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Understanding anti-obese potential of *Madhupaka* prepared with *Triphala Rasa vs Jala* through their chemical constituents

Kavita M. B.* and Pooja Hassan G

Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Hassan, Karnataka 573201, India

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This study aimed to ascertain the chemical parameters and nutritional profile of the preparation inferring on the possible component(s) of it. Honey was collected from the local market (Hassan, India), stored in ambient temperature for a year (OH). *Madhupaka* was prepared with water alone (MPJ) and with *Triphala rasa* (MPT). All the three samples viz., OH, MPJ, and MPT were tested for their chemical constituents viz., proximate analysis (total protein, total lipid, total carbohydrate, energy value), ash content, moisture content, total solids, HMF level, reducing sugars, total phenols, and total flavonoids. MPT had highest total dissolved solids (79.5%), total phenolics (560.34 mg of gallic acid equivalents/mL), total flavonoids (50.35 mg of quercitin equivalents/ 100g) and HMF (65.15 mg/kg). It had the lowest carbohydrate (60%) and reducing sugars (34.47 g/100 mL). MPT markedly reduces carbohydrates and reducing sugars indicating its action as *atarpaka* (non-nourishing). With its high total phenolic and flavonoid content, *Triphala madhupaka* can act as a potent antioxidant for regular consumption. MPT increases the amount of HMF only within the recommended daily allowance indicating towards its non-toxic effects and safety for human consumption on daily basis in the prescribed dose.

Key words: Flavonoids, Honey, *Madhupaka*, Phenols, *Puranamadhu*, *Triphala*.

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Introduction:

Madhu (honey) is a saccharine liquid prepared from the nectar of the flowers by the hive-bee *Apis mellifera* and bees of other species of *Apis.* Because of its sweetness, many a time artificial invert sugar is replaced with honey[1]. Honey is used along with many medicines as *Anupana* (adjuvant) to increase the effectiveness of the medicine and to make the bitter medicines palatable. Honey is extensively used because of its antioxidant activity which destroys free radicals[2]. *Ayurveda* emphasizes on raw honey (unprocessed) and has given individual importance to *Naveena Madhu* (freshly collected) and *Purana Madhu* (after one year of storage)[3].

Ancient India rarely witnessed apiculture as business and so, honey was usually collected from wild. The collection of nectar could also be done from poisonous flowers in the wild and hence heating honey, directly or indirectly, was contraindicated as it could act toxic when consumed.

Triphala is a combination of *haritaki* (*Terminalia chebula*), *bibitaki* (*Terminalia belerica*) and *amalaki* (*Emblica officinalis* Garten.) in the ratio of either 1:1:1[4] or 1:2:4[5]. It has a wide range of indications[6] among which are its *rasayana* (antioxidant/ rejuvenation), *medohara* (anti-obese/hypolypidemic), *prameha* (diabetes) are of interest. It is mainly consumed in the form of *churna* (powder) and *kvatha* (decoction). The way of making *triphala rasa* from its dry form is by boiling it[7]. The classical texts of *Ayurveda* mention that when *Paka* of *Madhu* (cooking honey on fire) is made on adding *Triphala Rasa* (juice of *Triphala*), it attains anti-obese property[8-9].

There is much research conducted on different varieties of honey and its chemical constituents. No published data is available on researches on *Madhupaka* (cooking honey adding with different liquids) and hence the knowledge is completely lacking in modern scientific terms. This study aimed to ascertain the chemical parameters inferring on the possible component(s) of it which may have anti-obese property.

Materials and Methods

Materials used

All chemicals and standards mentioned in the test were of analytical grade and purchased from an ISO, FDA & GMP certified Indian Laboratory. Honey was collected from the local market (Hassan, India).

*Correspondent author
Email: mbkhsn77@gmail.com

Triphala was collected from Sri Dharmasthala Manjunatheshwara Pharmacy (Udupi, India).

Sampling and sample preparation

Honey was stored separately in a dark room at room temperature for one year to make old honey. Old honey is said to be useful in *Medoroga* one of which is obesity (Sthoulya), a part of the same was used in the preparation of *Madhupaka* with *Triphala Rasa* and that with water.

Ingredients

The ingredients of the samples prepared are given in Table 1.

Preparation of *triphala rasa*[7]

The *Triphala quatha churna* was added to RO filtered water with quantities as mentioned in Table 1. This was boiled on the medium flame for four hours, reduced to one fourth and filtered to have 2000 mL of *triphala rasa* as the final product.

Preparation of *madhupaka*

There were two varieties of *madhupaka* prepared. *Madhupaka* prepared by adding water alone was used as standard compare to *madhupaka* prepared by adding water and *Triphala rasa*.

***Paka lakshana of madhupaka*[10]**

The *madhupaka* is said to be done if it attains the following features: a) sinks when dropped in water, b) smooth and sticky to touch, and c) when applied over a cloth, it sticks to the cloth.

***Preparation of madhupaka with water only*[11]**

The two ingredients as mentioned in Table 1 were taken in a wide-mouthed steel vessel with a thick bottom and mixed well. It was then heated over low flame for 1.20 hours and stirred continuously to avoid charring till honey attained *paka lakshanas*. The yield was 600 mL. This was used as a standard and compared along with old unprocessed honey.

***Preparation of madhupaka with triphala rasa*[11]**

Table 1 — The preparations and quantity of their ingredients

S. No.	Name of preparation	Name of ingredients	Quantity
1	*Triphala rasa*	*Triphala kwatha churna*	500 g
		Water	8000 mL
2	*Madhupaka* (Honey cooked with only water) - MPJ	Old honey	800 mL
		RO filtered water	900 mL
3	*Madhupaka* with *triphala rasa* -MPT	Old honey	800 mL
		RO filtered water	900 mL
		Triphala rasa	1700 mL

The three ingredients were taken in a wide-mouthed steel vessel with a thick bottom and mixed well. It was heated over a mild flame for three hours and stirred continuously to avoid charring till it attained *paka lakshana*. The yield was 600 mL.

All the experiments were conducted with the following sample prepared, i) old honey purchased from the local market (OH), ii) *madhupaka* prepared with water only (MPJ), and iii) *madhupaka* prepared with water and *triphala rasa* (MPT).

All the three samples were tested for their chemical constituents viz., proximate analysis (total protein, total lipid, total carbohydrate, energy value), ash content, moisture content, total solids, HMF level, reducing sugars, total phenols and total flavonoids.

Analysis of proximate composition[12] (AOAC – 1990)

***Proteins*[13-14]**

Percentage of protein content in the sample was assessed using a protein analyzer and was calculated by N/P (nitrogen/ protein) method.

Lipid Analysis

Lipid extraction was done using Folch method[15]. Total lipid content was calculated using the formula,

$$\text{Total lipid} = \frac{t2 - t1}{W} \times 100$$

where SW is sample weight, t_1 was pre-weighed test tube and t_2 was re-weighed test tube after complete evaporation of the solvent.

Carbohydrate

Total carbohydrate was calculated by using the formula

$$\text{Carbohydrate} = 100-[\text{Moisture} + \text{Fat} + \text{Protein} + \text{Ash} + \text{Fibre}]$$

The carbohydrate value was expressed in percentage per 100 g.

Moisture

The moisture content was tested by AOAC method[12]. The moisture content present in the sample was calculated using the following formula and expressed as percentage per 100 g.

$$\text{Moisture} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Weight of the sample taken}} \times 100$$

Ash

The ash content was measured by AOAC method[12]. The total ash content present in the sample was calculated by using the following formula and expressed in percentage per 100 g.

$$\text{Total Ash} = \frac{(\text{Weight of the crucible} + \text{ash}) - \text{Weight of the crucible}}{\text{Weight of the sample taken}} \times 100$$

Total solids

Total solids present in the sample were calculated using the following formula and was expressed in percentage per 100 g.

Total solids = 100 – Moisture

Energy Value

Total energy value of the samples was calculated by using the formula and expressed as Kcal per 100 g.

Energy value= [4×Protein%] + [9×Lipid%] + [4×Carbohydrate%]

Reducing sugars

Assessment of reducing sugar was done by dinitro salicylic acid (DNS) method[16]. Glucose was used to calculate the standard curve (200, 400, 600, 800, and 1000 µg/mL, r^2=0.996). The results were expressed as mg/mL.

Total Phenols/Phenolics

Phenolic compounds were detected by spectrophotometric Folin-Ciocalteumethod[17]. Gallic acid was used to calculate the standard curve (5, 10, 15, 20, and 25 µg/mL, r^2=0.999). The absorbance was read at 760 nm. The results were reported as the mean±standard deviations and were expressed as mg of Gallic acid equivalents per mL.

Total flavonoids

The total flavonoid contents were determined using aluminium chloride colourimetric method[18]. Quercetin was used to draw the standard curve in the concentration range of 100 to 1200 µg/mL. Absorbance was read at the wavelength of 437 nm. The results were reported as the mean±standard deviations and were expressed as mg of Quercitin equivalents per 100 g.

HMF (Hydroxy methyl furfural)

HMF was assessed through spectrophotometric method[19]. Total HMF was calculated using the formula,

$(OD_{443} - 0.055) \times 28.4$ = µmol total HMF per 100 g of dry matter of sample.

Results and Discussion

The results of the analysis of proximate components are tabulated in Table 2.

Proximate analysis

Proximate analysis is very important to know the beneficial effect of honey before and after processing. Here the honey was processed in two different combinations viz., with water alone as well as water and *Triphala rasa*. The proximate components such as water content (moisture), total protein, total fat, total carbohydrates, total ash and energy value of old honey sample from the market and heat-processed honey with water as well as that with water and Triphala rasa was assessed. The values of the entire proximate component are presented in Table 2.

Total protein

Protein was found only in MPT (4%). It was not detected in OH and MPJ. This may be due to microfiltration of honey done to remove the pollens which are the main source of proteins in honey. The other marketing reason is to avoid it's tracing to the floral source from which honey is formed[20,21]. Barthakur *et al.*[22] reported that *Terminalia chebula* which is among *Triphala* has high protein content. Addition of *Triphala rasa* might have added protein in MPT.

Total lipid

Lipids were found only in MPT (10%). There was no lipid detected in OH and MPJ. As such, there is no lipid present in raw honey but 10% of lipid content found in MPT might have been derived from *Triphala rasa*. Supporting to this, Onial *et al.*[23] reported that *Terminalia chebula* has 41.14% of fatty oils which includes both saturated and unsaturated fatty acids. Molla *et al.*[24] reported 22.57% of lipid content in *Terminalia belerica*. Meena *et al.*[25] reported that *Emblica officinalis* has 18% or more amount of fixed oil in it.

Total carbohydrate

The lowest carbohydrate content was found in MPT (60%) when compared to MPJ (74%) and OH (79%). The reduction of the carbohydrate content in MPT was mostly due to processing by adding *Triphala* and water. Tannins in *Triphala* are responsible for its astringent and bitter taste. Chung *et al.*[26] reported that increased tannin content in foods is of the less nutritional value as it is

Table 2 — Proximate analysis of samples (per 100 g)

S. No.	Parameter	Groups		
		OH	MPT	MPJ
1	Protein (%)	ND	4	ND
2	Lipid (%)	ND	10	ND
3	Carbohydrates (%)	79	60	74
4	Moisture content (%)	15.5	24	17
5	Ash (%)	0.5	1	1
6	Total solids (%)	79.5	79.5	78
7	Energy value (KCal)	316	346	296

ND = Not detected, OH (Old Honey), MPT (*Madhupaka* with *triphala rasa*) and MPJ (*Madhupaka* with *jala*) responsible to decrease food intake and efficiency in converting the absorbed nutrients to new body substances. Erejuwa *et al.*[27] reported that fructose and glucose in honey have the same molecular formula but different structural formula compared to others due to which their absorption is slow in the intestine which will elongate the duration of contact and interaction between fructose and intestinal receptors that play a key role in satiety. This allows more macronutrients to be passed into the large intestine, thereby limiting their intestinal absorption. The low carbohydrate state of MPT supports the clinical indication of MPT in obesity. The feeling of satiety if caused as mentioned above makes it *Guru* (heavy to digest) and prevention of macronutrients absorption in intestine makes it *Atarpaka* (non-nourishing) as required in obesity management[28].

Moisture content

Moisture was more in MPT (24%) when compared to MPJ (17%) and OH (15.5%). Ideal moisture content as per the WHO standard is less than 20%[29]. Reports of Moniruzzaman M *et al.*[30] and Chirife et al.[31] highlight that excessive moisture content causes fermentation in honey on storage by the action of osmotolerant yeasts, which results in the formation of ethyl alcohol and carbon dioxide. The heat processing of honey in market samples are probably done to maintain the moisture content below 20% as witnessed in the moisture content of OH. Increased moisture in MPJ and MPT are mostly due to the addition of water and *Triphala rasa*.

Ash

Ash content was found to be lesser in OH (0.5%) when compared to MPT (1%) and MPJ (1%). Though there is no significant difference in the ash content of three samples, the processed honey in the form of *madhupaka* had higher ash content. Ash content referred to the inorganic residue with mineral as its primary content. The higher the level of ash content, the higher the level of mineral content would be[32]. This would probably help supplementation of micronutrients which is a requirement in obesity management programme. This too supports MPJ as a probable anti-obese food.

Total solids

Total dissolved solids were found to be equal in both OH and MPT i.e., 79.5% slightly higher than MPJ (78%). Highest TDS is indicative of rich organic and inorganic substances in samples which support anti-oxidant, anti-bacterial, and wound healing actions of honey[31]. Obesity is one of the disorders related to oxidative stress. Presence of higher antioxidants indicates the removal of free radicals from the body, a requirement in obesity management. The higher TDS in MPT makes it a possible anti-obese food.

Energy value

Total energy value was found to be high in MPT (346 Kcal/100 g) in comparison OH (316 Kcal/100 g), and MPJ (296 Kcal/100 g) respectively. Compared to OH there is only a slight increase in energy value in MPT, thus both OH and MPT provides adequate energy. This slight increase in MPT is due to processing with *Triphala* which added 4% of protein and 10% lipid to it. Though the energy is higher in MPT, it is due to the presence of protein and lipid which are not harmful to obese.

Other chemical analysis

Other than proximate principles, reducing sugars, total phenols, total flavonoids and HMF were also analyzed. The comparative tabulation is presented in Table 3.

Sugars

Reducing sugar was found to be lowest in MPT (34.47 g/100 mL) when compared to MPJ (60.42 g/100 mL) and OH (69.09 g/100 mL). A lower amount of reducing sugar indicates lesser simple sugars which if not used for energy requirements, will be stored as fat. The MPT has the least amount of reducing sugar of the three samples indicating it's anti-obese potential. Processing with *Triphala rasa* has effectively reduced the total reducing sugars in MPT. The lower amount of total sugar could be due to

Table 3 — Results of other chemical analysis

S. No.	Parameter	OH	MPT	MPJ
1	Reducing sugars (g/100 mL)	69.06	34.47	60.42
2	Total phenols (mg/100 mL)	266.13	560.34	93.6
3	Total flavonoids (mg/100 g)	5.85	50.35	0.25
4	Hydroxymethylfurfural HMF (mg/kg)	41.17	65.15	52.12

OH (Old Honey), MPT (*Madhupaka* with *triphala rasa*) and MPJ (*Madhupaka* with *jala*) the conversion of sugar into inorganic acid. The storage of honey for very long periods or heating of honey samples during processing can lead to the conversion of sugars[33] to HMF as observed in MPT and MPJ. Supporting to this, a study by Sharma *et al.*[34] reported that a decrease in sugar content was observed with the rise in heating temperature in all varieties. Lowered reducing sugar means a reduction of sweetness. This probably indicates the lesser calorie by the carbohydrates.

Total phenols

Total phenolics were high in MPT (560.34 mg of gallic acid equivalents/mL) compared to OH (266.13 mg of gallic acid equivalents/mL) and MPJ (93.6 mg of gallic acid equivalents/mL) respectively. The heat processing of honey increases its amount of total phenols[35]. The increase of total phenols in MPT can also be due to the addition of phenols by *Triphala*. It is supported by an analytical study conducted by Naik *et al.*[36] which showed that *triphala* is rich in phenols/polyphenols (38±3%). Higher total phenolic content indicates a higher antioxidant activity of MPT. This hints towards a stronger anti-obese potential of MPT.

Total flavonoids

Flavonoid content was found to be highest in MPT (50.35 mg of quercitin equivalents/100 g) compared to MPJ (8.26 mg of quercitin equivalents/ 100g) and OH (5.85 mg of quercitin equivalents/ 100 g) respectively. Flavonoids in honey may originate from nectar, pollen or propils[37]. The market sample of honey was probably processed to remove the pollen and other allergens before bottling which is seen in OH. *Triphala*, in MPT is a good source of flavonoids[38]. This may be a reason for MPT showing a comparatively higher value of total flavonoids. Heating up to 120 ^{o}C increases the flavonoid content but higher than that destroys it[34]. Higher flavonoids mean higher antioxidant activity of MPT which makes it a possible anti-obese food.

HMF content

HMF content was found to be highest in MPT (65.15 mg/kg) when compared to MPJ (52.12 mg/kg) and OH (41.17 mg/kg) respectively. HMF is naturally present in honey, which is produced by the action of acidity in honey on reducing sugars and sucrose usually at room temperature[39]. The standard limit for HMF in unprocessed honey is 40 mg/kg[40] whereas honey of declared origin from regions with tropical climate and blends of these is 80 mg/kg[29]. The reason for OH having HMF value exceeding the general limit can be attributed to its origin in India, a tropical country. Cooking with fire (heat processing) adds to the increase of HMF value[33] in MPJ and MPT. Tosun *et al.*[41] in their study reported that HMF formation increases on storage. The estimated amount for the daily intake of HMF is 30-150 mg/kg[42]. Abraham *et al.*[43] in an animal experiment reported that there were no ill effects observed at a daily dose of HMF in the range of 80–100 mg/kg. Tamanna *et al.*[44] reported that HMF and other Millard reaction products in low amounts acts mainly as anti-oxidants. In the present study, the HMF is detected within the normal limits in all three samples. Thus MPT too can be regarded as non-toxic for oral administration though it has the highest amount of HMF among the three samples.

Conclusion

Madhupaka when prepared with *triphala rasa* markedly reduces total carbohydrates and reducing sugars contents honey. It also increases the total phenolics, flavonoids, and HMF contents in it. While the lower total carbohydrate and reducing sugar contents render it non-nourishing (*atarpka*), its high total phenolic and flavonoid content suggest that *madhupaka* when prepared *triphala rasa* possibly acts a potent antioxidant for regular consumption. Though *madhupaka* when prepared *triphala rasa* increases the amount of HMF, it still falls within the recommended daily allowance limit indicating it is safe for human consumption on daily basis in the prescribed dose. The study concludes that based on the chemical parameters between the three, *madhupaka* prepared with *triphala rasa* has possible components viz., total carbohydrates, reducing sugars, total phenolic content, total flavonoids, and HMF which may act as anti-obese agents.

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Conflict of interest

The work was part of post-graduation dissertation work submitted to RGUHS, Bengaluru. No competing financial interests exist.

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