Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences
ISSN- 0975-1491 Vol 6, Issue 8, 2014

Original Article

EVALUATION OF ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF STINGLESS BEE PROPOLIS (TETRAGONULA IRIDIPENNIS) OF TAMILNADU, INDIA

S. KOTHAI[1], B. JAYANTHI[2*]

[1]Department of Chemistry, Ethiraj College for Women (Autonomous), Chennai 8, Tamilnadu, India, [2*]Department of Chemistry, A.M.Jain College, Meenambakkam, Chennai 114, Tamilnadu, India.
Email: jayvarth@gmail.com

Received: 12 Jun 2014 Revised and Accepted: 18 Jul 2014

ABSTRACT

Objective: Propolis is a mixture of plant resins and bee secretions. This study is to evaluate the

antioxidant and antimicrobial activity of stingless bee propolis (Tetragonulairidipennis), rearedfromPudukottai region of Tamilnadu, India and also to determine the total phenol and total flavonoid contents of the sample responsible for these properties.

Methods: Stingless bee propolis was extracted by ultra sonication method and was characterized by UV-Visible, FT-IR and SEM analysis. Total Phenolic and flavonoid contents were determined by using Folin-Ciocalteu spectrophotometric method and Aluminium chloride colorimetric method respectively. DPPH radical scavenging assay was used to find the antioxidant activity of the sample. Antibacterial activity was determined by using standard agar well diffusion method.

Results: Total polyphenol content of the sample was 150µg/ml of Gallic acid equivalent (GAE) and the flavonoid content was 6mg/g of Quercetin equivalent (QE). The antioxidant potential of stingless bee propolis is found to be 83 %. The sample showed significant antimicrobial activity against various human pathogens.

Conclusion: Stingless bee propolis (Tetragonulairidipennis) collected from Pudukottairegion, Tamilnadu is a potential natural antioxidant source and is a promising antimicrobial drug for various bacterial infections.

Keywords: Stingless bee propolis, Tetragonulairidipennis, Sirutheni, Ultrasonic extraction, Polyphenols, Flavonoids, Antioxidant activity, Antimicrobial activity, Activity index.

INTRODUCTION

Beekeeping involving several species of native honey bees is a very important enterprise with a long tradition. Honey bees include stinged and sting-less. Stingless bees belong to the Apidae family. They are exclusive to tropical and sub-tropical areas. Their size ranges from 2mm, and not more than 5mm and they have no stinger [1]. In Tamil, these are called siruthenikkal. They are reared for harvesting small quantities of highly prized medical honey, wax and propolis.

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. Since stingless bees do not have sting and lacks defense organs, it protects the medicinally important rich food resources 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 [2-4].

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 against external invaders such as spiders, flies, wasps, ants, lizards etc., and also to protect against wind and rain [5,6].Propolis has a wide spectrum of pharmacological activities such as antibacterial [7-9],antimicrobial [10], antioxidant [11-14], anti-herpes [15], antiulcer [16], antihypertensive [17],anti-inflammatory [18] and also possess anticancer properties [19].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. Method of extraction of Propolis also plays a role. Various methods of extraction such as Maceration method, Microwave assisted extraction method and Ultrasonic extraction method were reported [22]. Compared to other traditional methods, ultrasonic irradiation (sound waves having frequency in the range of 20 KHz to 10 MHz) provides an unusual reaction condition [23- 25]. Ultrasonic waves do not directly interact with the components, because acoustic wave lengths are much larger than molecular interactions.

Thus, no direct molecular level interaction takes place between ultrasound waves and chemical compounds. When liquid samples are irradiated with ultrasound, the alternating expansive and compressive acoustic waves create bubbles and make the bubbles to oscillate. The oscillating bubbles can accumulate ultrasonic energy effectively and grows to a certain size. Under right conditions, the bubbles overgrow and subsequently collapse releasing the concentrated energy stored in the bubble within short time [26, 27]. This cavitation implosion (bubble collapse) provides an excellent solvent penetration in to the propolis sample and releases the bioactive components in to solvent medium quickly. Owing to the pharmacological importance, extensive studies on propolis were made worldwide, over various regions [28-30]. In India, a few studies were reported onthe regions of Maharashtra, Karnataka, Gujarat and Uttar Pradesh [31-33]. In Tamilnadu, studies over propolis are scarce. So, it is necessary to explore the bio medical uses and composition of propolis of different origin and various regions of Tamilnadu [34]. In this study, ultrasound intensification technique wasused to extract the stingless bee propolis collected from Pudukottai region of Tamilnadu, India. The total phenolics and flavonoids present in the sample were determined and the sample was also evaluated for its antioxidant activity and antimicrobial activity against various human pathogens.

1) Stingless bee (Tetragonulairidipennis) 2) Foraging on medicinal plant 3) stingless bee keeping. 4&5) Colony under study 6) stingless bee propolis taken for study

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.

Coordinates: 10.38° N 78.82° E

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

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.

To understand the chemical nature of the sample and to know the effect of ultra sonication, the ultrasonicated ethanolic extract of stingless bee propolis (USEESBP) was characterized by UV-VIS, FT-IR and SEM analysis.

UV-Visible Spectral Analysis

Sample: The sample (0.5ml) was diluted with double distilled water to avoid errors due to high optical density of the solution.

Instrument: Shimadzu UV 1650pc Spectrophotometer.

Scanning Range:200nm to 500nm.

Fourier Transform Infrared Spectral Analysis (FT-IR)

Sample: The sample was grinded with KBr pellets, dried in infrared light and then subjected to FT-IR measurement.

Instrument: Shimadzu FT-IR Spectrophotometer.

Spectrum Rang**e:** Spectrum was recorded in the range of 4000-400cm^{-1}.

SEM Analysis

A thin film of the sample was prepared on a carbon coated grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid was allowed to dry by placing it under mercury lamp for 5 minutes.

Determination of total polyphenols

Total polyphenolic content of ethanolic extract of stingless bee propolis was determined using Folin-ciocalteu reagent [35].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 inµg of gallic acid equivalents (GAE) per ml of the extract.

Estimation of total flavonoid

Aluminum chloride colorimetric technique was used for Flavonoids estimation [36]. 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).

Evaluation of antioxidant activity

1,1–diphenyl-2-picryl hydrazyl radical (DPPH) was used for the determination of free radical scavenging activity of the USEESBP sample. BHT (Butyl Hydroxyl Toluene)was used as standard (Sigma-Aldrich, MO, USA). The experiment was performed in triplicates. 100µl of ultra sonicated ethanolic extract of stingless bee Propolis was added to three tubes containing 3.7ml of absolute methanol. To this 200µl of DPPH reagent was added and the reaction mixture was kept at room temperature for 30 minutes. The absorbance of the reaction mixture was recorded at 517nm using a UV-visible spectrophotometer. Same procedure was done for the standard BHT and its absorbance was also measured.

$$\% \text{ Antioxidant activity} = \frac{(\text{Absorbance of blank}) - (\text{Absorbance of test})}{\text{Absorbance of blank}} \times 100$$

Assay of antibacterial activity

Preparation of inoculum

Stock cultures were maintained at 4°C on Nutrient Agar Slant. Active cultures forthe 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 negative control.

RESULTS AND DISCUSSION

FTIR measurement was carried out to identify the possible biomolecules responsible for the pharmacological activities of stingless bee propolis sample. Peaks at 3423, 3412, 3379 cm^{-1} corresponds to hydrogen bonded O-H, N-H, C-H stretching vibrations of alcohols, phenols, amides and alkanes. A Peak at 2929 cm^{-1} indicates the presence of very strong C-H stretching vibrations of aromatic rings, methylene, methyl and O-H group of acids. The peak at 2664cm^{-1} corresponds to symmetric stretching vibration of N-H bond.

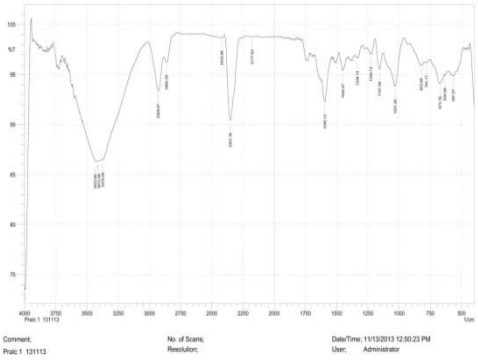


Fig. 1: FT-IR spectrum of stingless bee propolis sample

The peak 1595 cm^{-1} corresponds to asymmetric C=O frequency and to the overtone frequency of N-H bonds. The Peak at 1450cm^{-1} corresponds to asymmetric C=O, C-N of amines. The Peak at 1337cm^{-1} indicates the presence of symmetric C=O, Symmetric C-H, N=O bonds. Peak at 1031cm^{-1} corresponds to fundamental C-C bond peaks. In general FT-IR Spectrum of the sample shows the presence of fundamental vibrations such as aromatic C-C, C-H, C-O, N-H and O-H bonds[38] which correspond to the presence of alcohols, acids, esters and aliphatic amines. These groups are responsible for the biological activity of the sample.

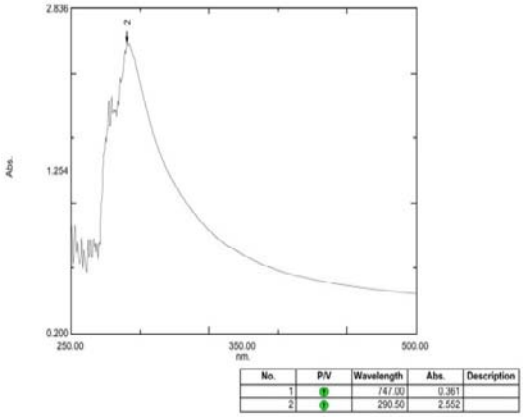


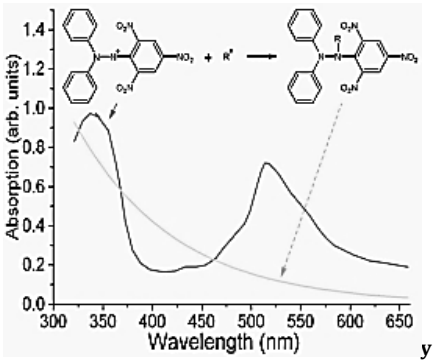
Fig. 2: UV-absorption spectra of stingless bee propolis

A strong absorption peak at 290.50nmis attributed to the presence of phenols and flavonoids. The higher absorbance value indicates the rich composition of polyphenols and flavonoids in the sample. From the absorbance value, quality of the stingless bee propolis sample can be assessed[6]. The total polyphenol content in the sample was determined by Folin-Ciocalteu's method using Gallic acid as the standard[35] and the total Flavonoid content in the sample was determined by Aluminium Chloride Colorimetric method using Quercetin as the standard[36]. The results of the study are given in Table 1.

Table 1: Total phenolic and flavonoid contents

Content	Amount
Total phenolic content	150µg/ml GAE
Total flavonoid content	6mg/g QE

A positive correlation was observed between the total phenolic and flavonoid contents of the sample with its free radical scavenging activity. The antioxidant potential of the sample was assessed by its DPPH radical scavenging ability. DPPH is a stable free radical compound, deep violet in colour and shows an absorbance maximum at 517nm. When DPPH is added to the extract, it gets reduced and a free radical is formed from the scavenger. The reaction of DPPH is monitored by measuring the decrease in absorbance of its radical at 517nm.Upon reduction of this radical by an antioxidant, the absorbance at 517nm disappears. The antioxidant activity of the standard BHT (Butyl Hydroxy Toluene) was also measured. The results of the study are given in Table 2.

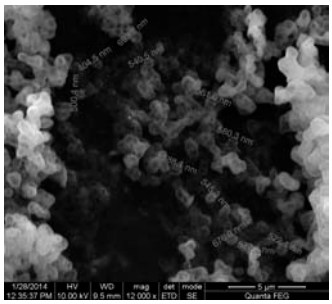


y

Table 2: Antioxidant activity assay

Sample	OD	% DPPH
Ultrasonicatedethanolic extract of stingless bee propolis (Conc -1mg)	0.33	82.06
	0.31	83.15
	0.31	83.15
Standard (BHT) (Conc-1mg)	0.013	99.29
	0.014	99.23
Blank OD is 1.84	0.013	99.29

The results of the antioxidant assay, showed that the ethanolic extract of stingless bee propolis sample has an antioxidant potential of 83%.Thus, the stingless bee propolis extract is a potential natural antioxidant source and can be used to treat diseases associated with oxidative stress.



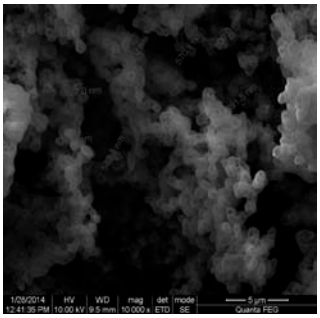


Fig. 3: SEM images stingless bee propolis sample

The effect of ultrasonication is seen from the surface morphology. The bio active components are found to be in the nano regime size. The average size is 550nm.Nano sized bio active components could interact more with themicro-organism and can exhibit enhanced biological activity. Thus, extraction procedure also plays an important role. Antibiotic resistance among microbes urgently necessitates the development of novel antimicrobial agents, especially from safe, harmless natural products.

The antibacterial activity of ultrasonicated ethanolic extract of stingless bee propolis sample was tested against certain gram +ve bacteria viz., Staphylococcus, Vibrioparahaemolytics, Bacillus and certaingram–vebacteria namely E.Coli, Vibriospp, Salmonella, Aeromonas, Klebsiella, proteusspp etc. The results of the anti-microbial activity at various concentrations 500 µg/ml, 750µg/ml and 1000µg/ml are shown in **Table 3**. Among these concentration, maximum zone of inhibition was observed for all the microbes at 1000 µg/ml or 1mg/mlconcentration level.The results are expressed as Mean ±S.D where n=3.

Table 3: Antimicrobial activity at various concentrations

S. No.	Organism	Zone of inhibition(mm) Concentration (µg/ml) 500	750	1000	Antibiotic ampicillin 1mg/ml	DMSO (20 µl)
1	E.Coli (gram –ve)	4 ±0.05	5±0.01	7 ±0.4	10±0.51	--
2	Staphylococcus (gram +ve)	1±0.03	4 ±0.07	6±0.2	15±0.18	--
3	Vibrio spp (gram –ve)	5±0.1	9 ±0.02	12±0.3	13±0.07	--
4	Vibrio parahaemolytics(gram +ve)	1±0.06	3 ±0.03	5 ±0.01	15±0.02	--
5	Salmonella (gram –ve)	5±0.05	6 ±0.4	9 ±0.06	13±0.09	--
6	Bacillus (gram +ve	1±0.01	3±0.7	6 ±0.90	14±0.65	--
7	Aero monas (gram –ve)	1±0.03	3±0.2	6 ±0.04	10±0.57	--
8	Klebsiellaspp(gram –ve)	2±0.2	4±0.01	7 ±0.05	11±0.03	--
9	Proteus spp (gram –ve)	4±007	6±0.3	7 ±0.02	13±0.05	--

Comparing the results of the antimicrobial activity of stingless bee propolis sample 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.

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

From the above graph, it is seen that the stingless bee propolis sample shows maximum sensitivity towards gram negative bacteria Vibrio spp, almost equivalent to chemical antibiotic ampicillin and shows the least sensitivity towards Vibrio Parahaemolytic, which is a gram positive bacteria. In general, the results of the above study showed that, the Stingless bee propolis reared from Pudukottai region of Tamilnadu was found to be more active against gram –ve bacteria than gram positive bacteria Since the antibacterial effect towards all the organism increased with increase in the concentration of the sample, at slightly higher concentration level, the natural extract of stingless bee propolis sample can be a very good substitute for ampicillin antibiotic.

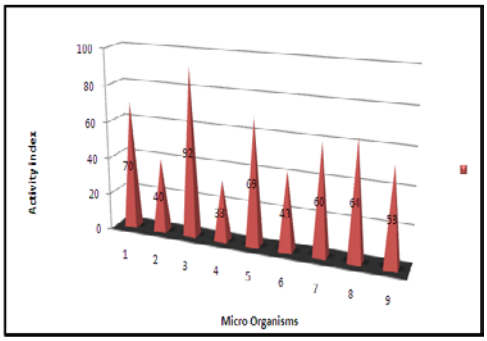


Fig. 4: Antibacterial activity Index against pathogens

CONCLUSION

To our knowledge, this is the first study, which gives a detailed report on the Total phenolic and flavonoids contents, spectroscopic

characterization, antioxidant and antibacterial activities of stingless bee propolis (Tetragonulairidipennis) sample, extracted by a sophisticated process of ultrasonication. This study has combined the advantages of both sono chemistry and green chemistry. We found that the stingless bee propolis reared from Pudukottai region of Tamilnadu is a potential natural source of antioxidant and is a promising antimicrobial agent.

CONFLICT OF INTERESTS

Authors declare that there is no conflict of interest.

ACKNOWLEDGEMENT

We are thankful to Thiru. Tamilselvan, Anavayal, Pudukottai, Tamilnadu for providing a rare stingless bee propolis sample. We are also thankful to the Management of both Ethiraj College for Women (Autonomous)Chennai-8, and A.M. Jain College, chennai-114 for their support in completing this study. A Special thanks to Instrumentation Centre of Ethiraj College for the instrumental facilities provided to us.

REFERENCES

1. Suresh Kumar M, Ranjit Singh AJA, Alagumuthu G. Traditional beekeeping of stingless bee (Trigon sp) by Kani tribes of Western Ghats, Tamilnadu, India. Indian J of Traditional Knowledge 2012;11(2):342-5.
2. Claus Rasmussen. Stinglessbees (Hymenoptera: Apidae: Meliponini) of the Indiansubcontinent:Diversity, taxonomy and current status of knowledge. J Zootaxa 2013;3647 (3):401-28.
3. Mathivanan V. Nabi Shah GH, Manzoor M, Mir GM, Selvisabhanayakam. A Review on propolis-As a novel Folkmedicine. Indian J of Sci 2013;2 (30):23-30.
4. Bankova V. Recent trends and important developments in propolis Research. J Evid Based Compl Alt Med 2005;2 (1):29-32.
5. ToretiVC, Sato HH, Pastore GM, Park YK. Recent Progress of propolis for its Biological and Chemical Compositions and its Botanical Origin. J Evid Based Compl Alt Med 2013;2013:697390.
6. Liviu AL, Marghitas, Daniel S Dezmirean, Otillia Bobis. Important Developments in Romanian Proposes Research. J Evid Based Compl Alt Med 2013;2013:159392.
7. Kujumgiev A, TsevetkovaI, Serkedjieva Y, Bankova V, Christov R, Popov S. Antibacterial, antifungalandantiviral activity of propolis of different geographicorigin. J of Ethnopharmacol 1999;64 (3):235-40.
8. Seidall V, Peyfoon E, Waston DG, Rearley J. Comparative study of the antibacterial activity of propolis from different geographical and climatic zones. J Phytother Res 2008;22:1256-63.
9. Preethikalia, Neelima RK, KusumHarjai. Phytochemical screening and antibacterial activity of different extracts of propolis. Indian J of Pharm and Biological Res 2012;3 (6):219-22.
10. Park YK, Koo MH, Abreu JAS, Ikegaki M, Cury JA, Rosalen PL. Antimicrobial activity of propolis on oral microorganisams. J Current Microbiology 1998;36 (1):24-8.
11. LaskarR A, Ismail SK. Roy N, Begum N A. Antioxidant activity of Indian propolis and its chemical constituents. J Food Chem 2010;122:233-7.
12. Alexandra CH, Juliana C, Leticia C, Ivan PA, Ademilson E, PatricaiaV. Composition and antioxidant activity of propolis from three species of Scaptotrigona stingless bees. J of Apisproduct and Apimedical Sci 2009;(2):37-42.
13. Kumazawa S, Hamasaka T, Nakayama T. Antioxidant activity of propolis of various geographic origins. J Food Chem 2004;84(3):329-39.
14. Nagai T, Inoue R, Inoue H, Suzuki N. Preparation and antioxidant properties of water extracts of propolis. J Food Chem 2003;80:29-33.
15. Vynograd N, Vynograd I, Sosnowski Z. A comparative multi centre study of the efficacy of propolis, acyclovir and placeo in the treatment of genital herpes (HSV). Phytomedicine 2000;7(1):1-6.
16. Iyyampillai S, M Kandaswamyand Subramanian S. Antiulcerogenic and ulcer healing effects of Indian propolis in experimental rat ulcer models. J of Apiproduct and Apimedical Sci 2010;(1):21-8.
17. Maruyama H, Sumitou Y, Sakamoto T, Araki Y, Hara H. Antihypertensive effects of flavonoids isolated from brazillian green propolis in spontaneously hypertensive rats. J Biol Pharm Bull 2009;32:1244-50.
18. Mc Lennan SV, Bonner J, Milne S, Chariton A *et al*. The anti-inflammatory agent propolis improves wound healing in a rodent model of experimental diabetes. J Wound Repair Teagen 2008;16:706-13.
19. Kimoto T S, Arai M. Apoptosis and suppression of tumor growth by artepillin C extracted from Braziliznpropolis. J Cancer Detect Prev1998;22:506-15.
20. Massaro F C, Brooks P R, Wallace H W, Nsengiyuma V, Narokai L, Russell F D. Effect of Australian Propolis from stingless bees (TetragonulaCarbonaria) on Pre-Contracted Human and Porcine Isolated Arteries. J PLOS ONE 2013;8(11) 1-10. e81297.
21. Magro Filho O, de Carvalho AC. Application of propolis to dental sockets and skin wounds, J. Nihon Univ. J School Dentistry1990;32(1);4-13.
22. Boryna T, Dorina T, Bankova V. Different extraction methods of biologically active components from propolis:preliminary study. J Chem Cent 2007;1:13.
23. Mason T J, Colings A, Sumel A. Sonic and ultrasonic removal of chemical contaminants from soil in the laboratory and on a large scale. J Ultrason Sonochem 2004;11:205.
24. Gedanken A. Using sonochemistry for the fabrication of nanomaterials. J Ultrasonics Sonochemistry 2004;11(2):47-55.
25. Suslick K S, Bang J H. Applications of ultrasound to the synthesis of Nanostructured materials. J Adv Mater 2010;22:1039-59.
26. Kumar V, Nigam K D P. Process intensification in green synthesis. J Green process syn 2012;1:79-107.
27. Sanpa S, Sutjarittangtham K, Tunkasiri T, Sukum E, Chantawannakul P. Ultrasonic extraction of Thai propolis for antimicrobial and antioxidant properties. J Adv Mater Res 2012;506:371-4.
28. Sforcin J M, Fernandes A, Lopes CA, Bankova V, FunariSR. Seasonal effect on Brazilian propolis and antibacterial activity. J Apitherapy 2000;23(5):314-6.
29. Rebiai A K, Milanex T, Belfar M L. Total polyphenol contents, radical scavenging and cyclic voltammetry of Algerian propolis. Academic Science, Int J of Pharmacy and Pharm Sci 2014;6(1):395-400.
30. Katircioglu H, Mercan N. Antimicrobial activity and chemical compositions of Turkish propolis from different regions. African J of Biotech 2006;5 (11):1151-3.
31. Shivaraje N, Surendra G, Bhushanam M, Ravikumar HG. Antimicrobial activity of propolis of Trigona sp. and ApisMellifera of Karnataka, India. PJMR 2012;2 (2):80-5.
32. Choudhari MK, Punekar SA, Ranade R, Paknikar KM. Antimicrobial activity of stingless bees (Trigon sp.) propolis used in the folk medicine of Western Maharashtra,India. J Ethnopharmacol 2012;141:363-7.
33. Thirugnanasampandan R, Raveedran SB, Jaya Kumar R. Analysis of chemical composition and bio active property of evaluation of indianpropolis. Asianpac J Trop Biomed 2012;2 (8):651-4.
34. Kumar N, Ahmad M, Dang R, Husain A. Antioxidant and antimicrobial activity of propolis from Tamil Nadu Zone. J Med Plant Res 2008;2(12):361-4.
35. McDonald S, Prenzler P.D, Autolovich M, Robards K. Phenolic Content and Antioxidant Activity of Olive Extracts. J Food Chem 2001;73(1):73-84.
36. Chia-Chi C. Ming-Hua Y, Hwei-Mei W, Jiing-Chuan C. Estimation of Total Flavonoid content in propolis by two complementary Colorimetric Methods. J of Food and Drug Analysis 2002;10(3):178-82.
37. Kothai S, Gayathri K, kannappan V, Perumal P T, Mani Megalai K. Identification of Gallstones using Spectroscopic technique. IJCTR 2009;1(3):430-5.
38. G Aruldhas. Chapter Infrared Spectroscopy:Molecular Structure and Spectroscopy. 2ndEdition. New Delhi:PHI Learning Private Ltd;2007. P 176-213.