous	8.50±0.31	4.91±0.57	0.92±0.17

Physicochemical evaluation of mulberry extract toothpaste, Betle Leaves Extract<sup>®</sup> toothpaste and Pepsodent<sup>®</sup> toothpaste were showed in Table 4. Formula 3 has the physicochemical character which is similar to the Betle Leaves Extract<sup>®</sup> toothpaste and Pepsodent<sup>®</sup> toothpaste.

#### Microbiological test of mulberry leaves extract toothpaste

Result of antimicrobial activity of mulberry leaves extract toothpaste on dental plaque was showed in Table 5. This reveal that Formula 3 has a similar inhibition power to Betle Leaves Extract<sup>®</sup> and Pepsodent<sup>®</sup> toothpaste toward dental plaque.

**Table 5: Inhibition power of mulberry leaves extract toothpaste, Betle Leaves Extract<sup>®</sup> toothpaste and Pepsodent<sup>®</sup> toothpaste toward dental plaque**

Formula	Inhibition power (mm)
F0	15.83±0.24
F1	16.30±0.41
F2	16.83±0.39
F3	21.88±1.21
Betle Leaves Extract <sup>®</sup> toothpaste	23.88±1.07
Pepsodent <sup>®</sup> toothpaste	22.75±0.85

#### CONCLUSION

Herbal material of mulberry leaves can be successfully prepared by collecting the leaves, wet sorting, washing, cutting, dry sorting, packaging and storing. The prepared mulberry leaves herbal material were treated as chopped, grain and fine particles. The three kind of mulberry tea meet the requirement of green tea. The mulberry leaves extract toothpaste can be successfully prepared by mixing 1, 2, and 4 % of mulberry extract with basis of toothpaste. Formula 3 containing 4 % of mulberry extract has a similar inhibition power to Betle Leaves Extract<sup>®</sup> and Pepsodent<sup>®</sup> toothpaste toward dental plaque

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