

Study on Chemical and Bioactive Components of Different Floral Sources'

Honey in Nepal

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Honey samples of commercial honey bee (Apis mellifera) were collected from different bee keepers in Nepal. Total 16 different samples from Dang, Chitwan, Nawalparasi, Sarlahi, Makwanpur and Rautahat districts of Nepal were obtained, representing honey of 4 different floral sources 'Chiuri' (Diploknema butyracea), 'Rudhilo' (Pogostemon plectranthoides), Mustard (Brassica napus), and Buckwheat (Fagopyrum esculentum). Chemical composition and bioactive components of the honey samples were studied. Moisture content, pH, total acidity of the examined honey samples was found to be in the range of 19.30 ± 0.87 to 20.15 ± 1.39 %, 3.35 ± 0.63 to 4.80 ± 0.15, 109.25 ± 2.06 to 191.25 ± 14.73 meq/kg, respectively. Antioxidant activity, polyphenol, and flavonoid content were found to be in the range of 51.51 ± 4.95 to 97.84 ± 3.75 %, 17.82 ± 1.61 to 59.34 ± 2.77 mg GAE/100g, 1.22 ± 0.65 to 3.86 ± 0.80 mg GAE/100g, respectively. TSS, reducing sugars and HMF content ranged from 77.5 ± 0.46 to 78.0 ± 0.91 °Bx, 64.06 ± 1.99 to 70.76 ± 1.26%, and 49.5 ± 4.50 to 214 ± 39.20 mg/kg respectively.

Key words: Antioxidant, Floral sources, Honey, Hydroxymethylfurfural, Polyphenol

Introduction

Honey is the sweet and thick liquid food product produced by honeybees by collecting nectar of blossoms or from secretions of any part of plants and specially transforming into honey in honeycomb (NS, 2017 and DFTQC, 2016). Honey's main components are sugars and generally honey comprises approximately mixture of monosaccharide's fructose and glucose as well as other substances such as organic acids, enzymes and solid particles derived from honey collection. The color of honey differs from nearly colorless to dark brown. Honey could be in viscous fluid or crystallized state. The flavor and aroma vary, but are derived from the plant origin, (CAC, 2001).

Honey contains phenolic compounds which acts as natural antioxidants and promotes human health. It is suggested that flavonoids decrease risk of coronary heart disease by three major actions: improving coronary vasodilation, decreasing the ability of platelets in the blood to clot, and preventing low-density lipoproteins (LDLs) from oxidizing (Khalil and Sulaiman, 2010). The antioxidants present in honey include both enzymatic: catalase, glucose oxidase, peroxidase and non-enzymatic substances: ascorbic acid, tocopherol, carotenoids, amino acids, proteins, organic acids, Maillard reaction products, and more than 150 polyphenolic compounds, including flavonoids, flavonols, phenolic acids, catechins, and cinnamic acid derivatives (Ferreira et al., 2010). Flavonoids have important benefits in cardiovascular health, mainly on blood pressure and in the prevention of the damage due to lipid peroxidation of the low density proteins. Other therapeutic aspects of polyphenols are the protection of gastric mucus, liver mucus, anti-inflammatory and anti-neoplastic behaviour, antimicrobial activity (including the selective inhibition of some immunodeficiency viruses and the biochemical effects on enzymes and hormones (Uthurry,

Hevia and Gomez-Cordoves, 2011). Nepal has great potential on production of high quality natural honey due to its diverse natural resources and floral conditions. The honey production in Nepal is increasing every year as number of people involving on beekeeping sector is increasing every year throughout the country. Nepal Government and different NGOs/INGOs like GIZ, ICIMOD, MEDEP, and FNCCI etc. are actively working to develop this sector. According to data (2013/2014) of Ministry of Agricultural Development (MoAD) there are 170000 bee hives in Nepal and 1650 metric ton honey is produced annually which is increasing significantly every year. According to the International Trade Centre (ITC) the total export of honey in the world is estimated to be around 493,573 MT with a value of over USD 1.7 billion.

But Nepali honey is not being able to penetrate the international honey market on its full potential due to lack quality certification. Lack of updated scientific information regarding the quality, freshness, physicochemical properties and bioactive components of Nepali honey is a major constraint for hindering the attraction of Nepali honey in the international market. Similarly, there have not been enough research on honey quality according to different floral sources, honey bee species and production locations.

Materials and methods

Materials

Honey samples

Honey samples of commercial honey bee (*Apis mellifera*) were collected from different bee keepers in Nepal. Total 16 different samples originating from different parts of Nepal were obtained, representing honey of 4 different floral sources 'Chiuri' (*Diploknema butyracea*), 'Rudhilo' (*Pogostemon plectranthoides*), Mustard (*Brassica napus*), and Buckwheat

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(*Fagopyrum esculentum*). All the sample honey was monofloral honey as derived from at least 55 % pollen contribution from one floral source (Von der Ohe et al., 2004). All the samples were kept safe in an air tight bottle under normal room temperature until complete analysis at laboratory of Food Research Division (FRD), Nepal Agricultural Research Council (NARC) for analysis.

Chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich Company, USA. Phenol reagent was purchased from Finar Limited, India. Gallic acid (purity 99.5%) was purchased from LOBA Chemie India. Methanol, Petroleum ether were purchased from Fisher Scientific, India. sodium carbonate, aluminiumtrichloride and other required analytical chemicals were purchased from Merck limited, India. All chemicals used were of analytical grade.

Equipment

The spectrophotometer used was of model Cary 60 UV-Vis Agilent Technologies USA. Similarly, the refractometer and pH meter used were Abbe mat 3200, Anton Paar Opto Tec Germany and pH 211 Microprocessor pH meter Hanna Instruments.

Methods

Determination of refractive index and moisture content

Refractive index and moisture content were determined according to method described in AOAC, 2005. Refractive index of honey samples were measured at 20°C using digital refractometer. Then moisture content were correlated from the refractive index and water content correlation standard table.

Total soluble solid (°Bx)

Total Soluble Solid was determined according to method described in Rangana (2005) using Atago hand refracto meter and the values were expressed as °Bx.

pH

pH of the honey sample was determined according to the method recommended by International Honey Commission (Bogdanov, 2009). A digital pH meter was used to measure the pH of a 10% (w/v) solution of honey prepared in distilled water.

Total ash content and minerals (Iron, Phosphorous, and Calcium)

Total ash content was determined according to the methods of (AOAC, 2005); five grams of honey was placed in silica crucibles, which required preheating to darkness with hot plate to prevent honey foaming. Then, the samples were incinerated at 550°C in a muffle furnace for 5 hours. After cooling to room temperature, the obtained ash was weighed and calculated. Then, minerals (iron, phosphorous, and calcium) were analyzed from the ash solution according to the method described in Rangana (2005).

Acidity (free, lactone, and acidity as formic acid)

Free, lactone, total acidity and acidity as formic acid were determined by titrimetric method (AOAC, 2005). Ten gram honey samples were dissolved in 75 ml distilled water in a 250 ml beaker. The electrode of digital pH meter was immersed in the solution, stirred and titrated with 0.05 N NaOH to pH 8.5 (free acidity). Then, the addition of NaOH was stopped;

immediately 10 ml of 0.05 N NaOH was added and without delay back-titrated with 0.05 N HCL to pH 8.3 (lactone acidity). Total acidity resulted from addition of free and lactone acidities. The results were expressed as miliequivalents per kg (meq/kg).

Hydroxymethylfurfural (HMF)

Hydroxymethylfurfural (HMF) was determined by UV-Spectrophotometric method as described in AOAC method (2005). Five gram honey sample was weighed and mixed with 25 ml water then transferred to a 50 ml volumetric flask. 0.5 ml carrez solution I was added and mixed. Again 0.5 ml carrez solution II was added, mixed and diluted to volume with distilled water. Then, filtered through filter paper, discarding the first 10 ml filtrate, 5 ml filtrate were taken to two test tubes and 5 ml water distilled water was added to the sample while 5 ml of 0.2% sodium bisulfite was added to the reference. Contents in each tubes were mixed properly using vortex mixer, sample absorbance at 284 nm, reference absorbance at 336 nm was measured, and HMF (mg/100 g) was calculated.

Polyphenol, flavonoid, and antioxidant activity

Extract preparation

The extracts of honey were prepared according to the method described by Dimitrijević, Kostić, Stojanović, Mitić, Mitić, and Đorđević, (2014) with some modification. Five grams of sample was mixed with 80 % methanol (30 mL) and this was kept under continuous shaking for 20 minutes and then filtered through Whatmann no. 1 filter paper. The residues were again submitted to two more extraction cycle for 20 minutes each, totalizing 60 minutes of extraction time. The filtrates were combined in volumetric flask, and the volume was made up to 100 mL with 80 % methanol. The extracts were stored in refrigerator for analysis of polyphenol, flavonoids, and antioxidant activity.

Polyphenol content

The total phenol content of sample extracts were measured by using Folin-Ciocalteu method, as described by Mahadavi, Nikniaz, Rafraf, and Jouyban, (2010). In a 25 ml volumetric flask containing 9 ml of distilled water, 1 ml of extract or gallic acid standard solution (100 µg/ml-1000 µg/ml) was added. 1 ml of Folin-Ciocalteu reagent was added to the mixture and were shaken. After 5 minutes, 10 ml of 7 % Na₂CO₃ solution was added and the solution was diluted to volume with distilled water and mixed well. After incubation for 90 min at room temperature, the absorbance against prepared reagent blank (distilled water) was measured using an UV- VIS spectrophotometer at wavelength of 765 nm. Standard solutions of gallic acid was used to obtain standard curve. The results were expressed as mg of gallic acid equivalents (GAE) per 100 g dw (dry weight) of sample.

Flavonoid content

The total flavonoid content (TFC) was determined as (Samatha, Shyamsundarachary, Srinivas and Swamy, 2012) and (Walvekar and Kaimal, 2014) using the aluminum trichloride assay through spectrophotometry. An aliquot (0.5 ml) of extracts was taken in different test tubes then 2 ml of distilled water was added followed by the addition of 0.15 ml of sodium nitrite (5 % NaNO₂, w/v) and allowed to stand for 6 min. Later 0.15 ml of aluminiumtrichloride (10 % AlCl₃) was added and incubated for 6 min, followed by the addition

Table 1

Influence of floral sources on moisture, pH, total ash, acidity and total soluble solids content of honey.

Sample (Floral sources)	Moisture %	pH	Total ash %	Acidity % (as formic acid)	TSS(°Bx)
Chiuri	19.85 ± 0.50	4.80 ± 0.15	0.95 ± 0.02 ^a	0.06 ± 0.00 ^b	77.9 ± 0.52
Rudhilo	20.15 ± 1.39	3.83 ± 0.09	0.14 ± 0.02 ^b	0.20 ± 0.01 ^a	78.0 ± 0.91
Mustard	19.30 ± 0.25	3.35 ± 0.63	0.16 ± 0.01 ^b	0.08 ± 0.01 ^b	78.0 ± 0.20
Buckwheat	19.95 ± 0.44	3.90 ± 0.43	0.10 ± 0.01 ^b	0.22 ± 0.03 ^a	77.5 ± 0.46

The values are mean values of four replicate samples (n=4) ± standard error of mean and the values with different alphabetical letters represents values significantly different at the 0.05 level of probability according to the Tukey test.

of 2 ml of sodium hydroxide (NaOH, 4 % w/v) then volume was made up to 5 ml with distilled water. After 15 min of incubation at room temperature the mixture turned to pink color then absorbance was measured using UV- VIS spectrophotometer at wavelength 510 nm. Distilled water was used as blank. The calibration standard curve was prepared by preparing gallic acid solutions and results were expressed as mg of Gallic acid equivalents per 100 g DW of samples.

Antioxidant activity

The antioxidant activity was determined by the DPPH radical scavenging method as described by (Walvekar and Kaimal, 2014) and (Rajan, Gokila, Jency, Brindha and Sujatha, 2011). DPPH solution (0.004 % w/v) was prepared in 95 % methanol. The samples were mixed with 95 % methanol in 1:9 ratios so as to make final volume 10 mL, thus the extract was prepared. Equal volume of extract and freshly prepared DPPH (0.004 % w/v) were mixed and the tubes were incubated at room temperature in dark for 10 minutes, the absorbance was taken at wavelength 517 nm using a UV-Vis spectrophotometer using 95% methanol as blank. The scavenging activity of the extract against the stable DPPH was calculated using the following equation:

$$\text{Scavenging activity (\%)} = \frac{A - B}{A} \times 100$$

where, A is absorbance of DPPH and B is absorbance of DPPH and extract combination.

Determination of sugars

Reducing sugars, total reducing sugars and sucrose

Estimation of reducing sugar, total reducing sugar and sucrose was done according to method described in AOAC (20005). One gram honey sample was weighed and transferred to 250 ml volumetric flask, 10 ml neutral lead acetate solution (20%) was added, diluted to volume with distilled water and filtered. 25 ml of the filtrate was transferred to 500 ml volumetric flask with 100 ml distilled water. Few drops of potassium oxalate solution (10%) were added until there were no further precipitations, made up to volume, mixed well and filtered. The filtrate was then used for further titration. 5-5 ml of Fehling A and B was taken with 10 ml distilled water in a 250 ml conical flask, heated to boiling and titrated with the sample solution to determine reducing sugars content. For determination of total reducing sugars, 50 ml of the clarified, de-leaded filtrate was transferred to a 100 ml volumetric flask, 5 ml conc. HCL was added and allowed to stand at room temperature for 24 hours. Neutralized with conc. NaOH followed by 0.1N NaOH, made up to the

volume and transferred to burette for titration with Fehling A and B as in the titration process for estimation of reducing sugar. Sucrose content was calculated by formula given below:

$$\text{Sucrose \%} = (\text{Total reducing sugars \%} - \text{Reducing sugars \%}) \times 0.95$$

Glucose, fructose and fructose: glucose ratio

Glucose % was determined iodimetrically in a weak alkaline medium and the value was subtracted from reducing sugars % to arrive at fructose% and fructose: glucose ratio (Pearson, 1976). Weighed 2 g honey sample in a 250 ml volumetric flask and made up to volume, mixed well and 25 ml aliquot transferred to a 250 ml iodine flask. Pipetted 50 ml of 0.1N iodine, 50 ml of 0.2N sodium bicarbonate solution, added to the aliquot, mixed well and allowed to stand in dark for 2 hours. Acidified with 25% H₂SO₄ and titrated with standard sodium thiosulphate using starch solution (1%) as an indicator. Similarly, blank titration was carried out and calculations were done by formulas given below:

$$\text{Glucose \%} = \frac{N \text{ of thiosulphate} \times (\text{blank} - \text{titre}) \times 0.009005 \times 100}{2 \times 0.1N \times \text{weight of sample}}$$

$$\text{Fructose \%} = \text{Reducing sugars \%} - \text{Glucose \%}$$

$$\text{Fructose: Glucose ratio} = \frac{\text{Fructose \%}}{\text{Glucose \%}}$$

Statistical analysis

The research was performed in Completely Randomized Design (CRD). The collected samples were analyzed in four replicates. The analysis of variation was done by using software Statistical Package for Social Sciences (SPSS) version 20. One way anova was performed between different parameters and floral sources. The mean value and standard error of mean were calculated and posthoc test was performed by using Tukey test at 5% significance level.

Results and Discussions

Moisture content

Honey moisture content depends on the environmental condition and manipulation by beekeepers at the harvest period, and it can vary from season to season and from year to year (Acquarone *et al.*, 2007). Honey quality and shelf life is affected by moisture content The higher the moisture content is the higher probability of honey fermentation (Sohaimy *et al.*, 2015).

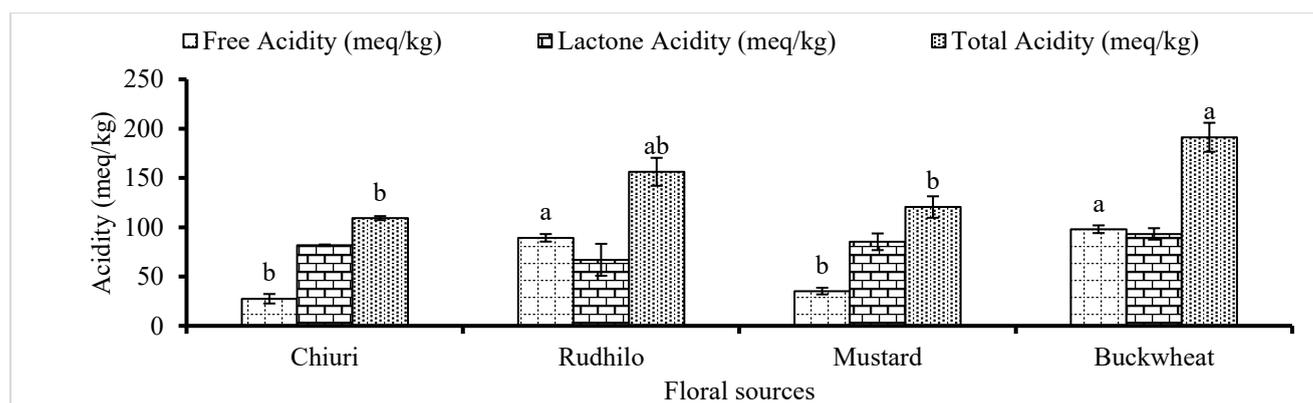


Fig. 1 Influence of floral sources on free, lactone, and total acidity value of honey. The values are mean values of four replicate samples (n=4). Vertical error bars represent \pm standard error of mean and the values with different alphabetical letters represents values significantly different at the 0.05 level of probability according to the Tukey test.

In the present study, the moisture contents of the examined honey samples were found to be in the range of 19.30 ± 0.87 % to 20.15 ± 1.39 % (Table 1). Statistically there was not any significant differences ($P > 0.05$) in moisture content among honey from different floral sources. The moisture contents were within the allowed range of ($\leq 21\%$) according to the international regulations of quality (CAC, 2001) and not more than 23% (FNCCI/AEC, 2006), (DFTQC, 2016). In our study the values were similar to those previously reported for different kinds of honey whose corresponding values ranged from 18.7% to 21.8% (Manresa, 2005).

pH

The pH is a parameter correlated with honey storage and with microorganism growth that could change the texture and honey stability (Feas, Pires, Estevinho, Iglesias, and Araujo 2010). The pH values in the studied samples were found to be in the range of 3.35 ± 0.63 to 4.80 ± 0.15 (Table 1) and was found to be within the standard limit of pH 3.40 to 6.10 (CAC, 2001). Statistically there was not any significant differences ($P > 0.05$) in pH among honey from different floral sources.

Acidity (free, lactone, total acidity, acidity expressed as formic acid)

Organic acids like gluconic acid in equilibrium with their corresponding lactones or internal esters, and inorganic ions such as phosphate, sulphate and chloride are responsible for acidity in honey (Terrab *et al.*, 2004). Fig.1 represents free, lactone and total acidity, and Table 1 acidity as % formic acid. Acidity as % formic acid ranged from 0.06 ± 0.01 to 0.22 ± 0.03 %. Highest was observed in buckwheat flower honey while lowest was observed in *chiuri* flower honey. Statistically there was a significant difference ($P < 0.05$) in acidity among honey from different floral sources. The acidity expressed as formic acid observed in this study were within the acceptable limit of 0.2% (DFTQC, 2016 and NS, 2017).

Free acidity, and lactonic acidity which is considered as the acidity reserve when the honey becomes alkaline, while the total acidity is the sum of free and lactonic acidities (Habib *et al.*, 2014). Free acidity was found to be in the range of 27.50 ± 1.55 to 98 ± 12.24 meq/kg. Highest free acidity was observed in buckwheat flower honey while lowest was

observed in *chiuri* flower honey. Statistically there was a significant difference ($P < 0.05$) in free acidity among honey from different floral sources. Lactone acidity was found to be in the range of 67 ± 16.22 to 93.25 ± 5.81 meq/kg. Highest lactone acidity was observed in buckwheat flower honey while lowest was observed in *rudhilo* flower honey. Statistically there was not any significant difference ($P > 0.05$) in free acidity among honey from all four floral sources.

Total acidity was found to be in the range of 109.25 ± 2.06 to 191.25 ± 14.73 meq/kg. Highest total acidity was observed in buckwheat flower honey while lowest was observed in *chiuri* flower honey. Statistically there was a significant difference ($P < 0.05$) in total acidity among honey from different floral sources. The total acidity observed in this study were above the acceptable limit of less than 50 meq/kg (CAC, 2001), indicating the presence of undesirable fermentation or it may be attributed to harvesting season and floral sources (Habib *et al.*, 2014).

Total soluble solid content

Total Soluble Solid (TSS) content ranged from 77.5 ± 0.46 °Bx to 78.0 ± 0.91 °Bx (Table 1). Highest TSS content was observed in *rudhilo* flower honey while the lowest was observed in buckwheat flower honey. Statistically there was no significant difference ($P > 0.05$) in TSS content among honey from different floral sources. Similar observation of TSS in the range of 79 to 84.1°Bx was reported by Habib *et al.*, (2014) and Silva *et al.*, (2009).

Antioxidant activity

Antioxidant activity shown by extract of concentration 10 mg/ml ranged from 51.51 ± 4.95 to 97.84 ± 3.75 % DPPH radical scavenging as shown in Fig. 2. Highest antioxidant activity was observed in mustard flower honey while the lowest was observed in *rudhilo* flower honey. Statistically there was a significant difference ($P < 0.05$) in antioxidant activity among honey from different floral sources. Antioxidant activity was found to be highest in *chiuri* flower honey.

Similar antioxidant activity in range of 40 to 95 % DPPH radical scavenging was reported by Neupane *et al.*, (2015) in the study of antioxidant properties of honey from different

Table 2

Influence of floral sources on reducing sugars, total reducing sugars, sucrose, glucose and fructose glucose ratio of honey.

Sample (Floral Sources)	Reducing sugars %	Total reducing sugars %	Sucrose %	Glucose %	Fructose/ Glucose ratio
<i>Chiuri</i>	64.06 ± 1.99 ^b	70.38 ± 2.21	6 ± 0.35	30.39 ± 1.92	1.12 ± 0.07
<i>Rudhilo</i>	66.41 ± 0.92 ^{ab}	74.03 ± 1.32	7.24 ± 0.77	30.28 ± 1.04	1.2 ± 0.07
Mustard	70.76 ± 1.26 ^{ab}	75.72 ± 0.38	4.71 ± 0.89	32.69 ± 1.96	1.19 ± 0.17
Buckwheat	66.72 ± 1.07 ^a	71.83 ± 1.11	4.86 ± 1.68	32.8 ± 0.96	1.04 ± 0.07

The values are mean values of four replicate samples (n=4) ± standard error of mean and the values with different alphabetical letters represents values significantly different at the 0.05 level of probability according to the Tukey test.

altitudes of Nepal. The antioxidant activity shown by honey is mainly attributed to the phenolic substances present in it. Honey has been found to contain significant antioxidant activity the antioxidants being glucose oxidase, catalase, ascorbic acid, flavonoids, phenolic acids, carotenoid derivatives, organic acids, Maillard reaction products, amino acids, proteins. Recent results show that high-molecular-mass melanoidins found in honey are also potent antioxidants (Brudzynski and Miotto, 2011).

Polyphenol content

Polyphenol content in the honey samples used in this study ranged from 17.82 ± 1.61 mg GAE/100g to 59.34 ± 2.77 mg GAE/100g as shown in Fig. 2. Highest polyphenol content was observed in buckwheat flower honey while the lowest was observed in *chiuri* flower honey. Statistically there was a significant difference (P<0.05) in polyphenol content among honey from different floral sources. Slightly higher polyphenol content than our study, in the range of 41.90 to 72.14 mg GAE/100g was reported by Neupane *et al.*, (2015) in the study of antioxidant properties of honey from different altitudes of Nepal. Similar polyphenol content in range of 78.96 to 166.1 mg GAE/100g in several honeys from various floral sources were reported in literatures (Silva *et al.*, 2013; Gheldof & Engeseth, 2002; Beretta *et al.*, 2005; Bertoneclj *et al.*, 2007). Several investigations have found a significant level of phenolic compounds in the honey samples of different floral origins (Meda *et al.*, 2005; Ferreres *et al.*, 1991; Gil *et al.*, 1995; Martos *et al.*, 2000; Balsa *et al.*, 2006). Polyphenols are another important group of compounds with respect to appearance and functional properties. 56 to 500 mg/kg total polyphenols were found in different honey types, depending on the honey type (Gheldof and Engeseth, 2002). Polyphenols in honey are mainly flavonoids (e.g. quercetin, luteolin, kaempferol, apigenin, chrysin, galangin), phenolic acids and phenolic acid derivatives which are responsible for the antioxidant properties of honey.

Flavonoid content

In this study flavonoid content ranged from 1.22 ± 0.65 mg GAE/100g to 3.86 ± 0.80 mgGAE/100g as shown in Fig.2. Highest flavonoid content was observed in mustard flower honey while the lowest was observed in *chiuri* flower honey. Statistically there was no significant difference (P>0.05) in flavonoid content among honey from different floral sources. Several Investigations have found a significant level of flavonoid in the honey samples of different floral origins (Meda *et al.*, 2005; Ferreres *et al.*, 1991; Gil *et al.*, 1995; Martos *et al.*, 2000; Balsa *et al.*, 2006). The flavonoid content can vary between 2 and 46

mg/kg of honey and was higher in samples produced during dry season with high temperatures (Tomas *et al.*, 2009).

Reducing sugars content

Reducing sugars content of honey samples were found to be ranged from 64.06 ± 1.99% to 70.76 ± 1.26% as shown in Table 2. Highest reducing sugars content was observed in mustard flower honey while the lowest was observed in *chiuri* flower honey. Statistically there was a significant difference (P<0.05) in reducing sugars content among honey from different floral sources. The reducing sugars content observed in this study except *chiuri* flower honey were in compliance with DFTQC standard, Nepal standard and Codex standard which is minimum and 65% for blossom honey (DFTQC, 2016), (NS, 2017), (CAC, 2001). While the reducing sugars content of *chiuri* flower honey was slightly lower than the standard. Rai *et al.*, (2009) had reported the reducing sugars content of Nepali honey from different floral sources to be in the range of 72.72 to 81.8%, which was higher than our findings.

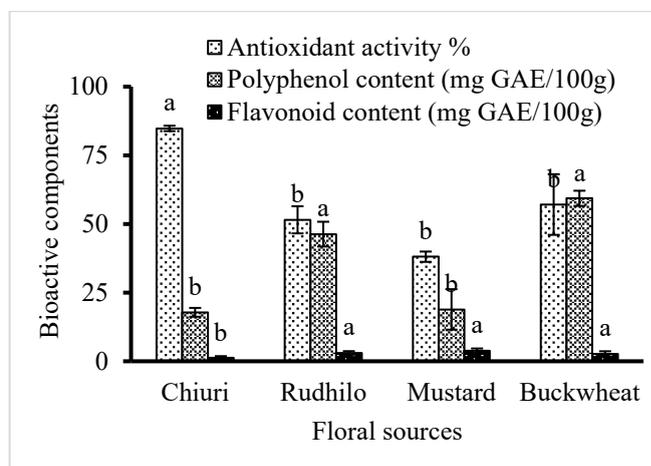


Fig. 2 Influence of floral sources on bioactive components of honey. The values are mean values of four replicate samples (n=4). Vertical error bars represent ± standard error of mean and the values with different alphabetical letters represents values significantly different at the 0.05 level of probability according to the Tukey test.

However, Pathak (2015) had reported the reducing sugars content of Nepali honey from different floral sources to be in the range of 64.77 to 74.02%, which was similar to our findings. Similarly, Shahnawaz *et al.*, (2013) reported the reducing sugars content of Pakistani honey from different floral sources in the range of 69 to 75%, which was similar to our observation.

Total reducing sugars content

In this study total reducing sugars content ranged from $70.38 \pm 2.21\%$ to $75.22 \pm 0.38\%$ as shown in Table 2. Highest total reducing sugars content was observed in mustard flower honey while the lowest was observed in *chiuri* flower honey. Statistically there was no significant difference ($P>0.05$) in total sugars content among honey from different floral sources. Similar observation was reported by Pathak (2015), where the total reducing sugars content of different honey samples ranged from 66.94 to 75.47 %.

Sucrose content

Sucrose content of different type of honey samples used in this study is shown in Table 2. Sucrose content ranged from $4.71 \pm 0.89\%$ to $7.24 \pm 0.77\%$. Highest sucrose content was observed in *rudhilo* flower honey while the lowest was observed in mustard flower honey. Statistically there was no significant difference ($P>0.05$) in sucrose content among honey from different floral sources. Sucrose content of *chiuri* and *rudhilo* flower honey were slightly higher than the Nepal standard of maximum 5 % for blossom honey (DFTQC, 2016 and NS, 2017). While, the sucrose content of mustard and buckwheat flower honey were within the standard value. Similar observation was reported by Rai *et al.*, (2009) who reported sucrose content in the range of 1.94 to 8.02 % in the study of different Nepali honey samples.

Glucose content

In the study of glucose content, glucose content ranged from $30.28 \pm 1.03\%$ to $32.8 \pm 0.96\%$ as shown in Table 2. Highest glucose content was observed in buckwheat flower honey while the lowest was observed in *rudhilo* flower honey. Statistically there was no significant difference ($P>0.05$) in glucose content among honey from different floral sources. Similar observation was reported by Habib *et al.*, (2014) who reported glucose content in the range of 27.78 to 32.35 % in the study of different honey samples from arid regions.

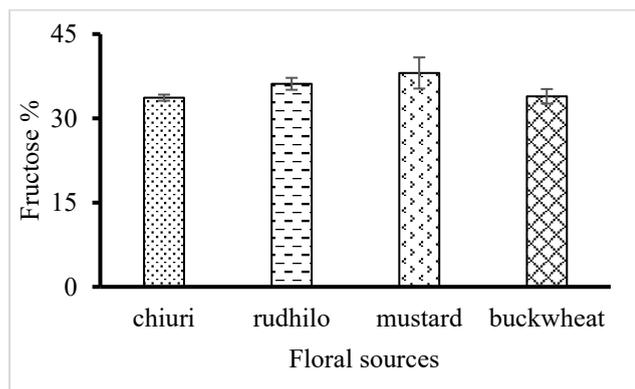


Fig. 3 Influence of floral sources on fructose content of honey. The values are mean values of four replicate samples ($n=4$). Vertical error bars represent \pm standard error of mean.

Fructose content

Fructose content ranged from $33.67 \pm 0.55\%$ to $38 \pm 2.77\%$ which is shown below in Fig. 3. Highest fructose content was observed in mustard flower honey while the lowest was observed in *rudhilo* flower honey. Statistically there was no significant difference ($P>0.05$) in fructose content among honey from different floral sources. Fructose content was found to be higher than glucose content in honey samples

from all four different floral sources. Similar observation was reported by Habib *et al.*, (2014) who reported fructose content in the range of 32.36 to 42.42 %.

Fructose glucose ratio

Fructose glucose ratio of the studied honey samples ranged from 1.04 ± 0.06 to 1.20 ± 0.07 (Table 2). Highest fructose glucose ratio was observed in *rudhilo* flower honey while the lowest was observed in buckwheat flower honey. Statistically there was no significant difference ($P>0.05$) in fructose glucose ratio among honey from different floral sources. Since, the fructose glucose ratio was greater than 0.95 in honey from all floral sources, it was within the mandatory standard of minimum 0.95 (DFTQC, 2016 and NS, 2017). The concentration of fructose was higher in honey from all floral sources. Honey containing less amount of glucose than fructose has ability to fluid (Ouchemouk *et al.*, 2007). Furthermore, honey crystallization depends on other factors such as other sugar contents (e.g. sucrose, maltose), insoluble substances (e.g. dextrin, colloids, pollen) and storage temperature (Buba *et al.*, 2013).

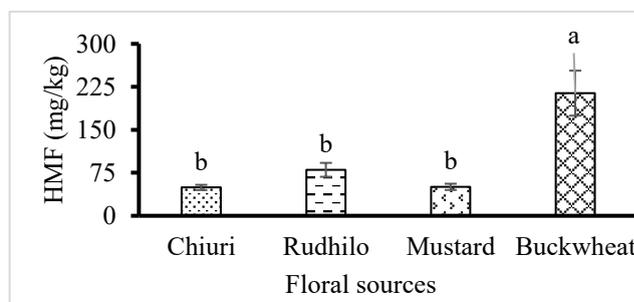


Fig. 4 Influence of floral sources on HMF content of honey. The values are mean values of four replicate samples ($n=4$). Vertical error bars represent \pm standard error of mean and the values followed by different letters are significantly different at the 0.05 level of probability according to the Tukey test.

Hydroxymethylfurfural (HMF) content

The hydroxymethylfurfural (HMF) is one of the important parameter to determine the freshness of honey. This compound is mainly produced from simple sugars, especially fructose, by the action of acids. Several factors have been reported to influence the levels of HMF such as temperature and time of heating, storage conditions, pH and floral sources, so it provides indication of overheating and storage in poor condition (Fallico *et al.*, 2006). HMF content ranged from 49.5 ± 4.50 mg/kg to 214.0 ± 39.2 mg/kg (Fig. 4). Highest HMF content was observed in buckwheat flower honey while the lowest was observed in *chiuri* flower honey. Statistically there was a significant difference ($P<0.05$) in HMF content among honey from different floral sources. HMF content in all the samples studied were higher than Nepal standard and other international standards, which is maximum up to 40 mg/kg (DFTQC, 2016, NS, 2017 and CAC, 2001). HMF content of buckwheat honey was significantly different than other floral sources as was found to be 214.0 ± 39.2 mg/kg.

Total ash and minerals content

Total ash content is a criterion for botanical and geographical origin of honey (Sohaimy *et al.*, 2015). In the present study

total ash content ranged from 0.16 ± 0.00 mg/100g to 0.95 ± 0.01 mg/100g (Table 1). Highest ash content was observed in *chiuri* flower honey while the lowest was observed in mustard flower honey. Ash content of all samples were within acceptable range (0.6-1.2 g/100g) accepted by (CAC, 2001). These results referred to the rich content of pollen source surrounding the apiary yard during honey production (Sahinler *et al.*, 2004).

Iron content

Iron content in the studied honey samples ranged from 5.82 ± 0.12 mg/100g to 9.11 ± 0.20 mg/100g as shown in Fig 5. Highest iron content was observed in *rudhilo* flower honey while the lowest was observed in mustard flower honey. Statistically there was a significant difference ($P < 0.05$) in iron content among honey from different floral sources. Similar observation was reported by Habib *et al.*, (2014) who reported iron content in the range of 0.12 to 11.08 mg/100g in the study of different honey samples from arid regions. Iron content obtained in this study is higher than the value (0.42 mg/100g) reported by USDA (1999), higher iron content could be due to variation in geographical location, as soil mineral profiles vary from one place to another altering the mineral contents.

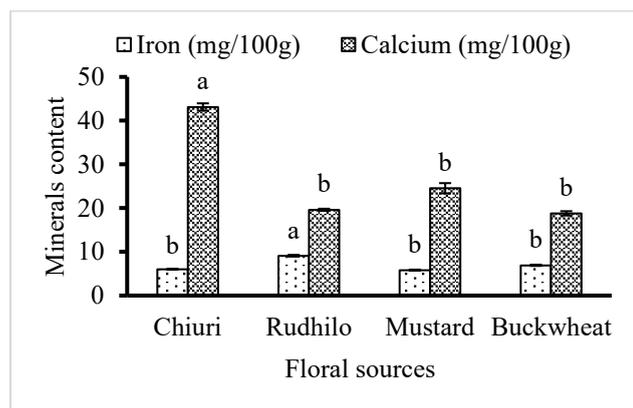


Fig. 5 Influence of floral sources on iron and calcium content of honey. The values are mean values of four replicate samples ($n=4$) \pm standard error of mean and the values with different alphabetical letters represents values significantly different at the 0.05 level of probability according to the Tukey test.

Calcium content

Calcium content ranged from 18.80 ± 0.50 mg/100g to 43.2 ± 0.85 mg/100g as shown in Fig. 5. Highest calcium content was observed in *chiuri* flower honey while the lowest was observed in buckwheat flower honey. Statistically there was a significant difference ($P < 0.05$) in calcium content among honey from different floral sources. Slightly lower calcium content to our study was reported by Habib *et al.*, (2014) who reported calcium content in the range of 0.79 to 24.82 mg/100g in the study of different honey samples from arid regions. Calcium content obtained in this study is higher than the value (6 mg/100g) reported by USDA (1999) higher calcium content could be due to variation in geographical location, as soil mineral profiles vary from one place to another altering the mineral contents.

Phosphorous content

Phosphorous content ranged from 0.05 ± 0.00 mg/100g to 0.29 ± 0.02 mg/100g as shown in Fig. 6. Highest phosphorous content was observed in buckwheat flower honey while the lowest was observed in mustard flower honey.

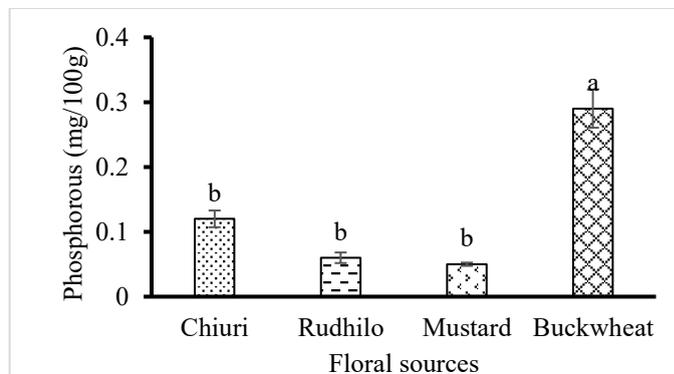


Fig. 6 Influence of floral sources on phosphorous content of honey. The values are mean values of four replicate samples ($n=4$). Vertical error bars represent \pm standard error of mean and the values with different alphabetical letters represents values significantly different at the 0.05 level of probability according to the Tukey test.

Statistically there was a significant difference ($P < 0.05$) in phosphorous content among honey from different floral sources. Higher phosphorous content than in our study was reported by Habib *et al.*, (2014) who reported phosphorous content in the range of 0.90 to 26.40 mg/100g in the study of different honey samples from arid regions. Phosphorous content obtained in this study is less than the value (4 mg/100g) reported by USDA (1999) higher phosphorous content could be due to variation in geographical location, as soil mineral profiles vary from one place to another altering the mineral contents.

Conclusions

The chemical and bioactive compositions of Nepali honey from different floral sources were studied. The quality parameters like moisture, ash, reducing sugars, fructose glucose ratio, and acidity (as formic acid) were within the quality standards set by Nepal government and other international standards. However, parameters like total acidity, sucrose content and HMF were higher than the quality standards set by Nepal government and other international organizations. Highest antioxidant activity of 97.84 ± 3.75 % was shown by *Chiuri* honey, highest polyphenol content was shown by *Rudhilo* and buckwheat honey. There was a significant difference in antioxidant activity and polyphenol content according to floral sources but flavonoid content did not show any variation according to the floral sources. Mineral content was found to be highest in *Chiuri* honey where the calcium content was 18.80 ± 0.50 mg/100g. Highest reducing sugar was observed in mustard honey (70.76 ± 1.26 %), sucrose content of *Chiuri* and *Rudhilo* honey exceeded the mandatory standard of Nepal (5%). HMF content in all honey samples exceeded the mandatory standard (40 meq/kg), HMF content of *Chiuri*, *Rudhilo* and Mustard honey were near to the mandatory limit but the HMF content of Buckwheat honey was almost 5 times higher than mandatory

standard. High HMF content observed in the honey samples could be result of thermal treatment at higher temperature during processing of raw honey by the bee keepers or it may also be due to adulteration in honey.

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