

Comparison of Physical properties of different Propolis Samples from two different regions of India



Agriculture

KEYWORDS : Propolis , physical properties, melting point, solubility, comparison

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ABSTRACT

Variations in chemical composition of propolis may also affect its melting point The melting point of propolis samples collected in Srinagar (J&K) from different colonies, irrespective of period of collection varied between 81.04 to 83.05°C and Samples collected in Coimbatore (TN) varied between 81.92 to 86.63°C. Variations were also observed during different month from two different years. The melting point was 76.00°C of the samples from Srinagar collected in April and 81.31°C of the samples collected from Coimbatore likewise samples collected during March shows melting points 92.44 and 92.90 from two respective places. Such variations may be due to different sources of resin and type of impurities in the propolis samples solubility of propolis in 70% alcohol was more in the samples collected from Srinagar 45.79 to 47.93% and Coimbatore 47.05 to 49.26% using 18 and 26 mesh nylon nets respectively Compared to other traps 33.44 to 39.29 in Srinagar and 34.46 to 40.49 in Coimbatore which is indication of the effect of trapping method on the composition of propolis The solubility of propolis was same whether extracted for 10 or 17 days.

Introduction

Propolis or »bee glue« is a sticky, gummy, resinous product of honeybees (*Apis mellifica* L.) that is accumulated in hives. Bees collect propolis from various leaf buds and cracks in the bark of trees (Burdock; 1998) In the northern hemisphere (Europe, North and South America and western Asia), the tree sources are: *Populus* spp., *Betula* spp., *Ulmus* spp., *Quercus* spp., *Salix* spp., *Aesculus hippocastanum* L., *Picea* spp., *Fraxinus* spp, etc. (English *etal* 1992). These origins may account for the smell, color, constitution, and chemical composition of propolis. Propolis is a very complex mixture and, in general, it is composed of 50% balsams and resins, 30% wax, 10% essential oils, 5% pollen and 5% of various other substances like sugars, vitamins, etc. (Bankova *etal* 2000)

Propolis occurs in the form of a wax-like resin. Its melting point is usually in the 60–70 °C range, sometimes as high as 100 °C. Propolis consists mainly of resins (40–45%), waxes and fatty acids (25–35%), essential oils (10%), pollen (5%), and organic compounds and minerals (5%) (Krell 1996). The composition varies according to geographic location and climate, since vegetation varies with environmental conditions. Propolis from Brazil has attracted the most interest because of its high biological activity.

Materials and Methods

Selection of bee colonies

Eight bee colonies; four from each Srinagar -SRG₄₈, SRG₅₀, SRG₅₅ and SRG₆₀ (J&K) and Coimbatore -COM₄₀, COM₂₀, COM₃₅ and COM₄₅ (TN) were equalised in bee strength and brood area for collecting propolis samples for determining melting point .

To determine Solubility different propolis Traps were devised for collection of different propolis samples, the respective methods used for measuring the physical properties (Melting point and Solubility) of propolis are discussed below.

Determination of melting point of propolis

Melting point of propolis samples collected during different months was determined by capillary tube method (Bahl and Sharma, 1984). Small amount of propolis was taken and put inside a thin walled capillary tube, which was tied to a thermometer with the help of thread and heated in a well stirred wax bath. The temperature (°C) at which propolis began to melt (ini-

tial reading) and at which it liquefied completely (final reading) were recorded. The averages of these initial and final readings of three replicates represented the melting point of propolis sample.

Determination of solubility of propolis

For this purpose the known quantity of propolis (varying between 1-3 g) was weighed and mixed in known volume of 70 per cent ethyl alcohol. One part of propolis was crushed in nine parts of 70 per cent ethanol. This mixture was then placed in a close opaque dark coloured bottle and allowed to stand for 10 and 17 days for standardizing the period of extraction. Once no significant variations were found between the two periods of extraction, further extraction was restricted only to 10 days. The mixture was shaken two or three times a day. On the last day, the mixture was filtered through Whatman No. 01 filter paper with great care so as to obtain the extract. The filtrate was collected whereas, the residue was dried at room temperature and weighed. The residue represents the impurities (wax and mechanical impurities) present in the samples. By recording the differences in weight of propolis taken for extraction and the weight of residue, the percent solubility of propolis was determined. Impurities and extent of solubility of propolis samples taken from colonies having different bee population were also determined in the same way.

RESULTS AND OBSERVATIONS

Melting point of propolis

Melting points of propolis samples collected from different bee colonies during different months in Srinagar (J&K) and Coimbatore (TN) during 2012 and 2013 are shown in Table 01, Fig 01 and Table02, Fig02 respectively. The data revealed that irrespective of month of propolis collection, melting point of propolis samples collected from different colonies ranged between 81.04 to 83.05°C and 81.92 to 86.63°C respectively. Samples of propolis collected from colony number SRG₄₈, SRG₅₀ and SRG₅₅ from Srinagar (J&K) had almost same range of melting point (differences non-significant). However, samples taken from colony SRG₆₀ had significantly less melting point (81.04°C) than those from SRG₄₈ (83.05°C). Likewise Samples of propolis collected from colony number COM₄₀, COM₂₀ and COM₃₅ from Coimbatore (TN) had almost same range of melting point (differences non-significant). However, samples taken from colony COM₄₅ had significantly less melting point (81.92°C) than those

from Com₄₅ (86.63°C), and in comparison melting point of propolis samples taken from Srinagar (J&K) ranges between 81.04 to 83.05°C and Samples taken from Coimbatore (TN) ranges between 81.92 to 86.63°C respectively.

Variations were also observed in the average melting point of propolis collected during different months, irrespective of the colonies. The propolis collected during the month of June from Srinagar (J&K) had minimum average melting point (76.27°C). Whereas it was maximum of 92.44°C in sample collected during March. The average melting point of propolis samples collected during April and May was identical varying non-significantly between 76.00 to 76.88°C. The average melting point of propolis samples collected in March was significantly more (92.44°C) as compared to those of April (76.00°C). Thereafter it was between 76.00 to 84.21°C of the samples collected during April to July (differences significant). The average melting point of the samples collected during July to September was still significantly higher varying between 84.21 to 83.84°C as compared to those of August. Highest average melting point of 92.44°C was observed in samples collected during the month of March, decline significantly thereafter in April to 76.00°C. From the data, it is

also revealed that among the propolis samples collected during different months from different experimental colonies, average minimum melting point was 76.00°C and maximum 92.44°C.

The propolis collected during the month of June from Coimbatore (TN) in comparison with Srinagar (J&K) had average minimum melting point (77.34°C). Whereas it was maximum of 92.90°C in sample collected during March. The average melting point of propolis samples collected during May and June was identical varying non-significantly between 77.34 to 77.87°C. The average melting point of propolis samples collected in March was significantly more (92.90°C) as compared to those of April (81.31°C). Thereafter it was between 77.87 to 85.21°C of the samples collected during May to September (differences significant). The average melting point of the samples collected during July to September was still significantly higher varying between 85.17 to 85.21°C as compared to those of August. Highest average melting point of 92.90°C was observed in samples collected during the month of March, decline significantly thereafter in April to 81.31°C. From the data, it is also revealed that among the propolis samples collected during different months from different experimental colonies, average minimum melting point was 77.34°C and maximum 92.90°C.

Table01: Melting point (°C) of propolis collected from different bee colonies at Srinagar (J&K) during different periods of year-2012.

Bee Colonies	Melting Point(°C) Srinagar (J&K)				
	SRG. ₄₈	SRG. ₅₀	SRG. ₅₅	SRG. ₆₀	Mean
March	93.00(9.64)*	87.32(9.34)	96.15(9.80)	93.30(9.65)	92.44(9.61)
April	74.42(8.62)	76.00(8.71)	75.80(8.70)	77.79(8.82)	76.00(8.71)
May	78.00(8.83)	77.52(8.81)	76.00(8.71)	76.00(8.71)	76.88(8.76)
June	76.51(8.74)	76.00(8.71)	75.85(8.70)	76.71(8.75)	76.27(8.73)
July	85.24(9.23)	85.87(9.26)	81.43(9.02)	84.31(9.18)	84.21(9.17)
August	82.34(9.07)	81.33(9.01)	81.23(9.01)	85.56(9.24)	82.61(9.08)
September	81.29(9.01)	85.55(9.24)	80.88(8.99)	87.66(9.36)	83.84(9.15)
Mean	81.54(9.02)	81.37(9.02)	81.04(9.00)	83.05 (9.11)	
CD _{0.05}	4.67	7.18	6.73	7.37	

Figures in parentheses are Log+1 Transformed values

Fig 01: Melting point (°C) of propolis collected from different bee colonies at Srinagar (J&K) during different periods of year-2012

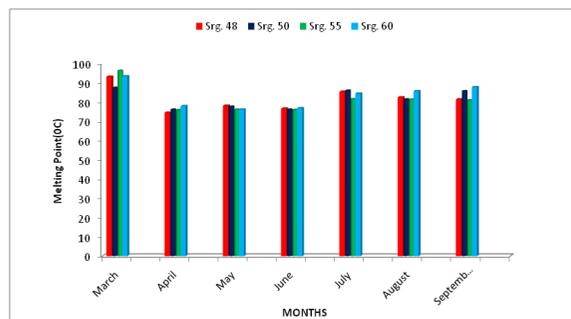


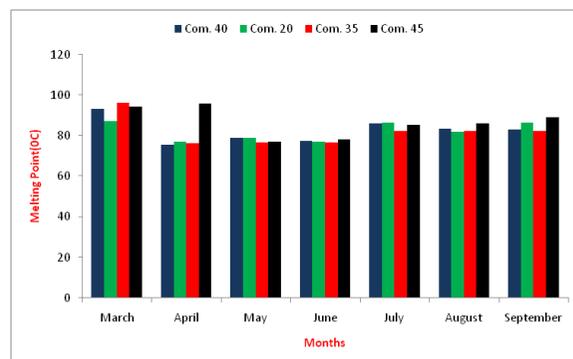
Table 02: Melting point (°C) of propolis collected from different bee colonies at Coimbatore (TN) during different periods of year-2013.

Bee Colonies	Melting Point(°C) Coimbatore (TN)				Mean
	COM. ₄₀	COM. ₂₀	COM. ₃₅	COM. ₄₅	
March	93.33(9.96)*	87.38(9.34)	96.36(9.81)	94.53(9.72)	92.90(9.63)
April	75.76(8.70)	77.00(8.77)	76.50(8.74)	96.00(9.79)	81.31(9.01)
May	79.00(8.88)	78.83(8.87)	76.66(8.75)	77.00(8.77)	77.87(8.82)
June	77.36(8.79)	77.00(8.77)	76.63(8.75)	78.40(8.85)	77.34(8.79)
July	86.16(9.28)	86.61(9.30)	82.56(9.08)	85.33(9.23)	85.17(9.22)
August	83.33(9.12)	82.00(9.05)	82.26(9.06)	86.20(9.28)	83.44(9.13)

September	83.00(9.11)	86.36(9.29)	82.50(9.08)	89.00(9.43)	85.21(9.23)
Mean	82.56(9.06)	82.17(9.08)	81.92(9.05)	86.63(9.30)	
CD _{0.05}	8.684	8.592	9.143	8.756	

Figures in parentheses are Log+1 Transformed values

Fig 02: Melting point (°C) of propolis collected from different bee colonies at Coimbatore (TN) during different periods of year-2013.



Solubility of propolis

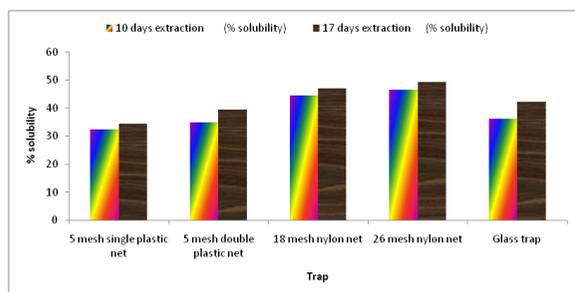
The data recorded on solubility of propolis from Srinagar (J&K) in 70 per cent ethanol (Table 03, Fig03) revealed that irrespective of duration of extraction, the solubility was significant higher of the samples collected using 26 (47.93%) and 18 mesh nylon net trap (45.79%). The solubility of propolis from other three traps varied non significantly between 33.44 to 39.29

per cent. Irrespective of type of propolis trap, per cent solubility was identical in samples extracted for 10 days (38.94%) or 17 days (42.55%) differences being non-significant. Keeping in view both extraction period and trap types, minimum solubility of 32.40 per cent was found of the samples collected using 5 mesh single plastic nets after 10 days of extraction and maximum of 49.31 per cent of the samples collected using 26 mesh nylon net and extracted for 17 days.

Table 03: Extent of solubility(%) of propolis in ethanol (70 %) collected from *Apis mellifera* colonies at Srinagar (J&K) during 2012 using different traps

Trap	Period of Extraction of propolis in ethanol (70 %) Srinagar(J&K)		Mean
	10 daysextraction (% solubility)	17 days extraction (% solubility)	
5 mesh single plastic net	32.40	34.49	33.44
5 mesh double plastic net	35.00	39.55	37.27
18 mesh nylon net	44.50	47.09	45.79
26 mesh nylon net	46.56	49.31	47.93
Glass trap	36.26	42.33	39.29
Mean	38.94	42.55	
CD _{0.05}	8.811	8.988	

Fig03: Extent of solubility (%) of propolis in ethanol (70 %) collected from *Apis mellifera* colonies at Srinagar (J&K) during 2012 using different traps

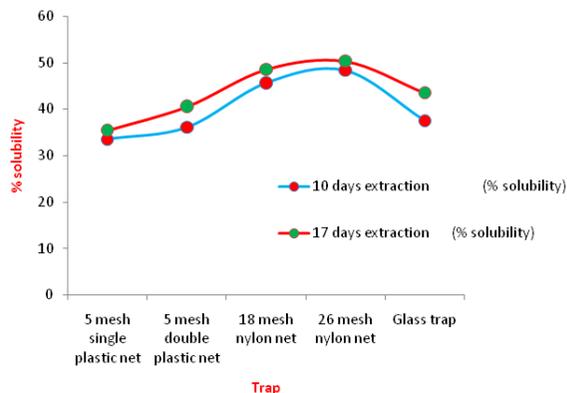


In comparison to the the data recorded on solubility of propolis from Coimbatore (TN) in 70 per cent ethanol (Table04, Fig04) revealed that irrespective of duration of extraction, the solubility was significant higher of the samples collected using 26 (49.26%) and 18 mesh nylon net trap (47.05%). The solubility of propolis from other three traps varied non significantly between 34.46 to 40.49 per cent. Irrespective of type of propolis trap, per cent solubility was identical in samples extracted for 10 days (40.14%) or 17 days (43.68%) differences being non-significant. Keeping in view both extraction period and trap types, minimum solubility of 33.47 per cent was found of the samples collected using 5 mesh single plastic nets after 10 days of extraction and maximum of 50.30 per cent of the samples collected using 26 mesh nylon net and extracted for 17 days.

Table 04: Extent of solubility (%) of propolis in ethanol (70 %) collected from *Apis mellifera* colonies at Coimbatore (TN) during-2013 using different traps

Trap	Period of Extraction of propolis in ethanol (70 %) Coimbatore (TN)		Mean
	10 days extraction (% solubility)	17 days extraction (% solubility)	
5 mesh single plastic net	33.47	35.46	34.46
5 mesh double plastic net	36.00	40.56	38.28
18 mesh nylon net	45.55	48.55	47.05
26 mesh nylon net	48.23	50.30	49.26
Glass trap	37.46	43.53	40.49
Mean	40.14	43.68	
CD _{0.05}	8.43	8.79	

Fig 04: Extent of solubility (%) of propolis in ethanol (70 %) collected from *Apis mellifera* colonies at Coimbatore (TN) during-2013 using different traps



Discussion

Variations in composition of propolis may also affect its melting point. Such variations were visible in the propolis samples collected from different colonies. The melting point of propolis samples collected in Srinagar (J&K) during 2012 from different colonies, irrespective of period of collection varied between 81.04 to 83.05°C and Samples collected in Coimbatore (TN) during 2013 varied between 81.92 to 86.63°C. Variations were also observed during different month from two different years. The melting point was 76.00°C of the samples from Srinagar collected in April and 81.31°C of the samples collected from Coimbatore likewise samples collected during March shows melting points 92.44 and 92.90 from two respective places. Such variations may be due to different sources of resin and type of impurities in the propolis samples. Krell (1996) has generalized that propolis liquefies at 60-70°C but for some sample melting point may be as high as 100°C. The melting point of propolis samples was thus within this range.

The pharmacologically active constituents of propolis are found in fractions in soluble solvents like alcohol (Schmidt and Buchmann, 1992).But beewax and pollen or other contaminants which are of little commercial value are mostly insoluble in such solvents and form the residue. In the present studies solubility of propolis in 70% alcohol was more in the samples collected from Srinagar 45.79 to 47.93% and Coimbatore 47.05 to 49.26% using 18 and 26 mesh nylon net respectively Compared to other traps 33.44 to 39.29 in Srinagar and 34.46 to 40.49 in Coimbatore which is indication of the effect of trapping method on the composition of propolis. Bratkowski and Wilde (2002) have also found that plastic screens were more effective in collecting high quality propolis. Lebedev (2006) reported that in small holed nets, bees fill propolis mixed with less quantity of wax as compare to large holed nets.

The solubility of propolis was same whether extracted for 10 or 17 days. The procedure described by Chi-Fang *et al.* (2002) was followed for extraction of propolis who advocated 8-10 days period for extraction. This is contrary to generalization made by Krell(1996) that the longer propolis is soaked in alcohol more ingredients will be dissolved. He further adds that soaking beyond 2or 3 weeks does not increase the extent of extraction. In the present studies extraction for ten days was sufficient to get the propolis extract. Most of workers have reported solubility of propolis in the range of 31 to 65 per cent (Nieva Moreno *et al.* 2000; Cunha *et al.* 2004). The solubility of propolis is well within this range.

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