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Study of the Physiochemical Properties of *Apis mellifera* L. Wax from the Two Regions of Madhya Pradesh, India

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ABSTRACT: Beewax is one of the most precious bee products which are produced by worker bee and used to construct their hive. Beeswax is a biodegradable, eco-friendly and valuable product that can provide a worthwhile income in addition to honey. It has several commercial, medical, and therapeutic uses that necessitate understanding its chemical makeup. The study analyzed the physicochemical properties of beeswax collected from different locations in Guna and Morena districts of Madhya Pradesh during 2021-22 and 2022-23. Eight beeswax samples were collected from local beekeepers apiary and evaluated. The result showed that the range of compositional content of beeswax *viz*, melting point (61.70 to 64.23°C and 61.57-64.03°C), refractive index (1.430 -1.442 and 1.433-1.445), specific gravity (0.955-0.975 and 0.955-0.973), ash content (0.033 to 0.078% and 0.032-0.079%), total volatile matter (0.327-0.583% and 0.377-0.543%), saponification value (91.67-99.33 and 92.33-98.67) acid value (18.79-22.87 and 19.12-22.53), ester value (72.88-76.47 and 73.03-76.27) and ester to acid ratio(3.38-3.90 and 3.40-3.87) during 2021-22 and 2022-23 respectively. However, except ash content all other parameters of beewax sample showed non-significant variation among the collected location. The overall results revealed that all the beeswax samples satisfied the acceptability criteria set by national standards. Based on these results, beeswax can safely be used as a food additive and subjected to the pharmacological industry.

Keywords: Beewax, physicochemical properties, *Apis mellifera*, acid value, ester value, refractive index.

INTRODUCTION

Beeswax is a creamy-colored substance and the oldest bee product by which bee build the comb that forms the structure of their hive. In particular, *Apis mellifera* Linnaeus (Am) and *Apis cerana* Fabricius (Ac) produce the most bees wax and are secreted by worker bees of 12 to 18 days of age from epidermal glands present on the ventral side of fourth to seventh abdominal segment and wax secretion starts diminishing as their age is increasing but regain its capacity during feed shortages (Brown, 2010; Carillo *et al.*, 2015). The bee hive is made up of hexagonal wax construction that the bees utilise to store nectar, honey, pollen and as a location to lay eggs and raise broods (Bradbear, 2009). Beeswax is initially liquid after secretion, but when exposed to air, it solidifies. Beeswax is an organic non-paraffin phase change material (PCM) that is made up of more than 300 different chemical components, including unsaturated hydrocarbons, free acids, hydroxyl polyesters, and long chain alcohols (Bogdanov, 2009, Bogdanov, 2004; Tulloch, 1980). Beeswax is resistant to many acids and soluble in the majority of organic solvents, however, it is insoluble in water due to this it is used as a coating on fruits and vegetables, for food preservation and as hydrophobic insulators. Wax is shiny and lustrous in nature this property exploited to make varnishes, polishes, and in the cosmetic industry. it also has healing and anti-inflammatory properties which are considered effective in the treatment of inflammation, burns, making ointment, also used in coating agent for drugs or pills, Consuming beeswax prevents the molecules inside from disintegrating before the medications reach the digestive tract (Al Waili, 2003; Ranjha, 2010; Fabra, 2009).

Recently, some researchers identified antibacterial properties in beeswax which provides a new area of investigation but a handful of research done on chemical composition and quality analysis of honeybee wax in India. The unraveling properties of beeswax make it important from the research point of view so the study is based on the physio-chemical properties, of *A. melifera* wax collected from the different apiaries of local beekeepers from Guna and Morena districts of Madhya Pradesh, India.

MATERIALS AND METHODS

Crude beeswax combs samples were collected from eight villages of hives of *Apis mellifera* honeybee in two districts (Guna and Morena) Madhya Pradesh India (Fig. 1). Accordingly, a total of three samples (L1-Sumaoli, L2-Mungavali and L3-Sahadpura villages of Morena district) and five sample from (L4-Padampura, L5-Barkhedahat, L6-Khumbraj, L7- Ramnagar and L8-Pahadiya villages of Guna districts) of Madhya Pradesh India from December 2021 to April 2022 and December 2021 to April 2022. All beeswax samples were collected, refined, purified and analysis based on the protocol of the Quality and Standards Authority of Ethiopia (QSAE, 2005).

Melting point (°C). Beeswax was initially melted by warming it in a water bath to a temperature just high enough to melt it. This was done in order to find the wax's melting point. The thermometer was then dipped and pulled out of the wax, thinly coating the bulb, and left for 24 hours. The melting point of the beeswax was determined after 24 hours by inserting a thermometer through a bored cork into the test tube, placing it in a water bath, and gradually raising the temperature by 1 ˚C every 3 minutes while observing the first drop of melted wax that formed.

Refractive index. The refractive index is measured by Abbe refractometer. To get rid of any contaminants and retaining moisture, the obtained wax samples were melted and run through a rapid filter paper. By recirculating the water from the water path, the refractometer's temperature was set to 75+1°C. The lower prism was covered with a few drops of the sample, and the prism was then closed, fastened, and left to stand for a couple of minutes. The refractive index was read and noted after the sample reached the test temperature. The refractive index was read and noted after the sample reached the test temperature

Specific gravity. The beeswax sample weighed around 2g and was melted in a porcelain crucible at roughly 100°C before being permitted to cool down to the ambient temperature. The material was weighed while suspended from a tarred string after being kept at a temperature of 20 ±1°C for two hours. The sample's mass was calculated first in air and then in rectified spirit kept at a constant 20 ±1°C. To calculate the specific gravity of the beeswax sample, the specific gravity bottle was used to measure the rectified spirit's specific gravity at 20°C/20°C

$$\text{Specific gravity} = \frac{M1.d}{M1 - M2}$$

where

M1 = mass in gram of the wax in air.

d = specific gravity of rectified spirit.

M2 = mass in gram of the matter in alcohol.

Ash content. The platinum dish was heated until it turned red, cooled in desiccators to the ambient temperature, then weighed. The wax was then carefully measured after being placed in a watch glass and weighing around 50g. Around 3/4 of this quantity was moved to the platinum dish and burned on a Bunsen burner to ensure that the wax burns gently at the furnace. We ceased heating the sample when about half of the material had burned, cooled it, and then added the remaining material. The substance was roasted once more until it was entirely scorched. It was then burned for an hour at 550°–650°C in a muffle furnace, cooled to ambient temperature in desiccators, and weighed. The incinerating, cooling, and weighing processes were repeated until there was less than one milligram of variation between each weighted.

Then, the formula was used to determine the ash content;

$$\text{Ash, percent by mass} = \frac{100\ M2}{M1}$$

Where,

M2= mass in g of the ash;

M1 = mass in g of the wax taken for the test.

Total volatile matter. In a suitable dish, 10 g of wax was precisely determined, dried, and weighed before being baked for six hours at 105 °C. After cooling the dish in a desiccator and weighing it with the lid on, we heated the dish again in the oven for a total of thirty minutes. We repeated this process until the difference in mass between two weightings was less than one milligram, at which point we recorded the lowest mass attained.

Total volatile matter at 105°C,

$$\text{percent by mass} = 100\ \frac{(M1-M2)}{M1-M3}$$

Where;

M1- mass in gram of the dish with the wax before heating;

M2- mass in gram of the dish after heating;

M3- mass in gram of the empty dish

Acid value, max. A conical flask of 250 m1 was filled with the substance after it had been well mixed and carefully weighed to a weight of about 5 g. 75 ml of a solution consisting of two parts benzene and one part rectified spirit was added. The sample was heated under refrigeration until it disintegrated, then it was allowed to cool to room temperature before being titrated with a standard potassium hydroxide solution using phenolphthalein as an indicator until a pink colour was seen. The following formula was used to calculate the acid value (in mg KOH/g).

$$\text{Acid value} = \frac{56.1VN}{M}$$

Where; 56.1 = equivalent weight of Potassium hydroxide (KOH),

V=volume in ml of standard potassium hydroxide solution used;

N=normality of standard potassium hydroxide solution and

M = mass in gram of the material taken for the test.

Saponification value. A tarred conical flask containing 2.0 g of wax was weighed, and 25 ml of methyl ketone and 25 ml of an alcoholic potassium hydroxide solution were then added. A few bits of pumice stone were then added, and the reflux condenser was attached to the flask. The flask was cooked consistently but gently for two hours in a water bath. The condenser interior was

cleansed with around 10 cc of rectified spirit after the flask and condenser had been cooled.

The final step was adding a small amount of phenolphthalein indicator solution and titrating it with regular hydrochloric acid. At the same moment, a blank determination was made. The amount of potassium hydroxide needed to hydrolyze 1g of sample beeswax is measured as the saponification value.

$$\text{Saponification value} = \frac{56.1\ (B-S)\ N}{M}$$

Where 56.1 is equivalent weight of Potassium hydroxide (KOH);

B = volume in ml of standard hydrochloric acid required for the blank;

S=volume in ml of standard hydrochloric acid required for the material;

N =normality of standard hydrochloric acid (HCL), and

M = mass in g of the beeswax

Ester value. The ester value, which reveals how much potassium hydroxide was used in the saponification of the esters, was calculated as the difference between the saponification and the acid values.

Ester to Acid Ratio. The ester to acid ratio was obtained by dividing the ester value by the acid value.

Statistical Analysis. Data of all physiochemical characteristics were analyzed by two factors without replication analysis of variance (ANOVA) using Microsoft excel. The significant difference was set at the level of 5%.

RESULT AND DISCUSSION

The data given in Table 1-2 and Fig. 2-3 revealed the physiochemical parameter of crude beeswax samples were collected from eight villages recorded for both the years 2021-22 and 2022-23 separately basis.

Melting point, (°C). The melting point of beeswax is an important physical parameter used to identify its purity. The result on average melting point in *A. mellifera* wax showed ranged 61.70°C (L5-Barkhedahat) to 64.23°C (L2-Mungavali) and 61.57 ^{0}C (L1-Sumaoli) to 64.03°C (L2-Mungavali) respectively during 2021-22 and 2022-23. The test results of beeswax melting point indicated no significant difference between the samples collected from the two districts in both years and these results were within the acceptable range 62-65^{o}C melting point of wax set by FSSAI (2017) standard similar findings of melting point obtained by Tesfaye *et al.* (2016) reported melting point of beewax varied 62.3 -62.56 °C in Bale natural forest Southeastern of Ethiopia and Naik and Pargunde (2020) obtained melting point of beewax 61 to 64 °C in Mumbai market, western ghats.

Refractive index. The data on mean refractive index of beeswax samples varied from 1.430 and 1.433 (L6-Khumbraj) to 1.442 and 1.445 (L4-Padampura) respectively, with no significant difference found among the sample of the study area during 2021-22 and 2022-23. The result concurred with the finding of Bihonegn *et al*. (2017) recorded the refractive index of beewax range from 1.4438-1.4439 in districts of South Wollo Zone.

Specific gravity. The specific gravity of the sample collected varied from 0.955 and 0.955 (L5-Barkhedahat) to 0.975 and 0.973 (L8-Pahadiya) respectively, during 2021-22 and 2022-23. However, there were no significant difference between beeswax samples collected from the study areas. The result concurs with the finding of Tesfaye *et al.* (2016) reported specific gravity range from 0.9565-9625 in Bale natural forest Southeastern Ethiopia.

Ash percent. The assessment of beeswax ash content is critical since it represents its mineral composition. The result of beewax collected from two districts obtained significant difference and maximum ash content was found 0.078 and 0.079%in (L6-Khumbraj) while a minimum 0.033 and 0.032% in (L2-Mungavali) during 2021-22 and 2022-23. The ash content represents the mineral present in wax and the significant variation due to the fluctuation in mineral composition of different localities. The study result within the maximum acceptable limit (0.50% by mass) set by FSSAI (2017) standard and fulfilling the national requirement. The present findings are supported by the work of Shegaw *et al.* (2020) found the ash content (0.0809-0.0970) in Southern Nations Nationalities and Peoples Region (SNNPR).

Total volatile matter (%). Volatile materials in beeswax are those components apart from moisture that is released as gas and vapour upon combustion in the dry oven away from air contact. The data of volatile matter show no significant difference and was ranging from 0.327(L3-Sahadpura) to 0.583 % (L8-Pahadiya) and 0.377(L2-Mungavali) to 0.543% (L8-Pahadiya) respectively, during 2021-22 and 2022-23. The current result falls within 0.75% set by FSSAI (2017) standard for the volatile matter of wax. The current result agrees with the finding of Bihonegn *et al*. (2017) recorded a total volatile matter 0.447-0.636% in districts of South Wollo Zone.

Saponification cloud value, min, (mgKOH/g). The result of saponification values of collected beewax ranged from 91.67 and 92.33 (L3-Sahadpura) to 99.33 and 98.67 (L2-Mungavali) and did not differ significantly among the eight villages of two districts during 2021-22 and 2022-23 respectively. The result within the required beeswax saponification value 85-105 set by the FSSAI (2017) regulation. The above findings supported the range of saponification value varied 81-104 mg KOH/g in the Mumbai market, western ghats (Naik and Pargunde 2020).

Acid value(mgKOH/g). The acid value was measured by the quantity of potassium hydroxide (KOH) needed to neutralise the free acids in one gram of the sample. The acid value of *Apis melifera* wax samples collected from the different locations of Morena and Guna district was found to be ranging from 18.79 and 19.12 (L3-Sahadpura) to 22.87 and 22.53(L2-Mungavali) respectively, during 2021-22 and 2022-23. However, the acid value observed no significant difference among the different location samples and it satisfies the FSSAI (2017) standard which is in the range of 17 to 24. The result confirms the finding of Bihonegn *et al*. (2017) found acid value18.87-19.62 mg KOH/g in South

Wollo Zone wax and Naik and Pargunde (2020)18-21 mg KOH/g in Mumbai market, western ghats

Ester value (mgKOH/g). The ester value is measured by the difference between the saponification and the acid values. The ester value collected beeswax ranged from 72.88 (L3-Sahadpura) to 76.47 (L2- Mungavali) and 73.03 (L4, Padampura) to 76.27 (L1-Sumaoli) respectively, during 2021-22 and 2022-23. However, no significant differences were shown among study locations. The current result confirmed by previous workers with of ester value within 65 to 77 was found by Tesfaye *et al.* (2016); Bihonegn *et al.* (2017); Naik and Pargunde (2020).

Ester to acid ratio. The value of ester acid ratio of the collected beeswax sample showed no significant differences and ranged from 3.38and 3.40 (L2-Mungavali) to 3.90 and 3.87 (L6, Sahadpura) respectively, during 2021-22 and 2022-23. The above result confirmed by previous workers with ester acid ratio range 3.2-4.0 was observed by Tesfaye *et al.* (2016) and Bihonegn *et al.* (2017).

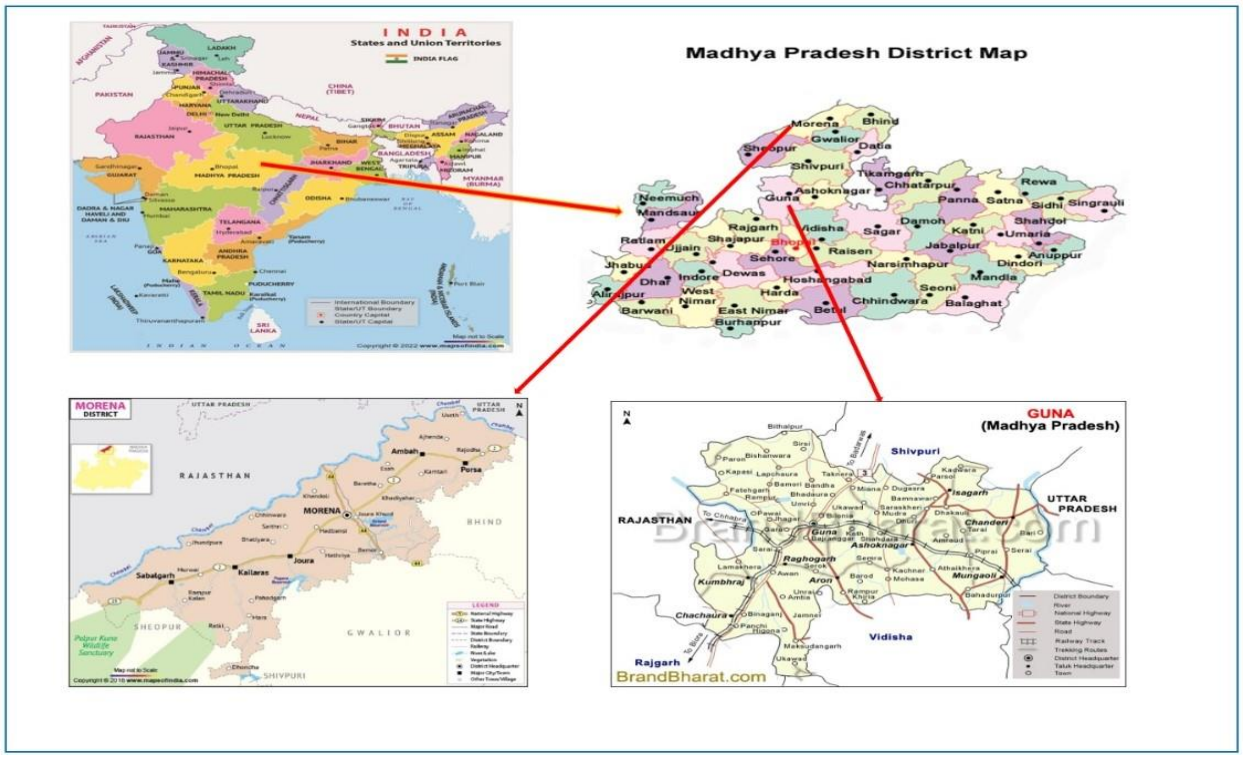

Fig. 1. Study area Guna and Morena district Madhya Pradesh, India.

Table 1: Physico-chemical characterization of wax collected from different locations of Guna and Morena districts (2021-22).

Sr. No.	Melting point (°C)	Refractive index	Specific gravity	Ash	Total volatile matter	Saponification Value	Acid value	Ester value	Ester to acid ratio
L1	61.93	1.432	0.965	0.037	0.417	98.00	21.63	76.37	3.54
L2	64.23	1.441	0.968	0.033	0.363	99.33	22.87	76.47	3.38
L3	63.33	1.431	0.960	0.034	0.327	91.67	18.79	72.88L	3.90
L4	62.90	1.442	0.961	0.050	0.403	93.67	20.27	73.40	3.63
L5	61.70	1.438	0.955	0.047	0.393	92.67	19.50	73.17	3.77
L6	62.97	1.430	0.959	0.078	0.520	95.67	20.03	75.63	3.80
L7	62.47	1.432	0.963	0.057	0.470	93.33	20.41	72.92	3.58
L8	63.37	1.437	0.975	0.061	0.583	95.00	21.57	73.43	3.42
S.Em ±	0.68	0.01	0.01	0.01	0.07	3.10	0.95	3.14	0.25
CD At 5%	NS	NS	NS	0.02	NS	NS	NS	NS	NS

Significant at 5 % level, NS- Non-significant

Table 2: Physico-chemical characterization of wax collected from different locations of Guna and Morena districts (2022-23).

Sr. No.	Melting point	Refractive index	Specific gravity	Ash	Total volatile matter	Saponification Value	Acid value	Ester value	Ester to acid ratio
L1	61.57	1.444	0.967	0.038	0.427	97.67	21.40	76.27	3.57
L2	64.03	1.439	0.965	0.032	0.377	98.67	22.53	76.13	3.40
L3	63.17	1.435	0.962	0.035	0.380	92.33	19.12	73.21	3.87
L4	62.43	1.445	0.966	0.052	0.407	94.00	20.97	73.03	3.48
L5	61.93	1.440	0.957	0.049	0.390	93.33	19.90	73.43	3.69
L6	63.13	1.433	0.955	0.079	0.533	96.33	20.27	76.07	3.77
L7	63.07	1.436	0.966	0.055	0.477	93.67	20.25	73.41	3.63
L8	63.50	1.434	0.973	0.058	0.543	95.67	21.30	74.37	3.50
S.Em ±	0.79	0.01	0.00	0.01	0.04	2.69	0.85	2.79	0.23
CD At 5%	NS	NS	NS	0.02	NS	NS	NS	NS	NS

Significant at 5 % level, NS- Non-significant

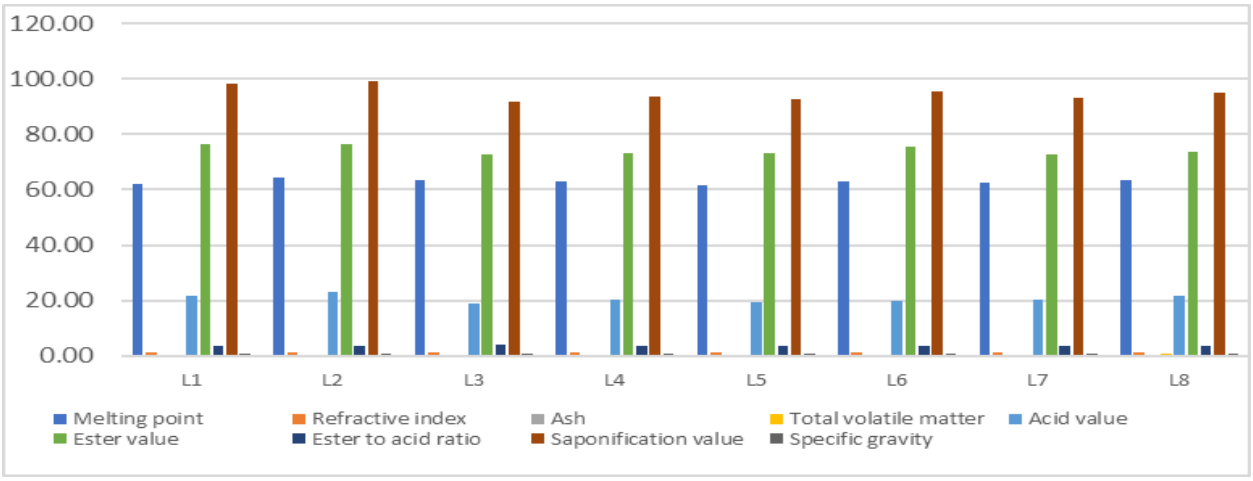

Fig. 2. Physico-chemical characterization of wax collected from different locations of Guna and Morena districts during 2021-22.

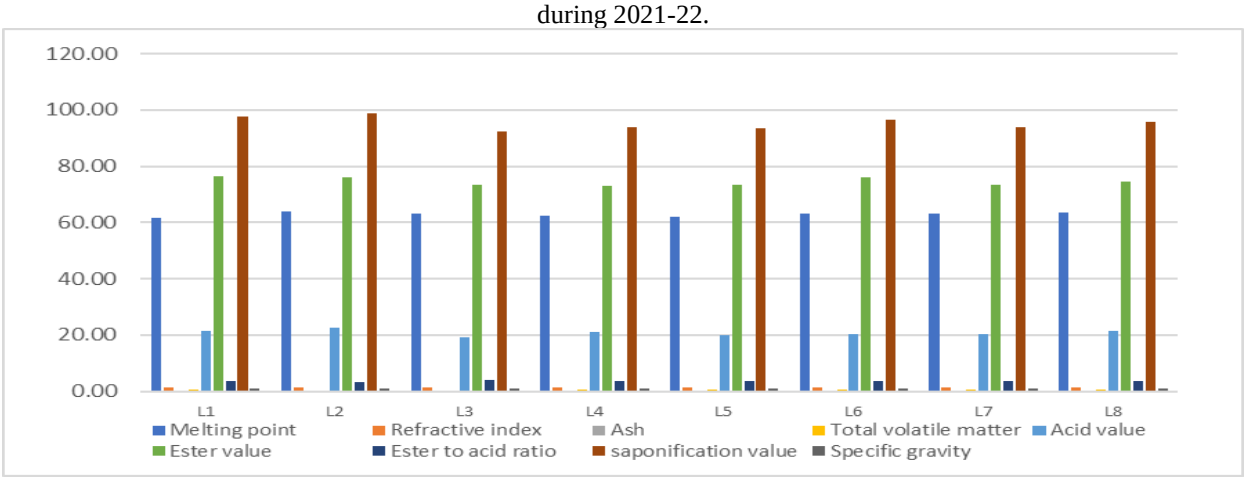

Fig. 3. Physico-chemical characterization of wax collected from different locations of Guna and Morena districts during 2022-23.

CONCLUSIONS

This research conclude that the physicochemical characteristics of beeswax produced in the Guna and Morena regions reflect acceptable quality when compared to national guidelines established to figure out the purity of the beeswax. According to the present results, the difference between the physicochemical properties of the beeswax samples was non-significant except ash content suggesting that geographical origin has no influence on natural beeswax. This research boosted information on the bee wax quality of this region and advised business expansion and employment creation strategy for the local community, as well as to increase the revenue potential of the sub sector enterprise.

FUTURE SCOPE

Bees generate beeswax, which is a biodegradable, economically viable, recyclable and valuable product that can provide a worthwhile income in addition to honey. The good quality of bee wax recognized in this study can be exploited to increase the demand for locally produced wax and also identify its property that make this product unique for utilization in a wide range of products and processes.

Author contributions. S.S*. A.S Y. S.P.S.T and S.T conceived and designed the analysis. S.S collected the data and contributed to analytical tools. S.S*. and A.S Y. performed the analysis. S.S.* wrote the manuscript. All authors read and approved the manuscript.

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Conflict of interest. None.

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