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Short communication

Propolis: Traditional uses, Phytochemical Composition and Pharmacological Properties

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Abstract

Propolis is a sticky material manufactured by bees to build and protect their hive. Propolis had been used worldwide for years in folk medicine. The aim of this paper is to give information about traditional uses, phytochemical screening and pharmacological studies of propolis in the world by using secondary references such as journals and articles. The traditional uses of propolis include home remedies, toothpastes, mouthwashes, creams, drops, dietary supplement, anti-putrefactive, anti-pyretic agent, antiseptic, wound healing agent, tuberculosis treatment, cold syndrome, treatment of burns, acne, herpes simplex and genitalis, neurodermatitis, anti-fungal activities in ocular and vaginal infections. Phytochemical screening had shown that propolis contains anthraquinones, alkaloids, fatty acids, flavonoids, glycosides, saponins, triterpenes, tannins and volatile oils. Propolis has pharmacological activities such as anti-inflammatory, antibacterial, antitumor, antioxidant and anti-cancer. Based on this article, propolis has many function and can be used in future as an alternative medicine.

Keywords: *Propolis, traditional uses, phytochemical screening, pharmacological studies.*

1. Introduction

A resinous material that bees collect from various plants to protect their hive are called as propolis [1]. Plant sources, geographical area and collecting season are effected the chemical compositions and biological activities of propolis [2,3]. Based on article [4], raw propolis contains resins (50%), waxes (30%), essential oils (10%), pollen (5%) and various organic compounds (5%). Bankova classified propolis in six main types namely: Brazilian green propolis, poplar propolis, birch propolis, Canarian propolis, red propolis and pacific propolis [5]. Raw material of propolis is shown in Figure 1.

Fig. 1: The propolis from stingless bee Heterotrigona itama species collected in UniSZA apiary, Besut, Terengganu, Malaysia.

2. Traditional uses

Propolis currently marketed by the pharmaceutical industry and had been used worldwide for years in folk medicine [6]. Back to c. 300 BC propolis recorded had been use in the form of home remedies, as a dietary supplement, toothpastes, creams, drops and continues until today [7]. Ayurveda has been reported the uses of propolis as an anti-fungal, anti-viral, anti-ulcer, anti-inflammatory, Acupuncture and Homeopathy [8]. Traditionally, propolis was used as an anti-pyretic and anti-putrefactive agent in Egypt. Romans and Greeks used propolis as an antiseptic, wound healing agent and mouth disinfectant. It became popular as an antibacterial agent in 17th and 20th centuries in Europe. Propolis was used for cold syndrome (upper respiratory tract infections, common cold, flu like infections), tuberculosis treatment, as mouthwashes and toothpastes to prevent caries, treat gingivitis and somatitis, anti-fungal activities in ocular and vaginal infections, wound healing, treatment of burns, acne, neurodermatitis, herpes simplex and genitalis in world war II [9].

3. Phytochemical screening

The purpose of qualitative phytochemical screening is to investigate the various classes of natural compounds which are present in the extracts. A number of structural groups was screened amongst which were anthraquinones, alkaloids, fatty acids, flavonoids, glycosides, saponins, triterpenes, tannins,

volatile oils, reducing substances, and coumarines [10]. According to a group of researcher, propolis has over 150 constituents of biochemical contents, such as phenolic, flavonoids, polyphenols, and ketones [11]. Additionally, the chemical composition of propolis is very complex and varies qualitatively and quantitatively; that confirmed by previous study which has mixture of different naturally chemical compounds with over 300 constituents [12]. Mostly, the raw propolis containing of resins (50 %), waxes (30 %), essential and aromatic oils (10 %), pollen (5 %), and various organic compound (5 %) that are vary depending on the diversity of location, plant sources and bee species [5, 13,14] and also plant vegetation, season, and time of collections, type of bees, and the concentration and nature of the solvents used for the extraction [15]. Thus, the composition of the plant of origin determines the chemical composition of propolis.

Most widely studies reported types of antioxidant which were flavonoids and phenolics compounds that have strong inhibitory effect against lipid oxidation by radical scavenging and have anticancer properties also potential to inhibit development of heart disease due to the bioactive compound contents [16]. Additionally, previous study indicated, chemical composition of propolis which depends on its origin, types of resin from the plants in their foraging area, that showed antioxidant properties of propolis by using scavenging of DPPH radical [17] and superoxide anion assay [18]. They claimed natural antioxidants can be phenolics such as flavonoids, phenolic acids, and tocopherols; nitrogen compounds such as amines, alkaloids, amino acids, and derivatives of chlorophyll; or ascorbics and carotenoids [11].

A study by Rosli and co-workers, which were analyse the phytochemical profile of propolis derived from local *T. apicalis* species was subjected to standard analytical methods namely High Performance Liquid Chromatography (HPLC) analysis to analyze its phytochemical profile. Meanwhile, its antioxidant activities of ethanolic extract of propolis by using DPPH radical scavenging assay, total phenolic compound (TPC) and total flavonoid compound (TFC). The results claimed, the presence of caffeic acid, p-coumaric, myricetin, quercetin, naringin, hesperetin, kaempferol, and baicalein using HPLC analysis. The antioxidant activities of propolis extract is dose dependent for DPPH radical scavenging assay. Additionally, correlation values of TPC and TFC against DPPH indicate that the antioxidant activities of propolis extract influenced by flavonoids and phenolics contents [11].

Due to the antioxidant properties of polyphenols, it has a higher potential biochemical activity to help reducing the risk of various diseases such as life-threatening and cardiovascular including anti-cancer activity [10]. Oxidation occurs when an imbalance between reactive oxygen species (ROS) generation and antioxidants occurs which can spread over targeting cells. Mechanisms of antioxidant action included removal or inactivation of oxygen reactive species, suppression of ROS formation and up-regulation or protection of antioxidant defenses. Major classes of phenolic compounds, which were flavonoids and phenolic acids, which have structure-antioxidant activity relationships in lipophilic systems also in aqueous; have a potential for antioxidant effects as hydrogen donating sources or as singlet oxygen quenchers, metal ion chelators, and as free radical scavengers [19]. Phenolic compounds have important role as antioxidants in scavenge free radicals due to the presence of aromatic compounds and hydroxyl substituents [20].

A study by Mendonca and members of the group was done by analysed the phytochemical profile of the ethanolic extract of red propolis (EEP) from brazilian propolis and its fractionation which were hexane, ethyl acetate, chloroform and methanol; antioxidant action of EEP and its fractions hexane, cloroform and ethyl acetate. The result for phytochemical screening showed, presence of flavonols catechins aurones, phlobaphene tannins, chalcones, pentacyclic triterpenoids flavonones, guttiferones and xanthones, Moreover, antioxidant percentages were showed in EEP and its fractions were obtained by liquid-liquid partitioning [21]. The presence of one specific compound of propolis might lead to the expectancy that the extract has the potential to show bioactivities potential in several diseases or anti-cancer properties [3].

3.1. Chromatographic Analysis

The term chromatography refer to the principle where molecules in mixture are separated from each other when it is applied onto surface or into solid and fluid stationary phase (stable phase) while moving with the aid of a mobile phase. The basic components in chromatographic are stationary phase, mobile phase and separated molecules. Chromatographic methods based on partition are used to separate and identify small molecules such as amino acids, carbohydrates, and fatty acids [22]. Gas chromatography-mass spectrometry (GC-MS) is a coupling chromatographic technique which is used to separate gas and liquid phase with the mass spectrometer as detector to identify various compounds presence in a test sample. GC is used to separate volatile and thermally stable compounds while GC-MS fragments the analyte based on its mass for identification of that particular analyte. GC-MS is widely used to analyze several of analyte due to its advantage which is faster analysis, significantly increased range of low volatile and thermally stable samples due to present of mass spectrometer and improved sensitivity of certain compounds that are hard to be identified [23]. Study by Thirugnanasampandan et al [24] had identified the following fatty acid in Indian propolis such as 9- octadecenoic acid, decanoic acid, 9,12 hezadecanoic acid, octadecadienoic acid methyl ester, 1-tetradecanol, octadecanol, 1-dotricontanol and 2,3 epoxy-5, 8-hectadecadien-1-ol. In the study article [25] revealed more than 50 individual compounds in the Mediterrean propolis from Greece which were flavonoids, fatty acids, sugars and 37 diterpenes. There were 20 of the diterpenes that had been identified for the first time that indicated the propolis was rich in diterpenes. The possible source plant for this propolis mainly came from conifer species of the Cupressaceae family due to these propolis samples had ferruginol, oxygenated ferruginol, totarol, totarol derivatives and semperviro which were characteristics from Cupressaceae. The GC analyses of propolis from north-eastern Brazil have identified the following fatty acid: oleic acid, palmitic acid, linoleic acid, and capric acid that were presence in higher concentration for ethanolic extract propolis (EEP) and hexane fraction propolis (H-Fr) [26]. This study was correlated with article [27] that has shown fatty acid was the main constituents in north-eastern Brazilian propolis for both EEP and H-Fr.

Another type of chromatography is high performance liquid chromatography (HPLC). HPLC is a form of liquid chromatography used to separate the compounds present in the sample. For liquid chromatography the choice of detection approach is very important in order for all the compounds to be identified. There are some types of detectors that can be used which are ultra violet (UV), photodiode array (PDA) and mass spectrometer (MS) [28]. HPLC is widely used for qualitative and quantitative analysis. The compound identification can be achieved based on retention time, molecular weight and spectral matching [29]. Previous study by [30] identified all extracts of green propolis from Brazil contained the artepilin C and p-coumaric acid by using HPLC with DAD as a detector. Based on article [31] also mentioned that the artepilin C and p-coumaric acid are the major active compounds in Brazilian propolis. Based on article [32] reported the major constituents in EEP samples by HPLC analysis with PDA and MS detection which are acid, 3,4-dimethoxycinnamic acid, ferulic acid, pinobanksin, pinobanksin 5-methyl ether, pinobanksin 3-acetate, cinnamylideneacetic acid, caffeic acid phenethyl ester, pinocembrin, chrysin, galangin, cinnamyl caffeate, tectochrysin. The propolis in this study was similar to poplar-type propolis because it rich in flavonoids [33]. Another study by [34] identified the pinobanksin-3--acetate as the main flavonoid and caffeic acid as the major phenolic acid in propolis from China (Beijing).

Table 1: Identification of compounds by chromatographic analysis.

Type of propolis	Country	Identified compounds	Type of analyses	References
Indian	India	9- octadecenoic acid, decanoic acid, 9,12 hezadecanoic acid, octadecadienoic acid methyl ester, 1-tetradecanol, octadecanol, 1-dotricontanol and 2,3 epoxy-5, 8-hectadecadien-1-ol	GC-MS	[24]
Mediterrean	Greece	More than 50 individual compounds which were sugars, flavonoids, fatty acids and 37 diterpenes.	GC-MS	[25]
Brazilian	Brazil	Oleic acid, palmitic acid, linoleic acid, and capric acid	GC-MS	[26]
Green	Brazil	Artepilin C and p-coumaric acid	HPLC-DAD	[30]
Poplar-type	China	Acid, 3,4-dimethoxycinnamic acid**,** ferulic acid**,** pinobanksin, pinobanksin 5-methyl ether, pinobanksin 3-acetate, cinnamylideneacetic acid, caffeic acid phenethyl ester, pinocembrin, chrysin, galangin, cinnamyl caffeate, tectochrysin	HPLC-PDA-MS	[32]
China	China	Caffeic acid as major phenolic acid and pinobanksin-3-O-acetate as the main flavonoid	HPLC-PDA-MS	[34]
Adamawa region, Cameroon	Tekel	Anthraquinones, alkaloids, fatty acids, flavonoids, glycosides, saponins, triterpenes, tannins, volatile oils, reducing substances, and coumarines	HPLC	[10]
T. apicalis	Malaysia	Caffeic acid, p-coumaric, myricetin, quercetin, naringin, hesperetin, kaempferol, and baicalein	HPLC	[11]
Brazilian Red	Brazil	Guttiferones, catechins, flavonones, phlobaphene tannins, flavonols, chalcones, pentacyclic, aurones, triterpenoids and xanthones,	LC-Orbitrap-FTMS	[21]

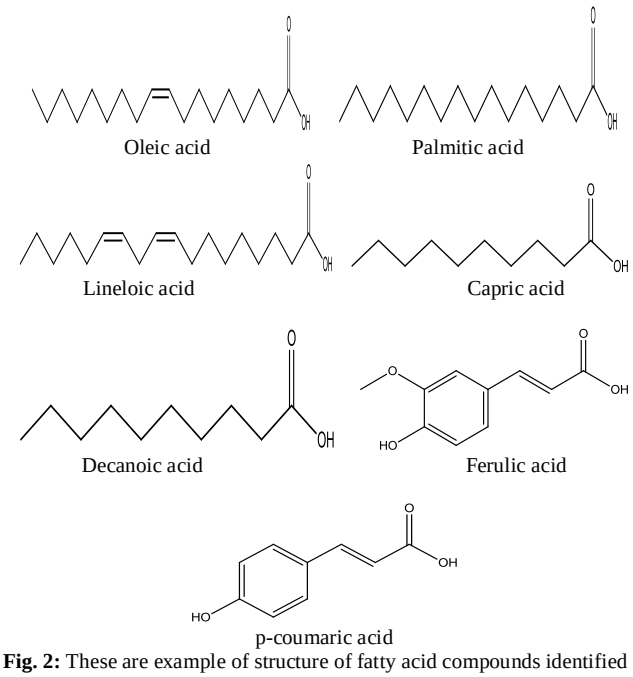

Fig. 2: These are example of structure of fatty acid compounds identified in propolis samples

4. Pharmacological studies

Many studies have proven that propolis have its versatility in pharmacological activities including anti-inflammatory effect, antibacterial activity against a wide range of pathogenic microbes, as well as the anticancer properties [35].

4.1. Anti-Inflammatory Effect

Anti-inflammation activity is the primary defenses carry out by the host to a variety of harmful threats including parasites, pathogen, toxic chemical substances, and physical damage to vascular tissues [36]. Caffeic acid phenethyl ester (CAPE), are the compound that has been recorded to possess anti-inflammatory properties. CAPE had been reported to be a influential inhibitor in T-cell receptor-mediated T-cell activation [37]. It is due the fact that T-cells play an important role in combatting inflammatory diseases. Besides, there are several compound recorded to carry out the same activity (Table 2).

Table 2: The bioactive compounds responsible for anti-inflammatory activity.

Bioactive compound	Propolis origin	References
Apigenin	Israel	[38]
Acacetin	Russia	[38]
Caffeic acid phenethyl ester (CAPE)	Cuba	[5]
Caffeic acid	Israel	[39]
Cinnamic acid	Europe	[38]
Acacetin	Brazil	[39]
Galangin	Cuba	[38]

4.2. Antimicrobial Activity

Propolis reportedly to has a notable and capable of inhibit the growth microorganisms such as bacteria, fungi and protozoa. Various studies have proven that propolis and its extracts have a positive outcome on Gram- positive bacteria yet restraint effect on Gram - negative bacteria. The most popular approach to determine the antibacterial effect of propolis is by using the bacterial susceptibility test whereas the nutrient agar were prepared and inoculated with selected bacteria culture and a propolis blank disc were placed on the top of it. The diameter of growth inhibition zone was measured after optimum incubation period and conditions in order to determine the antibacterial activity. Among of the factors that determine effectiveness of antimicrobial activities include the type of sample, dosage and the type of solvent used in the extraction of propolis [40]. Tables 3 illustrate some studies that show the application of propolis in microbial activity.

Table 3: The bioactive compounds responsible for anti-microbial activity.

Bioactive compound	Propolis origin	References
Lupenyl acetates	Yemen	[41]
Formononetin	Brazil	[42]
Guttiferones	Brazil	[42]
Flavones (apigenin)	Chile	[38]
Isoflavonoids	Brazil	[42]
Chalcone	Brazil	[42]

4.3. Anticancer Activity

Cancer can be classified by its properties whereas it capable of reproducing, undergo cell division and growth besides having the abilities to colonize and invade the territories of normal cells. It is mutated cells that exist in a form of masses of tissue or lumps. Cancerous cells can either be benign or metastasic tumor. A

benign cancer does increase in size yet it not necessarily invading the neighboring cells. As for metastasis cancerous, it has ability to proliferate, invade and colonize the neighboring cells. Studies and research had be done by many on how to combat cancer by analyze deeply on the anticancer activity. The anticancer activity is acts that inhibits halves and prevent the further existence, proliferation and metastasis of cancerous cells from colonize and invading the functional normal cells [43]. Among the many finding and studies on propolis, Artepillin C (3,5-diprenyl-4-hydroxycinnamic acid) had been reported to possess anticancer properties [44]. Besides, CAPE was recognized to have the significant anticancer activity properties [45]. Moreover, flavonoids from propolis had been recognized to have a potent role in anticancer activity [36]. The anticancer activity of propolis in all culture is mainly contributed by their ability to inhibit the DNA synthesis in cancerous cells, the crucial role in inducing apoptosis. Table 4 indicates the bioactive compound that has a potent role in anticancer activity.

Table 4: The bioactive compounds responsible for anticancer activity.

Bioactive compound	Propolis origin	References
CAPE	Germany	[46]
Guttiferones	USA	[42]
Elemicin	Cuba	[47]
Flavonoids	India	[36]
Isofluvonoids (pterocarpans)	Brazil	[48]
Triterpenoids (amyrl acetates)	Brazil	[49]

5. Summary

There are many traditional uses of propolis include home remedies, creams, drops, toothpastes, mouthwashes dietary supplement, anti-putrefactive, anti-pyretic agent, antiseptic, wound healing agent, tuberculosis treatment, cold syndrome, treatment of burns, acne, herpes simplex and genitalis, neurodermatitis, anti-fungal activities in ocular and vaginal infections. Phytochemical screening shown that propolis contains anthraquinones, flavonoids, glycosides, alkaloids, fatty acids, saponins, triterpenes, tannins and volatile oils. Propolis has pharmacological activities such as antibacterial, antitumor, anti-cancer, antioxidant and anti-inflammatory. Based on this article, propolis has many function and can be used in future as an alternative medicine.

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References

[1] Chang, H., Wang, Y., Yin, X., Liu, X. & Xuan, H. (2017) Ethanol extract of propolis and its constituent caffeic acid phenethyl ester inhibit breast cancer cells proliferation in inflammatory microenvironment by inhibiting TLR4 signal pathway and inducing apoptosis an autophagy. *BMC Complementary and Alternative Medicine* 17(471), 1-9.

[2] Popova, M., Bankova, V., Bogdanov, S., Tsvetkova, I., Naydenski, C., Marcazzan, G.L. & Sabatini, A.G. (2007) Chemical characteristic of poplar type propolis of different geographic origin. *Apidologie* 38, 306-311.

[3] Sforcin, J.M. & Bankova, V. (2011) Propolis: Is there a potential for the development of drugs?. *Journal of Ethnopharmacology* 133, 253-260.

[4] Kapare, H., Lohidasan, S., Sinnathambi, A. & Mahadik, K. (2017) Standardization, chemical profiling, *in vitro* cytotoxic effects, *in vivo* anti-carcinogenic potential and biosafety profile of Indian propolis. *Journal of Ayurveda and Integrative Medicine* 30, 1-8.

[5] Bankova, V. (2005) Chemical diversity of propolis and the problem of standardization. *Journal of Ethnopharmacology* 100, 114-117.

[6] Rufatto, L.C., Santos, D.A., Marinho, F., Henriques, J.A., Ely, M.R. & Moura, S. (2017) Red propolis: Chemical composition and pharmacological activity. *Asian Pacific Journal of Tropical Biomedicine* 7(7), 591-598.

[7] Kuropatnicki, A.K., Szliszka, E. & Krol, W. (2013) Historical aspects of propolis research in modern times. *Evidence-Based Complementary and Alternative Medicin*e, 1-11.

[8] Miguel, M.G. & Antunes, M.D. (2011) Is propolis safe as an alternative medicine?. *Journal of Pharmacy & BioAllied Sciences* 3(4), 479-495.

[9] Wagh, V.D. & Borkar, R.D. (2012) Indian propolis: a potential natural antimicrobial and antifungal agent. *Journal of Pharmacy and Pharmaceutical Sciences* 4(4), 12-17.

[10] Talla, E., Tamfu, A.N., Biyanzi, P., Sakava, P., Asobo, F.P., Mbafor, J.T., Tchuenguem, N.F.F. & Ndjouenkeu, R. (2014) Phytochemical screening, antioxidant activity, total polyphenols and flavonoids content of different extracts of propolis from Tekel (Ngaoundal, Adamawa region, Cameroon). *The Journal of Phytopharmacology* 3(5), 321-329.

[11] Rosli, N.L., Roslan, H., Omar, E.A., Mokhtar, N., Abdul, H.N.H. & Asem, N. (2016) Phytochemical analysis and antioxidant activities of *Trigona apicalis* propolis extract. *AIP Conference Proceedings*, 1791, 020018.

[12] Paulino, N., Dantas, A.P., Bankova, V., Longhi, D.T., Scremin, A., De Castro, S.L. & Calixto, J.B (2003) Bulgarian propolis induces analgesic and anti-inflammatory effects in mice and inhibits in vitro contraction of airway smooth muscle. *Journal of Pharmacological Sciences* 93(3), 307-313.

[13] Pietta, P.G., Gardana, C. & Pietta, A.M. (2002) Analytical methods for quality control of propolis. *Fitoterapia* 73(1), 7-20.

[14] Huang, S., Zhang, C.P., Wang, K., Li, G.Q. & Hu, F.L. (2014) Recent Advances in the Chemical Composition of Propolis. *Molecules* 19, 19610-19632.

[15] Mohamed, M., Usman, U.Z. & Abu, B.A.B. (2016) Phytochemical Screening and Comparison of Antioxidant Activity df Water and Ethanol Extract Propolis From Malaysia. *International Journal of Pharmacy and Pharmaceutical Sciences* 8(5), 413-415.

[16] Bankova, V.S., Castro, D. & Marcucci, M. (2000) Recent advances in chemistry and plant origin. *Apidologie* 31, 3-15.

[17] Russo, A., Cardile, V., Sanchez, F., Troncoso, N., Garbarino, A. & Vanellaand, J. (2004) Chilean propolis: antioxidant activity and anti-proliferative action in human tumor cell lines. *Life Science* 76, 545-558.

[18] Izuta, H., Narahara, Y., Shimazawa, M., Mishima, S., Kondo & Hara, H. (2009) 1,1-Diphenyl-2-picrylhydrazyl radical scavenging activity of bee products and their constituents determined by ESR. *Biological and Pharmaceutical Bulletin* 32, 1947-1951.

[19] Erica, W.T., Dejair, M., Giuseppina, N. & Antonio, S.P.C.S. (2008) Seasonal Variation, Chemical Composition and Antioxidant activity of Brazilian Propolis Samples. *eCAM* 7(3), 307-315.

[20] Mihai, C.M., Liviu, A.M., Daniel, S.D. & Lavinia, B. (2011) Correlation between Polyphenolic Profile and Antioxidant Activity of Propolis from Transylvania. *Animal Science and Biotechnologies* 44(2), 100-103.

[21] Mendonça, I.C.G., Moraes, P.I.G.C., Nascimento, T.G., Souza, N.S., Santos, O.J.M., Santos, A.R.E., Mousinho, K.C., Santos, A.F., Basílio, J.I.D., Parolia, A. & Barreto, F.S. (2015) Brazilian red propolis: phytochemical screening, antioxidant activity and effect against cancer cells. *BMC Complementary and Alternative Medicine* 15, 357-369.

[22] Coskun, O. (2016) Separation techniques: chromatography. *Northern clinics of Istanbul* 3(2), 156-160.

[23] Chauhan, A., Goyal, M.K. & Chauhan, P. (2014) GC-MS technique and its analytical applications in science and technology. *Journal of Analytical and Bioanalytical Techniques* 5(6), 1-5.

[24] Thirugnanasampandan, R., Raveendran, S.B. & Jayakumar, R. (2012) Analysis of chemical composition and bioactive property evaluation of Indian propolis. *Asian Pacific Journal of Tropical Biomedicine* 2(8), 651-654.

[25] Popova MK, Graikou K, Chinou I & Bankova VS (2010), GC-MS profiling of diterpene compounds in mediterranean propolis from Greece. J. Agric. Food Chem 58, 3167-3176.

[26] Castro ML, Vilela WR, Zauli RC, Ikegaki M, Rehder VLG, Foglio MA, Alencar SMD & Rosalen PL (2009), Bioassay guided purification of the antimicrobial fraction of a Brazilian propolis from Bahia state. *BMC Complementary and Alternative Medicine* 9(25), 1-6.

[27] Duarte, S., Rosalen, P.L., Hayacibara, M.F., Cury, J.A., Bowen, W.H., Marquis, R.E., Rehder, V.L., Sartoratto, A., Ikegaki, M. & Koo, H. (2006) The influence of a novel propolis on mutans streptococci biofilmes and caries development in rats. *Journal Archives of Oral Biology* 51, 15-22.
[28] Siddiqui, M.R., Al Othman, Z.A. & Rahman, N. (2017) Analytical techniques in pharmaceutical analysis: A review. *Arabian Journal of Chemistry* 10, 1409-1421.
[29] Smith, M., Thompson, K. & Lennard, F. (2017) A literature review of analytical techniques for materials characterisation of painted textiles—Part 2: spectroscopic and chromatographic analytical instrumentation. *Journal of the Institute of Conservation* 40(3), 1-16.
[30] Machado, B.A.S., Silva, R.P.D., de Abreu, B.G., Costa, S.S., Silva DFD, Brandão HN, Rocha JLCD, Dellagostin OA, Henriques JAP, Guez, M.A.U. & Padilha, F.F. (2016) Chemical composition and biological activity of extracts obtained by supercritical extraction and ethanolic extraction of brown, green and red propolis derived from different geographic regions in Brazil. *PLoS One* 11(1), 1-26.
[31] Tazawa, S., Warashina, T. & Noro, T. (2000) On the chemical evaluation of propolis. *Journal of Natural Medicines* 54, 306-313.
[32] Ahn, M.K., Kumazawa, S., Usui, Y., Nakamura, J., Matsuka, M., Zhu, F. & Nakayama, T. (2007) Antioxidant activity and constituents of propolis collected in various areas of China. *Food Chemistry* 101(4), 1383-1392.
[33] Fujimoto, T., Nakamura, J. & Matsuka, M. (2001) Diversity of propolis. Part 1. Propolis from the world. *Honeybee Science* 22, 9-16.
[34] Sun, C., Wu, Z., Wang, Z. & Zhang, H. (2015) Effect of ethanol/water solvents on phenolicprofiles and antioxidant properties of Beijing propolis extracts. *Evidence-based Complementary and Alternative Medicine*, 1-9.
[35] El-Guendouz, S., Al-Waili, N., Aazza, S., Elamine, Y., Zizi, S., Al-Waili, T., Al-Waili, A. & Lyoussi, B. (2017) Antioxidant and diuretic activity of co-administration of Capparis spinosa honey and propolis in comparison to furosemide. *Asian Pacific Journal of Tropical Medicine* 10(10), 974-980.
[36] Wagh, V.D. (2013) Propolis: a wonder bees product and its pharmacological potentials. *Journal of Advances in Pharmacological Sciences* 2013,1-11.
[37] Márquez, N., Sancho, R., Macho, A., Calzado, M.A., Fiebich, B.L., & Muñoz, E. (2004) Caffeic acid phenethyl ester inhibits T-cell activation by targeting both nuclear factor of activated T-cells and NF-κB transcription factors. *Journal of Pharmacology and Experimental Therapeutics* 308(3), 993-1001.
[38] Banskota, A.H., Tezuka, Y. & Adnyana, I.K. (2001) Hepatoprotective and anti-Helicobacter pylori activities of constituents from Brazilian propolis. *Phytomedicine* 8(1), 16-23.
[39] Toreti, V.C., Sato, H.H., Pastore, G.M. & Park, Y.K. (2013) Recent progress of propolis for its biological and chemical compositions and its botanical origin. *Evidence Based Complement Alternative Medical*, 1-13.
[40] Ugur, A. & Arslan, T. (2004) An in vitro study on antimicrobial activity of propolis from Mugla province of Turkey. *Medicinal Food* 7, 90-4.
[41] Ahmad, A.A., Nowfal, I.M., Bayaqoob, A., Rushdi, I., Yehya, A., Bernd, R.T., Simoneit, A.H., El-Mubarak, K. & Al-Mutlaq, F. (2017) Chemical compositions and characteristics of organic compounds in propolis from Yemen Saudi. *Journal of Biological Sciences* 24, 1094-1103.
[42] Almeida, E.T., Delgado, M.C., Oliveira, J.S., Kamiya, R.U., Arruda, R.E., Veiera, D.A., Silva, V.C., Escodro, P.B., Junior, I.D.B. & Nascimento, T.G. (2017) Chemical and microbiological characterization of tinctures and microcapsules loaded with Brazilian red propolis extract. *Journal of Pharmaceutical Analysis* 7(5), 280-287.
[43] Ooi, K.L., Tengku, S.T.M. & Shaida, F.S. (2013) Physalin F from Physalis minima L. triggers apoptosis- based cytotoxic mechanism in T47-D cells through the activation caspase-3- and c-myc-dependent pathways. *Journal of Ethnopharmacology* 150(1), 382-388.
[44] Lotfy, M. (2006) Biological Activity of bee propolis in health and disease. *Asian Pacific Journal of Cancer Prevention* 7(1), 22-31.
[45] Grunberger, D., Banerjee, R., Eisinger, K., Oltz, E.M., Efros, L., Caldwell, M., Estevez, V. & Nakanishi, K. (1988) Preferential cytotoxicity on tumor cells by caffeic acid phenethyl ester isolated from propolis. *Experientia* 44(3), 230-232.
[46] Lin, Y.H., Chiu, J.H., Tseng, W.S., Wong, T.T., Chiou, S.H. & Yen, S.H. (2005) Antiproliferation and radiosensitization of caffeic acid phenethyl ester on human medulloblastoma cells. *Cancer Chemotheraphy and Pharmacology* 57, 525-532.
[47] Cheynier, V. (2012) Phenolic compounds: from plants to foods. *Phytochemistry Reviews* 11, 153-77.
[48] Da Silva, F.C.O., Garcia, C.S.C., Gambato, G., de Souza, M.D.O., Salvador M, Moura S, Padilha FF, Seixas FK, Collares T, Borsuk S., Dellagostin, O.A., Henriques, J.A.P. & Roesch, E.M. (2013) Chemical characterization, antioxidant and cytotoxic activities of Brazilian red propolis. *Food and Chemical Toxicology* 52, 137-142.
[49] De Castro. I.V.F., Negri, G., Salatino, A., Bandeira, M.F.C.L. (2011) A new type of Brazilian propolis: Prenylated benzophenones in propolis from Amazon and effects against cariogenic bacteria. *Food Chemistry* 125, 966-972.