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# Physico-chemical Analysis of beeswax procured from Mumbai market, Western Ghats (Natural Habitat)

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## ABSTRACT

Beeswax is the substance that is obtained from the structure of a honey comb. The bees secrete wax to build the honey combs to store honey. It has rich hydrophobic property, the beeswax is in fact present within cosmetics and body products. Also, beeswax is used in food industry. Beeswax is a complex mixture of hydrocarbons, free fatty acid, esters of fatty acid. Beeswax plays an important role in Ayurvedic medicine, the traditional medicine name with *Madhushistha* (Beewax). The *Madhushistha* is used for the care of wound from abrasion or even from burns with topical application. Some references in Ayurvedic granth highlight the use of *Madhushistha* in combination with other herbal and mineral mixture, like *Sikta Taila*, *Mruddaharshringa*, *Jatyadi Taila*, etc. Beeswax is mainly of two types, natural beeswax and processed beeswax. Natural beeswax is clarified further heating in water with petroleum waxes. Because of high demand and shortage in market, adulteration of beeswax with cheaper materials are common like animal fat, plant oil and petroleum spirits (Paraffin wax). Hence authenticity of beeswax is major concern and that can be determined by using physico-chemical parameters such as- organoleptic test, solubility, Melting point, Specific gravity, Refractive index, Acid value, Ester value, Saponification value, volatile matter. With this justification the present study is expected to determine the quality status of four different beeswax samples procured from Mumbai market, western ghats (Natural habitat). The physico-chemical analysis revealed that volatile matter is detected in market sample (2) whereas market sample (1) shows higher melting point as compare to beeswax collected from the Natural habitat. Market sample (1,2) of beeswax shows slight adulteration hence natural habitat samples (1,2) of beeswax should be used in medicine preparation.

**Key words:** Beeswax, *Madhushistha*.

## INTRODUCTION

Beeswax is a natural wax produced by honey bees of the genus *Apis Cerana* and *Apis mellifera* and which are most bred by human. The wax is formed by worker bees, which is secreted from wax producing

mirror gland. The new wax initially is glass-clear and colourless, becoming opaque after mastication and adulteration with pollen by the hive worker bees.<sup>[1]</sup> It is rich in hydrophobic property. Beeswax is complex mixture of hydrocarbons, free fatty acid, esters of fatty acid, fatty alcohol and exogenous substance. Beeswax is insoluble in water and cold alcohol while completely dissolves in chloroform.<sup>[2]</sup> Beeswax is mainly of two types, natural beeswax and processed beeswax. Natural beeswax is clarified further by heating in water with petroleum waxes. Beeswax is used for more than 300 purposes like cosmetics and body products. Also, beeswax is used in food industry, traditional medicine.

Beeswax plays important role in Ayurvedic medicine having traditional name as *Madhuchhistha* (beeswax). Some references in Ayurvedic text highlight the uses of *Madhuchhistha* in combination with other herbal

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and mineral mixture like *Siktha Taila*, *Jatyadi Taila*, *Mruddarshringa Malahar* etc. *Madhuchhista* uses and properties of *Madhuchhista* are mentioned in *Dhanwanatri Nighantu* and *Bhavprakash Nighantu*. The *Madhuchhista* is used for the care of wound from abrasion or even from burns with topical applications. Many doctors use creams like cold cream, which contain olive oil, beeswax and rose water for the treatment of burns, wounds, cuts, bruises and fractures.<sup>[3]</sup>

The demand for beeswax is very high and it has been never satisfied. Because of its high demand and shortage in the market, adulteration of beeswax with cheaper materials like animal fats, plant oils and petroleum sprits (paraffin wax), it becomes a challenge for beeswax quality.<sup>[4]</sup> Hence authenticity of beeswax is major concern and that can be determined by applying physico-chemical parameters. With this justification the present study is expected to determine the quality status of four different beeswax samples procured from Mumbai market, western ghats (Natural habitat).

## AIM

1. To determine physico-chemical analysis of beeswax procured from Mumbai market, Western ghats (Natural habitat).

## OBJECTIVES

1. To collect different samples of beeswax from different area.
2. To analyze beeswax procured from Mumbai market, western ghats (natural habitat) physico-chemically.

## MATERIAL AND METHODS

- Market sample (1)
- Market sample (2)
- Original beeswax (1)
- Original beeswax (2)

Beeswax's market samples taken from the Mumbai market and original samples of beeswax collected from the natural habitat.

## Methods

1. Processing of beeswax
2. Analysis of beeswax samples

### Processing of beeswax

Wax was melted by boiling old comb in water. Beeswax was processed in two steps - in first step the wax was extracted from comb and cleaned. In second step it was purified. In purification method beeswax filtered through filter paper. Beeswax was stored in glass for best preservation and aroma. After melting and cleaning pleasant yellow coloured beeswax was obtained.<sup>[5]</sup>

### Analysis of beeswax samples

#### 1. Organoleptic parameter

- Description
- Colour
- Odour
- Taste

#### 2. Physicochemical parameter<sup>[6]</sup>

### Melting Point

The melting point of a wax is the temperature at which the wax changes its form from a solid to a liquid state. The beeswax was melted by warming it in water bath at a temperature just sufficient to melt it and the melting points were determined by the capillary tube method or the drop point method.

### Total Volatile Matter

To determine the total volatile matter of beeswax, 10 grams of the material was weighed in a suitable dish, previously dried and weighed, and was placed in an oven maintained at 105.2°C for 6 hours. The dish was cooled in desiccators and weighed with the lid on. It was heated again in the oven for 30 minutes. The process was repeated until the loss in mass between two successive weightings was less than one milligram. The result was recorded when the lowest mass has been obtained.

### Saponification Value

Dissolve 35 to 40 gms potassium hydroxide in 20ml water, and add sufficient alcohol to make 1,000ml. Allow it to stand overnight, and poured off the clear liquor. Weigh accurately about 2gm of beeswax in a tared 250ml flask, add 25ml of the alcoholic solution of potassium hydroxide, attach reflux condenser and boiled on a water bath for one hour, frequently rotating the content of the flask cool and added 1 ml of solutions of phenolphthalein and titrated the excess of alkali with 0.5N HCL. Note the number of ml required (a). Repeated the experiment with the same quantities of the same reagent in the manner omitting substance. Note the number of ml required.

### Acid Value

The acid value is the number of milligrams of potassium hydroxide required to neutralize a gram of the wax. It was determined by the titration of the wax solution in ethanol-toluene with 0.5 M potassium hydroxide. A blank was also titrated to correct solvent acidity. Two drops phenolphthalein was normally added as the titration indicator. The acid value (in mg KOH/g) was calculated.

### Ester Value

Ester value was determined by the difference between the saponification value and the acid value and it showed the amount of potassium hydroxide consumed in the saponification of the esters.

### Total Volatile Matter

To determine the total volatile matter of beeswax, 10 grams of the material were weighed in a suitable dish, previously dried and weighed and placed it in an oven maintained at 105.2°C for 6 hours. The dish was cooled in desiccators and weighed with the lid on. It was heated again in the oven for 30 minutes. The process was repeated until the loss in mass between two successive weightings was less than one milligram. The result was recorded when the lowest mass has been obtained.

## RESULTS

Result of organoleptic test and physico-chemical parameter for the four samples is given below.

### Organoleptic test

Organoleptic test	Market sample 1	Market sample 2	Original beeswax 1	Original beeswax 2
Description	Hard solid mass	Hard solid mass	Hard solid mass	Hard solid mass
Colour	Yellow	Yellow	Yellow	Yellow
Odour	Faint	Faint	Faint	Faint
Taste	Slightly better	Slightly better	Slightly better	Slightly better

### Physico-chemical parameter

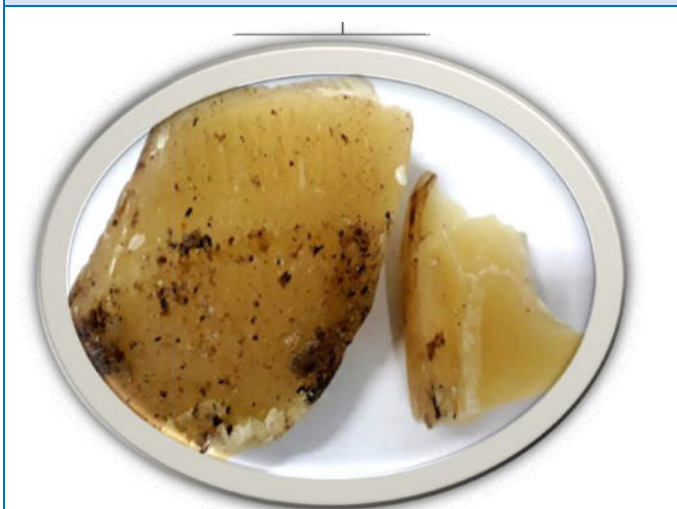
Physico-chemical parameter	Market sample 1	Market sample 2	Original beeswax 1	Original beeswax 2
Solubility	Insoluble in water, soluble in organic solvent when warm	Insoluble in water, soluble in organic solvent when warm	Insoluble in water, soluble in organic solvent when warm	Insoluble in water, soluble in organic solvent when warm
Melting range	63°C to 64°C	61 °C to 62°C	61°C	62°C
Specific gravity	0.96	0.94	0.95	0.96
Refractive index	1.440	1.444	1.42	1.43
Acid value	21	24	18	19
Ester value	75	72	72	75
Saponification value	104	100	82	81
Volatile matter	Nil	0.0021%	Nil	Nil



Market sample (1)



Original beeswax (2)



Market sample (2)



Original beeswax (1)

### DISCUSSION

**Melting point** - The melting point of beeswax is determined by measuring the temperature at which the first drop of liquid wax appears during heating. It should be between 61 and 64°C or preferably. The melting point of the examined samples ranged from 61.5°C to 64°C. all of the samples tasted were found within the acceptable limit but market sample (1) higher melting point about in between 63°C to 64°C as compared to other samples. Hence the market sample is contaminated with some foreign matter.

**Specific gravity** - Specific gravity of samples obtained varied from 0.95 to 0.96. However, there were no significant difference between beeswax samples collected from market samples and natural habitat.

**Acid value** - Acid value is the amount of KOH milligrams required to neutralize the free acidity present in one gram of bees wax. It is also determined by directly titrating the beeswax in an alcoholic medium against standard KOH solution. The acid value of the collected samples revealed that there was highly significant variations in market sample (1) - 21, market sample (2) - 24, original beeswax sample (2) - 19, original beeswax sample (1) - 19, original beeswax sample (2) - 19. The acid value variations of the beeswax samples collected from Mumbai market, western ghats indicate that original beeswax sample (1) is free from contamination.

**Ester value** - The ester value of beeswax is determined by the difference between saponification value and acid value which indicate the amount of KOH consumed during saponification of esters and the result must meet standard limit to show absence of contamination. there was no significant difference between beeswax samples collected from market samples and natural habitat.

**Saponification value** - Testing saponification value indicates the number of acids and ester group found in beeswax. The present study indicated significant difference between the beeswax samples collected from market sample (1)-104, market sample (2)-100, original beeswax sample (1)-82, original beeswax sample (2)-81. Market samples saponification value is higher than the original beeswax samples which are collected from natural habitat.

**Volatile matter** - Volatile matter is the weight loss percent of beeswax when heated in reducing atmosphere (out of air contact). It indicates the amount of volatile matter lost on heating. In all three samples volatile matter is nil but in market sample(2) - 0.0021% is present.

The results of some important physico-chemical analysis of beeswax melting point, specific gravity, refractive index, volatile matter, acid value, saponification value, ester value, and acid to ester ratio of the examined samples are presented. According to results are found the market samples saponification value acid value, melting point, volatile matter is higher than the original beeswax samples. Therefore original beeswax samples are more

preferable than market samples of beeswax for further medicinal purposes.

## CONCLUSION

The laboratory examination of the results of the study revealed that natural original beeswax samples met the quality parameter.

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