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ANALYTICAL EVALUATION OF MADHU WITH SPECIAL REFERENCE TO SAMYOGA AND SAMSKARA

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ABSTRACT

Madhu (Honey) is indicated in both *Sthoulya* and *Prameha* along with *Triphla* according to various Ayurvedic literatures. *Samskara* and Samyoga of *Madhu* with *Triphala Kashaya* revealed its role in the management in *Sthoulya* and *Prameha* respectively. Analytical evaluation of *Madhu* is carried out to understand the physico-chemical changes with respect to *Samyoga* and *Samskara* with *Triphala Kashaya*. Unprocessed *Purana Madhu*, *Jala samskarita Madhu*, *Triphala Kashaya samskarita Madhu* and *Triphala kashya mishrita Madhu* were subjected for various analytical procedures such as Sugar components, HMF, Total phenolic content, anti-oxidant assay and Electrophoresis. *Triphala Kashaya samskarita Madhu* contained high Phenolic content. *Tripahal Kashaya mishrtia Madhu* showed high anti-oxidant capacity despite having less phenolic content than Samskara sample. Present study thus establishes the role of anti-oxidant activity and high phenolic content in the management of Hyperglycaemia and Obesity respectively and also the current study stresses the need for characterization of phenolic content of pre and post processed samples of honey.

KEYWORDS: HMF (Hydroxy methyl furfural), Anti-oxidant, Electrophoresis, Phenolics and characterization

INTRODUCTION

Honey is a sweet food made by bees using nectar from flowers [1]. Since ancient times, honey has been known for its nutritive value and therapeutic values. The global production of honey is approximately 1.20 million tons per annum [2].

Ayurvedic literature describes *Madhu* as very much effective for *Kaphaja* disorders and other conditions like *Prameha* and *Sthoulya* [3]. Utility of *Madhu* is based on its unique quality called *Yogavahi*, by which it potentiates the efficacy of *Dravyas* used along with it either in the form of *Samyoga* or *Samskara* [4]. Indication as well as contraindication for utilization of heated honey is described in classical literature and sometimes in same books. While explaining *Ama* & *Pakwa Madhu Lakshana*, *Acharya Kaiyadeva* attributes different modalities of *Madhupaka* to achieve specific outcomes and one among such modality is processing honey with *Triphala Kashaya* [5].

Honey sold in the market is supposed to be processed (thermal treated) to increase its shelf life. Honey processing is commonly employed method for its commercial point of view. Processed honey is said to be more

stable with respect to its colour and other physico-chemical parameters. Pasteurization is also said to be another advantage of processing [6]. Neutralization of proteins/enzymes and destruction of fungal spores due to heating prevents fermentation of honey. Polyphenols and proteins are minor components of honeys. Binding affects function of both proteins and polyphenols. Environmental factors such as pH, temperature, ionic strength often modulate transient interactions toward formation of stable protein-polyphenols complexes with a long half-life [7].

Madhu has been tested experimentally for its anti-obesity and anti-hyperglycemic properties, which show different effects with respect to *Samyoga* and *Samskara*. *Triphala Kashaya Samskarita Madhu* has significant anti-obesity and anti-hyperlipidaemic potential [8] whereas *Samyoga* (unprocessed honey, just mixed with *Triphala Kashaya*) form is more effective in obesity induced hyperglycemia [9]. Changes pertaining to the nutritive and phyto-constituents of honey are thought to be responsible for such different efficacies, which demand detailed physico-chemical and phyto-chemical analysis. Hence present study is intended for analytical evaluation of *Madhu* with special reference to its *Samskara Anuvartana* [10] (Post-processing modulation).

MATERIALS & METHODS

Five litres of Honey was procured from Honey National Society, Marthandam, Nagercoil dist, Tamilnadu and was stored in Food grade plastic container for one year for maturation (as earlier pharmacological studies have been carried out using purana Madhu). Deseeded fruits of Haritaki (*Terminalia* chebula Retz), *Vibhitaki* (*Terminalia bellerica* Retz) and *Amalaki* (*Emblica officinalis* Geartn) were procured from Local market, Mysuru Karnataka and samples were authenticated by the subject expert by comparing with the voucher specimen maintained at *Dravyaguna* Museum, Department of *Dravyaguna*, JSSAMC, Mysuru.

Preparation of trial drug:

Triphala - Haritaki (Terminalia chebula Retz), Vibhitaki (Terminalia bellerica Retz) and Amalaki (Emblica officinalis Geartn) were pounded into coarse powder and mixed in equal proportions to make Triphala Churna [11]. Triphala Kashaya was prepared as per Kashaya Paka vidhi of Sharanghadhara Samhita [12].

Samskara with Triphala Kashaya:

Madhu and Triphala Kashaya were taken in equal proportion and mixed homogeneously and Paka was carried out over low flame (Mandagni) till Paka Lakshanas[13] were observed.

Jala Samskara:

Eight parts of Madhu was mixed with 1 part of water and was heated over Mandagni till volume was reduced to original volume of Madhu.[14]

Madhu was tested for its Physico-chemical parameters in four different forms (Table No 1).

1.Purana Madhu (KM)

2.Jala Samskarita Madhu (JSM)

3.Triphala Kashaya Samskarita Purana Madhu (TSM)

4.Purana Madhu mixed with freshly prepared Triphala Kashaya (No processing). i.e Samyoga (SYM). At the time of analysis, unprocessed Madhu was homogeneously

mixed with freshly prepared *Triphala Kashaya* and further diluted using distilled water as per the requirement of test protocol. *Triphala Kashaya Samskarita Madhu* & *Jala Samskarita Madhu* samples were diluted using only distilled water.

Physico chemical test parameters (Table No 1)		
SL.NO	**NAME OF TEST**	**PROTOCOL**[15]
A. Physico-chemical study of *Triphala* :		
1	Foreign matter	Ayurvedic Pharmacopoeia of India. Appendix Volume- 1
2.	Total ash content	Ayurvedic Pharmacopoeia of India. Appendix Volume- 1
3.	Acid insoluble ash	Ayurvedic Pharmacopoeia of India. Appendix Volume- 1
4.	Water soluble ash	Ayurvedic Pharmacopoeia of India. Appendix Volume- 1
5.	Extractive value	Ayurvedic Pharmacopoeia of India. Appendix Volume- 1
6.	Moisture content	Ayurvedic Pharmacopoeia of India. Appendix Volume- 1
*Above mentioned tests were conducted in Central Analytical Lab of JSSAMC, Mysuru.		
B. Physico-chemical Profile of *Madhu* :		
7.	Specific Gravity	IS-4941-1994
8.	Moisture content	IS-4941-1994
9.	Acidity expressed as formic acid	IS-4941-1994
10.	HMF (Hydroxy methylfurfural)*	IS-4941-1994
11.	Sucrose	IS-4941-1994
12.	Original Reducing Sugars(ORS)	IS-4941-1994
13.	Total Reducing Sugars(TRS)	IS-4941-1994
14.	Glucose, Fructose ratio	IS-4941-1994
15.	Protein content	IS-7219-1973
16.	Total Phenolic content	Spectro-photometric Folin Ciocalteu method.
17.	Anti-oxidant activity	Spectro-photometric Folin Ciocalteu method.
17. A.	STD Gallic acid IC_{50} value	
17. B.	Honey IC50 value as Gallic acid equivalent.	
*All these tests were conducted at Ganesh consultancy & Analytical Services, Mysuru.		
18.	GEL Electrophoresis	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS- PAGE) [16]
*Conducted at SJCE, Department of Bio-technology, Mysuru.		

OBSERVATIONS AND RESULTS

Pharmacognostical observations of Haritaki, Vibhitaki and Amalaki have been explained in Table 2. Observations regarding organoleptic test of Honey is explained in Table 3. Physico-chemical constants of Triphala are narrated in Table 4. Processed honey was dark brown coloured with more viscous consistency (Image 1). Physico-chemical, Total phenolic content, anti-oxidant assay and Electrophoresis (Image 2) results are mentioned in Table 5.

Table No 2 - Organoleptic tests of *Triphala*

Drugs	Color	Shape	Odour	Taste
Haritaki	Yellowish brown in colour with shiny and smooth texture.	Ovate and wrinkled longitudinally	Odourless	Astringent, slightly bitter and sweetish in the end.
Vibheetaki	Dark brown to black with hairy texture	Globular and obscurely 5 angled	Odourless	Astringent.
Amalaki	Black	Globose, curled pieces of pericarp of dried fruit	Odourless	Sour & Astringent.

Table No 3 - Organoleptic tests of *Madhu*

Drug and Tests	Colour	Odour	Taste
Purana Madhu	Light Brown	Characteristic odour	Sweet
Kashaya Samskarita Madhu	Dark Brown	Characteristic odour	Sweet & Astringent
Jala Samskarita Madhu	Light Brown	Characteristic odour	Sweet

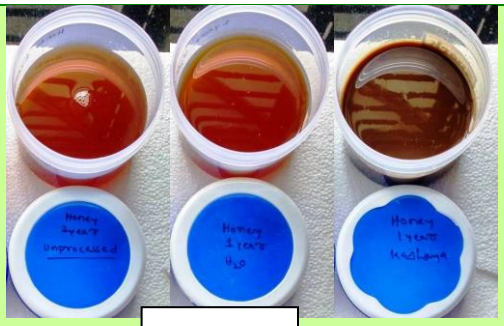

Image 1

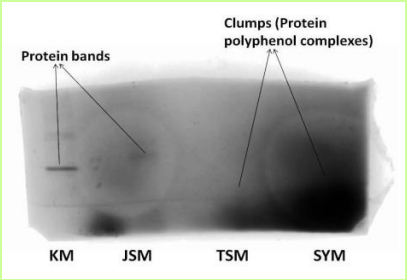

Image 2

Table No 4 - Physico-chemical tests of *triphala*

SL.NO	TEST	RESULTS
1.	Foreign matter	Absent
2.	Total ash	2.96 %
3.	Acid insoluble ash	0.14 %
4.	Water soluble ash	2.04 %

5.	Water extract	39.2%
6.	Moisture content	7.1%

Physico-chemical study of Honey Table No 5

SL. NO	TEST	KM	JSM	TSM	SYM
1.	Specific Gravity	1.3916g/ml	1.3751g/ml	1.3757g/ ml	
2.	Moisture content	21.5%	24.7%	20.7%	
3.	Acidity expressed as formic acid	0.13%	0.11%	1.49%	1.47%
4.	HMF(Hydroxymethyl furfural)	0.92mg/100g	6.87mg/100g	29.24mg/ 100g	15.57mg/ 100g
5.	Sucrose	1.07%	1.92%	5.08%	5.68%
6.	Original Reducing Sugars(ORS)	65.15%	62.03%	55.52%	54.35%
7.	Total Reducing Sugars(TRS)	66.28%	64.05%	60.87%	60.33%
8.	Glucose, Fructose ratio	0.78	0.87	0.06	0.27
9.	Protein content	1.58%	1.44%	1.65%	2.24%
10.	Total Phenolic content	214.39mg/kg	220.04mg/kg	47731.08mg/kg	38260.15 mg/kg
11.	Anti-oxidant activity: Honey IC_{50} value as Gallic acid equivalent	128.00µg	108.90µg	0.12µg	0.072µg

DISCUSSION

Madhu is one among naturally derived food product often regarded for its health benefits as well as usage in lifestyle related conditions like *Sthoulya* [17] and *Prameha* [18]. Both conditions are related with *Medodhatu* [19] and hence needs to be treated with *Purana Madhu*, which is said to possess *Lekhana karma* [20]. Samples selected for the present study was corresponding with the colour specifications of *Makshika Madhu* (*Taila Varna*) [21] as per the literature.

Previous studies conducted by author on anti-hyperlipidemic & anti-hyperglycemic activities of *Madhu* revealed potential benefits with *Purana Madhu* and hence analytical procedures were done after ageing

of *Madhu* for 1 year. Usually *Madhu* is utilized as a food product but the same can be used as *Anupana* along with the *Oushadhi Dravya* to potentiate the activity which is related to Yogavahitva [22]. Such Oushadhi Dravyas may be used either in *Samyoga* or subjected for *Samskara* to obtain specific outcomes. One among such combinations is *Triphala* & *Madhu* having common pharmacological activities such as *Sthoulya* (*Medho dhatu Vruddhi*) & *Prameha* where in the form of *Samskara* & *Samyoga* has been indicated respectively.

Previous studies have validated the concept of *Samyoga* and *Samskara* through experimental & clinical protocol. *Triphala Kashaya Samskarita Madhu* showed significant efficacy in obesity as well as Hyperlipidemia [8] but had very little effect on serum glucose levels where as unprocessed honey mixed with *Triphala Kashaya* exerted potent anti-hyperglycemic [9] potential among STZ induced hyperglycemic rats. Such differences in their respective efficacies are attributed to phyto-chemical, physico-chemical & nutritional aspects of *Madhu* and hence detailed analysis became essential.

Macroscopic & Organoleptic characteristics of *Triphala* were as per the standard observations of contemporary literatures & thus testify the quality of *Triphala*. Colour of *Purana Madhu* was slightly darker than the one which was freshly collected. Observations made during previous studies are suggestive of altered physical & chemical profile after ageing of honey which is mainly related to enzymatic & interactive reactions over sugars, proteins & phenolic compounds [23]. Darker honey [24] samples are often said to have health benefits compared to lighter ones & this justifies utility of *Purana Madhu* in *Chikitsa*.

Dark brown colour & characteristic organoleptic nature of *Kashaya Samskarita Madhu* can be related to added phyto-chemicals through *Triphala Kashaya* & heat effect which brings specific changes in the sugar components. Though many changes were not observed in *Jala Samskarita Madhu*, further alteration in terms, of organoleptic character were ceased. This can be related to enzymatic degradation [25] by heating process.

Specific gravity of all 3 samples were more less similar with slightly high value in KM which may be due to addition of water or *Kashaya*, which must have caused a slight dilution even after condensation process & this observation corresponds with the moisture content where JSM samples having highest moisture content followed by KM & TSM. Since TSM is processed until observation of *Paka Lakshana*, less moisture content is justifiable. Specific gravity is more attributed to the sugar content & in TSM molecules derived from *Kashaya* are also added to *Madhu* & hence might cause slight variation among S.G of KM & TSM.

The pH of honey is generally less than 7 & average pH is about 3.9 where as it can range from 3.4 to 6.1. In determination of acidity of the test samples value is expressed as formic acid constituent which showed higher values in TSM followed by SYM. Honey contains many kinds of organic & amino acids depending on the type of honey and these acids may be aromatic or non-aromatic. *Triphala* contains phenolic acids [26] like Chebuligic acid, Gallic acid and

Egallic acid, which are considered as week acids. Addition of Phenolic acids must have increased the acidity of honey in both *Samskara* & *Samyoga* form. Since both TSM & SYM are prepared using equal amount of *Triphala Kashaya*, acidity also remains similar. Studies have revealed that the potent anti-oxidant molecules like caffeic & gallic acids are not stable in high pH [27] & thus the low pH of honey seems to have a protective role & unimpaired pharmacological activity of phenolic acids can be ensured.

HMF is seen in most of the carbohydrate containing food substances & is said to be one of the causative factor for cancer manifestation. So HMF estimation is one of the quality parameters to ascertain the quality of honey [28]. Prolonged storage as well as heat process elevates HMF content & a range has been fixed to grade the honey samples as a superior (<40mg/100g) & Std (<80mg/100g) grade based on HMF content (FSSAI standards) [29]. All the samples had the HMF content below 40mg/1000g which is suggestive of superior grade of prepared product. TSM had comparatively higher proportions of HMF (29.24mg/100g) which indicates the influence of heating process [30] on HMF production.

All monosaccharides & few disaccharides (except sugars) are considered as reducing sugars but w.r.t honey the sucrose may present in invert form due to presence of invertase enzyme [31]. It is found that storage & heating process elevate invert sugar as reported in the previous works that heating Cane sugar (sucrose) is "inverted" by heating with a food acid [32] to the considerable extent & thus compared to original reducing sugars (mostly monosaccharides).

Usually the glucose fructose ratio among most of the natural products is one or slightly less than one. Less glucose fructose ratio indicates high fructose content which is evident in TSM (0.06) followed by *Samyoga (0.27*). More fructose content is seen to be caused when exposed to heat [33] because the fructose attached to the food can dissolve to the liquid & the glucose in the food can isomerize to fructose thus increasing measurable fructose.

Plant products also contain very less amount of proteins. Slight increase in protein content in *Samyoga* & *Samskarita* samples may be due to addition of proteins through *Triphala* whereas *Samyoga* form has fairly high (2.24%) of protein compared to 1.65% in *Samskarita Madhu* which is suggestive of heat effect & protein polyphenol complex [34] during heating process. This observation is further supported by Electrophoresis where a large clump was observed which could not separate effectively suggesting protein poly- phenol compounds.

Previous studies have established increase in phenolic content [35] after heating process and in the present study the same changes were observed. TSM exhibited highest phenolic content, i.e. 47731.08 mg/kg compared to 38260.15mg/kg in SYM sample. Even in Jala Samskarita Madhu slight increase in phenolic content has been observed.

Anti-oxidant activity is said to protect tissues & cells from oxidative stress & thus have a positive health benefits. It is generally believed that more the TPC (Total phenolic content), higher will be anti-

oxidant potential. Among 4 samples TSM has got highest TPC but when IC_{50} value as Gallic acid equivalent is concerned, anti-oxidant potential is less than that of SYM. The above results are suggestive of relationship between TPC & anti-oxidant activity but are not obvious as it depends on the type of sample [36]. The pool of the phenolic compounds can be low but the total anti-oxidant status can be very high [37]. Heating process though increases the phenolic content cannot exert potent anti-oxidant activity as observed by authors in the present study. Types & characterization of newly formed Phenolic compounds need to be further investigated through LCMS (Liquid chromatography and Mass spectrograph) procedure.

CONCLUSION

Madhu Samskara leads to elevation in fructose content. Heating has a potent role in increasing total phenolic content of *Madhu*. *Samyoga* of *Madhu* with *Triphala Kashaya* exerts the potent anti-oxidant activity compared to *Samskara*. Present study thus establishes that quantitative increase in TPC (Total phenolic content) will not increase anti-oxidant potential in all occasions. Heat effect brings about specific changes in pharmacological activities of honey in terms of anti-oxidant potential.

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