



ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(3): 4115-4120
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www.thepharmajournal.com

Received: 07-12-2022

Accepted: 16-01-2023

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Report on nutritional, antibiotic and physicochemical properties of honey from Garhwal region of Uttarakhand

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Abstract

Honey is a high-energy food enriched with medicinal properties. Its nutritional and medicinal properties depend upon its composition which is greatly influenced by the floral sources and the local environmental conditions. In the present study, two samples of honey from different parts of Uttarakhand Chamoli and Rudraprayag (Garhwal) region were evaluated in terms of quality factors at the Intertek Private India limited laboratory. The various parameters undertaken were physicochemical characteristics of the honey, including its moisture content, specific gravity, hydroxymethylfurfural content, acidity, total reducing sugars, and sucrose content and all these parameters followed all the specifications of FSSAI. Fiehe's test for both samples came back negative, proving that no adulteration. Both the samples were free from pesticide residue and antibiotic residue. Moreover, all nutritional testing was in compliance with industry standards.

Keywords: Kedar valley, Chamoli, physicochemical properties

Introduction

Beekeeping is both an intriguing science and an art. In India, beekeeping is primarily done as a full-time job and a fascinating hobby in order to provide a respectable revenue and table honey. Because beekeeping may be done for both their pollination services and their beloved products like honey, beeswax, propolis, bee venom, etc., honeybees are a wonderful gift to mankind^[17].

Due to its distinctive nutritional and therapeutic characteristics, which are ascribed to the interaction of the various component groups it contains, natural honey is one of the most in-demand goods. Honey is a naturally sweet substance that honey bees, *Apis mellifera*, produce from plant nectar (blossoms), plant secretions, or plant-sucking insects' excretions on living plants. Honeybees collect this sweet substance, transform it by mixing it with other substances, deposit it, dehydrate it, and then store it and allow it to ripen and mature in the honeycomb^[3,4]. The bees are said to produce honey in order to serve as their source of food in times of scarcity or during harsh weather conditions^[7].

Customers should feel confident in the honey they are purchasing in order for the government to be able to acquire foreign cash and restructure the national economy^[10]. The sensory, chemical, physical, and microbiological qualities of honey are what matter most.^[18]

Major chemical components of honey include sugars which represent the largest portion about 82% of honey composition^[6]. The composition of honey depends on the type of flowers visited by bees, climatic conditions in which the plants grow, and maturation.^[1,2] The physicochemical analysis of honey is important to the honey industry, as these factors are intimately related to storage quality, granulation, texture, flavor, and the honey's nutritional and medicinal qualities.

Importance of honey as a nutrient full of energy and prebiotic compounds and its usage in disease treatment^[15]. The purpose of this study, therefore, was to evaluate the nutritional properties of honey from *Apis mellifera*.

Although in India honey is produced and consumed on a large scale, there is a lack of information on the comparative biochemical properties of Indian honey. Therefore, the objective of the current study was to investigate the physicochemical properties of Chamoli and Rudraprayag region honey in Uttarakhand

Material and Method

1. Two honey samples were collected from different locations one from Rudraprayag and Chamoli (*Apis cerana indica* multiflora honey) and another from the Rudraprayag (*Apis mellifera* honey collected from the horse chestnut) and pooled into the two samples respectively, these two samples were processed and analyzed for the following properties.

Physicochemical properties

Method of testing for the physicochemical properties with the methodology mentioned in the result tables 5 and 6.

Nutritional test: The method of testing for nutritional tests

was done by the methodology mentioned in the result table 1 and 2.

Antibiotic test: The (LC-MS/MS) was used to conduct an antibiotic test, and the testing procedure is described in result tables 3 and 4.

Result: All the parameters (physicochemical properties, nutritional test and antibiotic properties) followed all the standards as per the specification of FSSAI and AOAC mentioned in the following tables.

Table 1: Nutritional Test of *Apis cerana indica* collected from Chamoli and Rudraprayag

S. No	Test Parameter	Units of measurement	Result	Specification	Limit of Quantification	Method of testing
1	Total energy	kcal/100gm	325.92	-	-	IFSG/SOP/CTE008
2	Carbohydrate	gm/100gm	81.48	-	-	IFSG/SOP/CTE013
3	Protein	gm/100gm	BLQ	-	0.5	IS: 7219:1973
4	Dietary fibre	gm/100gm	BLQ	-	0.5	IFSG/SOP/CTE007
5	Calcium	gm/100gm	8.36	-	0.05	IFSG/SOP/CTE140
6	Iron	mg/100kg	4.768	-	0.1	IFSG/SOP/CTE140
7	Vitamin A	gm/100gm	BLQ	-	15.0	IFSG/SOP/CTE046
8	Vitamin B1	gm/100gm	BLQ	-	0.03	IFSG/SOP/CTE045
9	Vitamin B2	gm/100gm	0.29	-	0.03	IFSG/SOP/CTE045
10	Vitamin B3	gm/100gm	BLQ	-	0.03	IFSG/SOP/CTE045
11	Vitamin B9	µg/100gm	BLQ	-	1.0	IFSG/SOP/CTE186
12	Vitamin B12	µg/100gm	BLQ	-	2.0	IFSG/SOP/CTE186
13	Vitamin D	gm/100gm	BLQ	-	0.5	IFSG/SOP/CTE047
14	Vitamin E	mg/100gm	BLQ	-	10.0	IFSG/SOP/CTE046

Table 2: Nutritional Test of *Apis mellifera* honey collected from Chopta Rudraprayag

S. No	Test Parameter	Units of measurement	Result	Specification	Limit of Quantification	Method of testing
1	Total energy	kcal/100gm	326.56	-	-	IFSG/SOP/CTE008
2	Carbohydrate	gm/100gm	81.64	-	-	IFSG/SOP/CTE013
3	Protein	gm/100gm	BLQ	-	0.5	IS: 7219:1973
4	Dietary fibre	gm/100gm	BLQ	-	0.5	IFSG/SOP/CTE007
5	Calcium	gm/100gm	2.65	-	0.05	IFSG/SOP/CTE140
6	Iron	mg/100kg	2.66	-	0.1	IFSG/SOP/CTE140
7	Vitamin A	gm/100gm	BLQ	-	15.0	IFSG/SOP/CTE046
8	Vitamin B1	gm/100gm	BLQ	-	0.03	IFSG/SOP/CTE045
9	Vitamin B2	gm/100gm	BLQ	-	0.03	IFSG/SOP/CTE045
10	Vitamin B3	gm/100gm	0.07	-	0.03	IFSG/SOP/CTE045
11	Vitamin B9	µg/100gm	BLQ	-	1.0	IFSG/SOP/CTE186
12	Vitamin B12	µg/100gm	BLQ	-	2.0	IFSG/SOP/CTE186
13	Vitamin D	gm/100gm	BLQ	-	10.0	IFSG/SOP/CTE046
14	Vitamin E	mg/100gm	BLQ	-	10.0	IFSG/SOP/CTE047

Table 3: Antibiotics (LC-MS/MS) of *Apis mellifera* honey collected from Chopta Rudraprayag

S. No.	Test parameter	Units of measurement	Result	Specification	Limit of Quantification	Method of testing
1.	Streptomycine, (including dihydrostreptomycin)	µg/kg	BLQ	Max 5.0	5.0	IFSG/SOP/C/TE/066
2.	Chloramphenicol	µg/kg	BLQ	Max 0.15	0.15	IFSG/SOP/C/TE/061
Nitrofurans Metabolites						
3.	Fuzadolidone Metabolite AOZ	µg/kg	BLQ	Max 0.5	0.5	IFSG/SOP/C/TE/064
4.	Furotodone Metabolite (AMOZ)	µg/kg	BLQ	Max 0.5	0.5	IFSG/SOP/C/TE/064
5.	Nitrofurantoin Metabolite (AHD)	µg/kg	BLQ	Max 0.5	0.5	IFSG/SOP/C/TE/064
6.	Nitrofurazone Metabolite (SEM)	µg/kg	BLQ	Max 0.5	0.5	IFSG/SOP/C/TE/064
Tetracyclines Max 0.5						
7.	Tetracycline (Sum of Tetracycline & 4-epi Tetracycline)	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/059
8.	Oxytetracycline (Sum of oxytetracycline & 4-epioxytetracycline)	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/059
9.	Chlortetracycline (sum of chlortetracycline & 4-chlortetracycline)	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/059
10.	Doxycycline	µg/kg	BLQ	Max 5.0	2.0	IFSG/SOP/C/TE/059

11.	Tylosine A	µg/kg	BLQ	Max 5.0	5.0	IFSG/SOP/C/TE/089
12.	Ampicillin	µg/kg	BLQ	Max 5.0	5.0	IFSG/SOP/C/TE/089
Sulfonamide and Trimethoprine Max 5.0						
13.	Sulfaguanidine	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
14.	Sulfaanilamide	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
15.	Sulfaacetamide	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
16.	Succinylsulfathiazol	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
17.	Sulfanazol	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
18.	Sulfaadimidine	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
19.	Sulfaadizine	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
20.	Sulfathiazol	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
21.	Sulfapyridine	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
22.	Sulfamethazine	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
23.	Sulfameter	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
24.	Sulfamethoxypradizne	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
25.	Sulfachloropyradizine	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
26.	Sulfamonomethoxine	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
27.	Sulfisoxazole	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
28.	Sulphamethoxazole	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
29.	Sulfadoxine	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
30.	Sulfaquinoxaline	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
31.	Sulfadimethoxine	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
32.	Sulfamoxole	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
33.	Sulfamethazole	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
34.	Sulfamerazine	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
35.	Sulfabenzamide	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
36.	Sulfachlozine	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
37.	Trimethoprine	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
Fluor quinolones						
38.	Ceprofloxacin	µg/kg	BLQ	Max5.0	2.0	IFSG/SOP/C/TE/086
39.	Enrofloxacin	µg/kg	BLQ	Max 5.0	2.0	IFSG/SOP/C/TE/086
40.	Erythromycin	µg/kg	BLQ	Max5.0	5.0	IFSG/SOP/C/TE/218
Heavy Metals						
41.	lead	mg/kg	BLQ	Max2.5	0.02	IFSG/SOP/C/TE/141
42.	Copper	mg/kg	BLQ	Max 3.0	0.50	IFSG/SOP/C/TE/141
43.	Arsenic	mg/kg	BLQ	Max1.1	0.10	IFSG/SOP/C/TE/140
44.	Tin	mg/kg	BLQ	Max 250	1.00	IFSG/SOP/C/TE/140
45.	Mercury	mg/kg	BLQ	Max 1.5	0.10	IFSG/SOP/C/TE/141
46.	Cadmium	mg/kg	BLQ	Max 1.5	0.05	
47.	Zinc	mg/kg	BLQ	-	1.0	IFSG/SOP/C/TE/140
48.	Methyl Mercury	mg/kg	BLQ	Max0.25	0.02	IFSG/SOP/C/TE/127
Pesticides						
49.	Pesticide	mg/kg	BLQ	-	0.01	IFSG/SOP/C/TE/110 ^A

Table 4: Antibiotics (LC-MS/MS) of *Apis cerana indica* honey collected Chamoli and Rudraprayag

S. No.	Test parameter	Units of measurement	Result	Specification	Limit of Quantification	Method of testing
1.	Streptomycine, (including dihydrostreptomycin)	µg/kg	BLQ	Max 5.0	5.0	IFSG/SOP/C/TE/066
2.	Chloramphenicol	µg/kg	BLQ	Max 0.15	0.15	IFSG/SOP/C/TE/061
Nitrofurans Metabolites						
3.	Fuzadolidone Metabolite AOZ	µg/kg	BLQ	Max 0.5	0.5	IFSG/SOP/C/TE/064
4.	Furotodone Metabolite (AMOZ)	µg/kg	BLQ	Max 0.5	0.5	IFSG/SOP/C/TE/064
5.	Nitrofurontoin Metabolite (AHD)	µg/kg	BLQ	Max 0.5	0.5	IFSG/SOP/C/TE/064
6.	Nitrofurazone Metabolite (SEM)	µg/kg	BLQ	Max 0.5	0.5	IFSG/SOP/C/TE/064
Tetracyclines Max 0.5						
7.	Tetracycline (Sum of Tetracycline & 4-epi Tetracycline)	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/059
8.	Oxytetracycline (Sum of oxytetracycline & 4 epioxytetracycline)	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/059
9.	Chlortetracycline (sum of chlortetracycline & 4 chlortetracycline)	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/059
10.	Doxycycline	µg/kg	BLQ	Max 5.0	2.0	IFSG/SOP/C/TE/059
11.	Tylosine A	µg/kg	BLQ	Max 5.0	5.0	IFSG/SOP/C/TE/089
12.	Ampicillin	µg/kg	BLQ	Max 5.0	5.0	IFSG/SOP/C/TE/089
Sulfonamide and Trimethoprine Max 5.0						
13.	Sulfaguanidine	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057

14.	Sulfaanilamide	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
15.	Sulfaacetamide	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
16.	Succinylsulfathiazol	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
17.	Sulfanazol	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
18.	Sulfaadimidine	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
19.	Sulfaadizine	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
20.	Sulfathiazol	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
21.	Sulfapyridine	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
22.	Sulfamethazine	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
23.	Sulfameter	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
24.	Sulfamethoxypradizne	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
25.	Sulfachloropyradizine	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
26.	Sulfamonomethoxine	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
27.	Sulfisoxazole	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
28.	Sulphamethoxazole	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
29.	Sulfadoxine	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
30.	Sulfaquinoxaline	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
31.	Sulfadimethoxine	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
32.	Sulfamoxole	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
33.	Sulfamethazole	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
34.	Sulfamerazine	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
35.	Sulfabenzamide	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
36.	Sulfachlozine	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
37.	Trimethoprime	µg/kg	BLQ	-	2.0	IFSG/SOP/C/TE/057
Fluoroquinolones						
38.	Ceprofloxacin	µg/kg	BLQ	Max5.0	2.0	IFSG/SOP/C/TE/086
39.	Enrofloxacin	µg/kg	BLQ	Max 5.0	2.0	IFSG/SOP/C/TE/086
40.	Erythromycin	µg/kg	BLQ	Max5.0	5.0	IFSG/SOP/C/TE/218
Heavy Metals						
41.	lead	mg/kg	BLQ	Max2.5	0.02	IFSG/SOP/C/TE/141
42.	Copper	mg/kg	BLQ	Max 3.0	0.50	IFSG/SOP/C/TE/141
43.	Arsenic	mg/kg	BLQ	Max1.1	0.10	IFSG/SOP/C/TE/140
44.	Tin	mg/kg	BLQ	Max 250	1.00	IFSG/SOP/C/TE/140
45.	Mercury	mg/kg	BLQ	Max 1.5	0.10	IFSG/SOP/C/TE/141
46.	Cadmium	mg/kg	BLQ	Max 1.5	0.05	
47.	Zinc	mg/kg	BLQ	-	1.0	IFSG/SOP/C/TE/140
48.	Methyl Mercury	mg/kg	BLQ	Max0.25	0.02	IFSG/SOP/C/TE/127
Pesticides						
49.	Pesticide	mg/kg	BLQ	-	0.01	IFSG/SOP/C/TE/110 ^A

Table 5: *Apis cerana indica* honey collected from the Rudraprayag and Chamoli districts

S. No	Test Parameters	Units of Measurement	Result	Specification as per FSSAI
1	hydroxymethylfurfural	mg/kg	47.24	NMT 80.0
2	Moisture	gm/100gm	18.4	NMT 20.0
3	Total ash	gm/100gm	0.12	NMT 2.0
4	Acidity	gm/100gm	0.05	NMT 0.2
5	Specific gravity at 27 degree celsius		1.41	NLT 1.35
6	Total reducing sugar	gm/100gm	77.65	Min 65.0
7	F-G ratio		1.2	0.95-1.50
8	Sucrose content	gm/100gm	BLQ	Below 5.0
9	Fiehe, s test		negative	-

Table 6: *Apis mellifera* Chestnut honey collected from the chopta region of Rudraprayag

S. No	Test Parameters	Units of Measurement	Result	Specification as per FSSAI
1	Hydroxymethylfurfural	mg/kg	2.4	NMT 80.0
2	Moisture	gm/100gm	18.23	NMT 20.0
3	Total ash	gm/100gm	0.13	NMT 2.0
4	Acidity	gm/100gm	0.06	NMT 0.2
5	Specific gravity at 27 degree celsius		1.42	NLT 1.35
6	Total reducing sugar	gm/100gm	77.89	Min 65.0
7	F-G ratio		1.14	0.95-1.50
8	Sucrose content	gm/100gm	BLQ	Below 5.0
9	Fiehe's test		negative	-

Discussion

All the physicochemical properties of both the samples were found to be as per the FSSAI standard and all the parameters followed the specification of FSSAI. The moisture of *Apis cerana indica* and *Apis mellifera* honey were 18.4 and 18.23 gm/100gm which were similar to the Malaysian honey where moisture was found to be $17.01 \pm 3.07\%$ and HMF of the *Apis mellifera* honey was 2.4 mg/kg and for the *Apis cerana indica* was 47.24 mg/kg, these quantities were found better and similar respectively, to the HMF 49.51 ± 0.12 mg/kg of Malaysian honey studied by the Moniruzzaman *et al.* (2013)^[8], where they also compared their Malaysian honey to the manuka honey. (Table 5) & (Table 6)

Specific gravity of the honey samples for both the species were more or less similar, 1.41 and 1.42 for *Apis cerana indica* and *Apis mellifera* honey respectively, these values were similar to the study carried out by Tiwari & Tiwari (2013)^[9] they also studied the specific gravity was found to be in the range of 1.36 to 1.41 of the honey collected from the different region of Uttarakashi of *Apis cerana indica*.

Total reducing sugar of both samples of *Apis mellifera* and *Apis cerana indica* were 77.89% and 77.65% respectively, which were more or less similar to the Manuka honey where total reducing sugar was 85% of the manuka honey collected from the *Lepospermum scoparium*^[11].

Ash content

The value obtained in total ash content of *Apis mellifera* and *Apis cerana indica* were 0.13 and 0.12 gm/100gm respectively, however our results were indicating clearness of honey samples and possibly lack of adulterations with molasses^[13] where they showed the value of total ash. 0.66% to 0.136% content below or within the limit allowed for honeys (0.6%) and not more than 2% as per the FSSAI specification.

Sucrose content

Value for sucrose content in both samples were below 5% which is as per the specification led by the FSSAI and our data for honeys from both the species (*Apis mellifera* and *Apis cerana indica*) similar study suggest the same result where the sucrose content value is also below the 5% that is 0.56 ± 0.43 to 1.66 ± 0.01 ^[12].

Antibiotics

In this report there is no residue was found in both the honey samples as all the antibiotics are below the level of quantification whereas Al-Waili *et al.*, (2012)^[13] revealed that antibiotics are found in honey because they are used in apiculture for treatment of bacterial diseases. Antibiotic residues originate mostly from the environment and improper beekeeping practices and D brand mentioned alphabetically which showed that Positive antibiotic residue values of honey. Enrofloxacin antibiotic residue was detected as 4.85 and 7.03 µg/kg in one sample in the G and H brands, respectively. Tetracycline and doxycycline antibiotic residues were also detected in the G brand as 1.36 µg/kg and 2.49 µg/kg, respectively, in only one sample. Among the brands, B brand had the highest dihydrostreptomycin antibiotic residue, and the lowest dihydrostreptomycin antibiotic residue was detected in H brand but as showed in the table 3 and 4 there is no antibiotic and pesticide residue were found which proves the honey of both sample is organic and safe which is easy to

evaluate that there is no possible risk to human health for being offered for the consumption in routine in limited dose.

Nutritional test

In *Apis mellifera* honey sample collected from Rudraprayag, calcium and iron was 2.65 and 2.66 respectively and in *Apis cerana indica* honey collected from the Rudraprayag and Chamoli honey, calcium and iron was 8.36 and 4.768 respectively whereas similar study was conducted by Ogidi and Ebenyam (2020)^[12], they reported that calcium and Iron (Fe) in *Apis mellifera* honey sample were found to be 5.06 ± 0.09 and 1.32 ± 0.0 respectively which showed the more or less similar results to this study.

Total energy and carbohydrate were found to be 325.92 and 81.48% respectively in *Apis cerana indica* honey collected Rudraprayag and Chamoli honey and 326.56 and 81.64 respectively in *Apis mellifera* honey collected from the Rudraprayag similar study was conducted by the where carbohydrate were found be 53.37% in *Apis dorsata*, 53.26% in *Apis florea* and 62.26% in *Apis mellifera* honey^[16]

Conclusion

This is the first of its kind report on physicochemical properties of Kedar valley and Chamoli honey. Our results indicated that some parameters of both honey sample were alike to the Malaysian and Manuka honey. This in turn can act as a boost for Uttarakhand honey to be promoted as brand and potentially be exported as these samples followed the the specification led by FSSAI and matched international brand honey samples like manuka.

Acknowledgement

We wish to thank the Department of horticulture and food processing Uttarakhand for their assistance during survey and analysis of the honey samples.

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