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Environmental Impact on Stingless Bee Propolis (*Tetragonula iridipennis)* Reared from Two Different Regions of Tamilnadu – A Comparative Study

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Abstract: Stingless beekeeping is very rare in Tamilnadu. Propolis of Stingless bees is a mixture of plant resins and bee secretions – it possesses a wide spectrum of medicinal properties. The chemical composition and the bio medical applications of stingless bee propolis vary depending on the geographicallocation of the region, itsClimate, season and the availability of the botanical sources from which the bees forage.This present study compares the impact of environment on the Total phenolic,Flavonoid contents and anti-bacterial activity of two stingless bee propolis sample, one reared from Pudukottai region of Tamilnadu and other one reared from the most commercial hub of Chennai region, namely Thyagarayar Nagar. Total polyphenol content of the Chennai sample is 10 µg/ml of Gallic acid equivalent (GAE) and the flavonoid content is 48µg /ml of Quercetin equivalent (QE). This is very low when compared to the pudukottai sample, total poly phenol of which is 150µg/ml of Gallic acid equivalent (GAE) and the flavonoid content is 6mg/g of Quercetin equivalent (QE). This is reflected in their antibacterial activity against different human pathogens also.This study shows the impact of highly polluted environment on the quality of the Chennai propolis sample and reveals that the environment is unsuitable for the existence of Stingless bees.
Key words: Stingless bees, Meliponiculture, Propolis, Phenolic Content, Flavanoid Content, Antibacterial activity.

Introduction

Bees are nature's gift to mankind.Honey bees pollinate nearly 80% of the flowering crops, which constitute one-third of everything we eat. The food cycle mainly relies on the pollination services of these untiring workers. Some of the important species of honey bees are Rock bees, Indian hive bees, little bees, Italian bees and Stingless or Dammer bees. Bee keepers rear Apis family bees like Apiscerana and Apismellifera for the commercial production of honey. Now a days, drastic changes in the climate, excessive

usage of chemical fertilizers and pesticides, global environmental issues pose a great problem to the survival of Honey bees. If they are devasted,we do not have substitute for the work that they only can do.

Honey bees include stinged and stingless. Stingless bees belong to the Apidae family. In Tamil, they are called as Siruthenikkal. They are exclusive to tropical and sub-tropical areas. Their size ranges from 2mm and not more than 5mm and they have non- functional sting[1]. Stingless bee keeping is called Meliponiculture.Honey produced by the Stingless bees has very high therauptic value, but the only drawback is they produce less honey.In an average a Stingless honey bee colony can provide only 600-700g of honey in a year, hence highly prized and is in great demand[2].Products of Stingless bees are highly medicinal because they collect nectar and pollen selectively from medicinally important small herbal plants and flowers such as Coco palm, banana, guava, papaya, mango tamarind, thumba-poo, thengen-boo, touch-me-not plant, jack fruit tree, tulsi, teak etc., They are the most important pollinators of treasured herbal plants. Normally stingless bees built their nest on trunk of trees, logs, wall crevices and under roofs.The unique feature of this nest is its multilayer arrangement[3]. Inside the nest there will be separate chambers for pollen storage, honey storage and brood rearing. These chambers are interconnected and the bees enter through a single opening. The combs are built in a horizontal or vertical pattern in the trunk of the tree or bamboo pole.In modern stingless bee keeping, a wooden structure with single narrow opening is used.

As the Stingless bees nest is rich in precious food resource, there is always adanger of invasion from predators like spiders, flies, wasps, ants, lizards etc. Since stingless bees do not have sting and lacks defense organs, it protects the medicinally important honey by covering the larger holes of the hive with wax like substance and seals the minute pores of the hive using a special type of resinous substance which it creates on its own by mixing its own body secretion from the salivary glands with the resins collected from the leaves, trees, plants, buds etc. This natural resinous substance produced by bee secretion and substances collected from plant parts is called propolis[4]. Due to its waxy nature and mechanical properties, bees use this propolis in the construction and repair of their hives, for sealing the openings and cracks and to smooth the internal walls so as to act as a protective barrier and also to protect against wind and rain[5,6]. Propolis also has a wide spectrum of pharmacological activities such as antibacterial[7-9], antimicrobial[10], antioxidant [11-13], anti-herpes[14], antiulcer[15], antihypertensive[16], anti-inflammatory[17] and also possess anticancer properties [18].The chemical composition and the bio medical applications of stingless bee propolis vary depending on the botanical sources with which the bees forage, the geographical location of the temperate region and its climate. Owing to the pharmacological importance, extensive studies on propolis were made worldwide, over various regions[19-21]. In India, a few studies were reported on the regions of Maharashtra, Karnataka, Gujarat and Uttar Pradesh[22-24]. In Tamilnadu, studies over propolis are scarce[25,26]. So, it is necessary to explore the bio medical uses and composition of propolis of different origin and various regions of Tamilnadu.Extraction of propolis also plays a important role.In this work, the bioactive components of the stingless bee propolis were best extracted by ultrasonication method[27]. Our previous study reported the chemical composition, spectroscopic characterization and bio-medical applications of stingless bee propolis of Pudukottai region in detail[28].

The objective of this study is to examine the physical texture, total phenolic and flavonoid contents of the Stingless bee propolis sample which we reared on our own in the region of Thyagarayar Nagar, Chennai. Its quality is studied and compared with the Stingless bee propolisof Pudukottai region of Tamilnadu through its anti-bacterial activity against certain gram positive and certain gram negative human pathogens.

Materials and methods

Stingless bee propolis collection

A bulk sample of stingless bee propolis was collected from the region of Patti Punkai, Anavayal, Pudukottai District, Tamilnadu. India.

(Sample A)

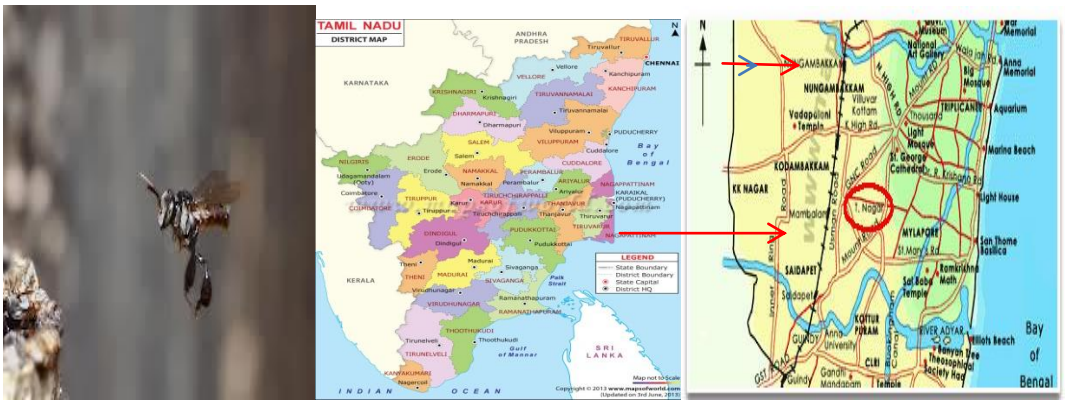
Coordinates: 10.38° N 78.82° E .

Stingless bees were reared and collected from the most commercial hub of Chennai, Thyagaraya Nagar**.**

(Sample B)

Coordinates : 13.08389° N 80.27000° E

The sample was kept in a freezer so that propolis could be handled easily.



Stingless bee District location of Chennai Geographical location of Pudukottai.T.Nagar, Chennai

Ultrasonic extraction of propolis

Instrument: Wensor Ultrasonic bath

20g of propolis was cut in to small pieces and was grounded well. 200 ml of a solvent mixture containing 140ml of ethanol and 60ml of distilled water in the ratio (7:3) was added in small lots with constant stirring. The solution was then filtered through whatman 41 filter paper. For effective extraction, the collected filtrate was subjected to ultra sonication for about three hours.

Determination of total polyphenols

Total polyphenolic content of ethanolic extract of stingless bee propolis was determined using Folici-ocalteu reagent [29]. The extract (100µl) was mixed with 2.5 ml of 1N Folin- Ciocalteu reagent and 2ml of 20% sodium carbonate solution. The mixture was allowed to stand for 15 minutes and then the absorbance was measured at 765nm against blank. Gallic acid was used as the standard. The data obtained were used to find the concentration of total phenol in the test sample by extrapolating the calibration curve obtained by plotting absorbance Vs various concentrations of gallic acid. The total phenolic content was expressed as µg of gallic acid equivalents (GAE) per ml of the extract.

Estimation of total flavonoid

Aluminum chloride colorimetric technique was used for Flavonoids estimation [30]. 0.5 ml of the Sample was mixed with 1.5ml of methanol, 0.1 ml of 10% aluminium chloride,0.1 ml of 1 M Potassium acetate and 2.8ml of distilled water. It was left at room temperature for 30 minutes, after which the absorbance of the reaction mixture was measured at 415 nm with a double beam UV-Visible spectrophotometer. The total flavonoid in the test sample was determined by extrapolating the calibration graph using Quercetin as the standard. Total flavonoid content was expressed in quercetin equivalents (QE).

Assay of antibacterial activity

Preparation of inoculum

Stock cultures were maintained at 4°C on Nutrient Agar Slant. Active cultures for the experiment were prepared by transferring a loop full of culture from the stock cultures into the test tubes containing nutrient broth and were incubated for 24hrs at 37°C.

Agar Disc Diffusion Method

Antibacterial activity of the extract was determined by disc diffusion method on Muller Hinton agar (MHA) medium. Muller Hinton Agar (MHA) medium was poured in to the petri plate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swabs moistened with the bacterial suspension. The discs were placed in MHA plates and 20 µl sample of Concentration 1000µg/ml, 750µg/ml and 500 µg /ml were placed in the disc. 20 µl of Standard ampicillin of concentration 1mg/ml was also placed in the disc. The plates were incubated at 37°C for 24 hrs. The quantification of microbial growth inhibition was

determined by measuring the diameter of clear zones of microbial growth around the wells in the agar. DMSO was used as a negative control.

Results and discussion

Stingless Beekeeping -Meliponiculture

Fig.1.Stingless beekeeping in Wooden box Stingless beekeeping in hollow pipe

Pudukottai regionT. Nagar,Chennai

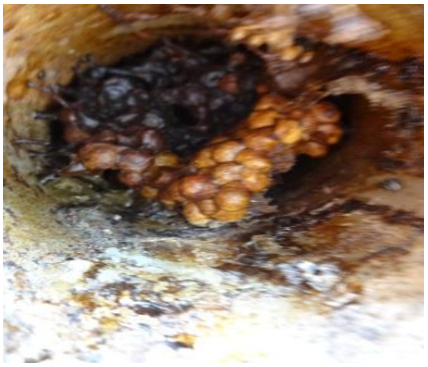


Fig.2.Separate chambers for Pollen, honeypots and propolis **After the removal of propolis Honey pots seen inside and pollens in the front**

Inside of the Colony is seen in **Fig.2**. Yellow colour pollens are collected in separate chamber, Honey pots are seen in separate chamber and propolis were seen and collected from the entrance of the bee hive.

Colour and Odour

The colour and odour of the propolis sample vary from region to region and from country to country. In general the Brazilian propolis appeared green in color and Taiwanese propolisappeared yellowish brown in colour, while colour of the chinesepropolis was dark brown[31]. Some propolis from Argentina, Australia, Brazil, Bulgania, Chile, South Africa, China, U.S, New Zealand and Thailand had a pleasant odour and were light yellow to dark brown in colour[32]. In our study, the sample collected from Pudukottai district of Tamilnadu was light honey brown in colour with a shiny texture looked like a Jaggery with a pleasant odour. The Chennai Sample was dark brown in colour and had no odour.

Fig.3. Propolis sample reared from pudukottai district (Sample A) **Propolis of T. Nagar region (Sample B)**

Effect of Ultrasonics Extraction

Ultrasonics extraction was one of the best method to extract propolis.Ultrasonication was carried out using ultrasonic waves of frequency 40 KHz.Theultrasonicationmechanism is due to acoustic cavitation[33].

Alternating expansive and compression of acoustic waves createbubbles.and it makes the bubbles to oscillate. The oscillating bubbles accumulate ultrasonic energy effectively andgrows. When the bubbles overgrow, they collape releasing the concentrated energy[34,35].This cavitation implosion provides an excellent solvent penetration in to propolis facilitating better extraction of bio- active components of propolis.

Total Phenolic content

The total polyphenol content of the ethanolic extracts of propolis samples A and B asdetermined by Folin-chicauteu's method using Gallic acid as the standard.is given Table.1.

Table 1: Total Poly phenolic content of propolis samples

Region	Amount
Sample A	150µg/ml GAE
Sample B	10µg/ml GAE

Total Flavonoid content

The Total flavonoid content of the samples A and B as determined by Aluminium Chloride Coulorimetric method using Quercetin as the standard is given in **Table 2**.

Table 2: Total flavonoid content of propolis Samples

Region	Amount
Sample A	6mg/ml of QE
Sample B	48 µg/ml of QE

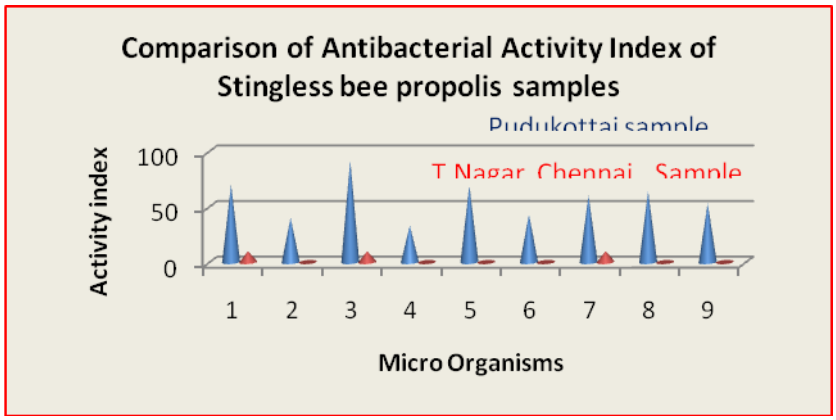
Table 3: Antimicrobial activity Samples A and B and zone of inhibition at various concentrations

			Zone of inhibition(mm)					Std
			Concentration (µg/ml)					
			500	750		1000		
No	Organism	A	B	A	B	A	B	
1	E.Coli (gram –ve)	4 ±0.05	-	5 ± 0.01	-	7 ±0.4	1	10
2	Staphylococcus (gram +ve)	1±0.03	-	4 ±0.07	-	6±0.2	-	15
3	Vibrio spp (gram –ve)	5±0.1	-	9 ±0.02	-	12±0.3	1	13
4	Vibrio parahaemolytics (gram +ve)	1±0.06	-	3 ±0.03	-	5 ±0.01	-	15
5	Salmonella (gram –ve)	5±0.05	-	6 ±0.4	-	9 ±0.06	-	13
6	Bacillus (gram +ve)	1±0.01	-	3 ±0.7	-	6 ±0.03	-	14
7	Aeromonas (gram –ve)	1±0.03	-	3 ±0.2	-	6 ±0.04	1	10
8	Klebsiella	2±0.2	-	4 ±0.01	-	7 ±0.05	-	11
9	Proteus spp (gram –ve)	4±007	-	6 ±0.3	-	7 ±0.02	-	13

From the results it is seen that the quality of Pudukottai District is much better than Thyagaraya Nagar, Chennai sample. Even though the Chennai bee keeping was purposely made near greeneries, the Poor quality of Chennai sample indicates that the environment prevailing in Thyagaraya Nagar,Chennai was not conducive for the survival of Stingless bees. The samples were tested for their anti-bacterial activity against certain gram +ve bacteria viz., Staphylococcus, Vibrio parahaemolytics, Bacillus and certain gram –ve bacteria namely E.Coli, Vibrio spp, Salmonella, Aeromonas, Klebsiella, proteusspp etc. The results of the antimicrobial activity at various concentrations 500 µg/ml, 750 µg/ml and 1000 µg/ml are shown in **Table 3**.

Comparing the results of the antimicrobial activity of stingless bee propolis samples with the antimicrobial activity of the standard ampicillin antibiotic at 1mg/ml concentration, antimicrobial activity Indices of the sample against the above mentioned human pathogens are calculated.

$$\text{Activity index} = \frac{\text{Activity of the sample}}{\text{Activity of the standard}} \times 100$$



From the anti- bacterial activity, stingless bee propolis sample reared from pudukottai district of Tamilnadu showed maximum sensitivity towards gram negative bacteria Vibrio spp, and least sensitivity towards Vibrio parahaemolytic which is gram positive bacteria. But Thyagaranagar sample showed only trace activity against E.Coli, Vibrio spp, Aeromonassppand showed almost nil activity for other microbes.This is due to highly polluted environment where the pollution level reported by the pollution control board is far beyond the permissible level and is entering in to danger zone day by day due to various commercial activities in the region. The results of this study reveals the effect of pollution on the quality of these small creatures that do untiring service to mankind.

Conclusion

This is the study of first kind in Chennai, which assessed the quality of stingless bee propolis reared from the most commercial hub of Chennai, popularly called as T.Nagar. High level of pollution than the permissible level is reflected inits almostnil antibacterial activity against various microbes.When compared to T.Nagar, Chennai sample, stingless bee propolissample of Pudukottai region is found to be better in its quality as well as in its antibacterial activity. It is necessary to provide proper environment for conserving these species as stinglee bees are one of the most important indigenous pollinators of the tropical region and to revive the traditional art of Meliponiculture, through the self employment group of Tamilnadu, as the products of Stingless bees are in great demand in export market.

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