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GC MS – Analysis of Bio Active Compounds from Propolis and Antibacterial Activity against *B.cereus* Isolated from Tasar Silkworm Cadavers

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Abstract
Propolis is a sticky, non toxic, soft resinous substance produced by honey bees. The chemical composition of propolis depends upon the collecting time, location and plant source resulting in the biological activity variation. The aim of this study was to analyze the chemical composition of propolis by using Gas chromatography – Mass spectrometer. Bee propolis is a natural medication applied topically and is considered to have antibacterial, antifungal, antiviral, antibiotic, antioxidant properties and is used for treatment of many infections. Propolis was found to be rich in alkaloids, saponins, tannins and resins. The chemical characterization revealed the presence of 9 distinct phytochemical compounds using GC – MS and the most predominant compound were S Methyl – L – Cysteine (62.36%) followed by Cis – 2,3 – Epoxyoctane (31.78%). The different concentrations of methanolic extracts of propolis showed varied antibacterial activities against *B. cereus* with highest zone of inhibition (19.0 mm).

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INTRODUCTION

The word propolis is Greek in origin "Pro" before, "polis" city of defender of the city entrance. Propolis is a territorially linked national product chemically characterized according to the region of the productions and seasons of the World. The best known primary products of honey keeping are honey wax, propolis, royal jelly, honey bees and their larvae.

Propolis is a mixture of substance used by bees to defend their hive, for filling the wall cavities of the hives, reducing the entrance during cold seasons, preventing the decay, mummification

and defend the intruders. Hence propolis is also known as bee glue.

Propolis is a sticky, non toxic, soft resinous substance which may be white, green, yellow or red colored on the basis of its plant resin source (Brown, 1989). The raw propolis is composed of plant resin (45 – 55%), essential oils (5 – 10%), wax (25 – 35%), aromatic oils (5%), pollen and other natural components (5%) (De Figueiredo *et al*., 2015) The chemical composition of propolis depends upon the collecting time, location and plant source.Hence its biological activity also varies (Wagh, 2013; Toreti *et al*., 2013).

Propolis contains more than 200 constituents, including benzoic acid, flavonoids and cinnamic acid derivatives (Negri *et al.,* 2003; Medana *et al*., 2008; Salatino *et al*., 2005). Propolis collected from temperate regions contains flavonoids, chrysine, ferulic acid, cynnamic acid and caffeic acid. The chemical constituents of propolis and its extracts has shown antimicrobial (Veliko *et al*., 2000; Barrientos *et al*., 2013) anti-inflamatory (Barbaric *et al*., 2011; Cavendish *et al*., 2015) antioxidant (Sawaya *et* al., 2009; Guimaraes *et* al., 2012; Campos *et al.,* 2014) and antifungal properties (Viuda-Martos *et al*., 2008).

Egyptians and Greeks recognized propolis for its healing properties as it is composed of essential oil, resins, phenols, flavonoids and aromatic compounds. Of all the constituents, flavonoids and caffeic acid play an important role in reducing inflammatory response.

Hydro alcoholic extract of Brazilian propolis showed antitumoral activity *invitro* and *invivo* (Pinheiro *et al*., 2014; Ribeiro *et al*., 2015; Lucarini *et al*., 2019). It attracted much as a potential substance in pharmaceutical and cosmetic industry due to its antimicrobial activity and exhibited therapeutical and biological activities which includes anti cancer, anti bacterial and anti fungal properties. Propolis is a chemical weapon of bees against human pathogen, microorganisms and viruses (Kujumgie *et al*., 1999; Bankota *et al*., 2001; Chen *et al*., 2003; Salamao *et al*., 2008).

MATERIALS AND METHODS

Chemicals and Reagents: All the chemicals and reagents used were of analytical grade purity, methanol and nutrient agar used for the experiment were of the highest quality and commercially available from market. *Bacillus cereus* was isolated from the tasar silkworm cadavers that were collected in Kakatiya University campus from the outdoor rearing batches.

Collection of Propolis sample: The propolises were collected from bee hives built on *Terminalia catappa* tree located in Lal Bahadur College, Warangal, Telangana. The hand collected propolises were stored at room temperature until its processing.

Figure 1: The image of crude propolis extract

Extraction procedure: The crude propolis were cut into small pieces and grounded in grinder then sieved to obtain adequate granulometry (approximately 0.250 mm) in order to increase

the surface area and improve the homogenization of the starting material in the extraction process. The sample was stored in air tight bottles and refrigerated until use.

Methanolic extract: The 50 gm of grounded propolis was dissolved in 100 ml of methanol for 3 days. The extract was filtered using Whatmann No.1 filter paper. A rotatory evaporation apparatus was used to remove the solvent and the remaining extracts were stored in bottles in the refrigerator until further use.

Qualitative phytochemical analysis: Phytochemical screening of methanolic extract of propolis *Terminalia catappa* showed alkaloids, phenols, flavonoids, terpenoids, tannin, saponins, essential oils and carbohydrates (Harborne, 1998; Misra *et al*., 2011; Gul *et al*., 2017)**.**

Identification and Quantification of Bioactive compounds using GC – MS

The methanolic extract of propolis was analyzed through GC – MS to determine the chemical profile. The GC – MS analysis was performed using a Shimadzu Qp 2010 Gas Chromatography – Mass Spectroscopy. It employed fused silica column packed with Elite – 5ms (5% Diphenyl 95% Dimethyl poly siloxane, 30 mm x 0.25 mm x 0.25 µm df) and the components were separated using helium as carrier gas at constant flow of 1 ml/min. Then 2 µl sample extract was injected into the instrument it was detected by the turbo gold mass detector with aid of turbo mass 5.2 software. The oven temperature program during the process was maintained with the temperature of 110 ^{0}C with 2 min holding with the injector temperature maintained to 200 ^{0}C and source temperature was 200 ^{0}C. The mass spectra were taken at 70ev, a scan period of 0.5 and fragment from 45 – 450 Da. The MS detection was completed in 36 min. interpretation on mass spectrum GC – MS was conducted using the database of National Institute Standard and Technology (NIST and WILEY) having more than 62,000 patterns (Mohan *et al*., 2016; Prakash *et al*., 2015)**.**

Isolation of bacteria

Bacterial isolation was carried out in sericulture lab, Kakatiya University, the pathogen were collected from whole body of the tasar silkworm cadavers collected from rearing sites (KU) using motor and pestle. The homogenate was filtered, centrifuged for 10 minutes. The supernatant was discarded and bacteria culture pellets were used for further experimentation after re suspending in distilled water (Aneja, 2003).

Antibacterial screenig

Antibacterial activity of propolis was evaluated by paper disc diffusion method using bacterial strain, *Bacillus cereus* using 18 hours culture in 200 ml of nutrient broth at 37 ^{0}C. The cultures were adjusted to approximately 10^{-5} CFO/ml in a sterile saline solution. 28 ml of the nutrient media was poured into sterilize petri dish and solidified at 37 ^{0}C in laminar air flow with no water drop formation to ensure antimicrobial growth. Innoculums from 10^{-3} dilutions were streaked on the solidified agar nutrient media with help of sterile cotton swab under aseptic conditions to study the antibacterial activity of different concentrations of methanolic crude extracts of propolis. 2 to 5 ml of the extracts were coated on sterile filter paper (Whatman's No. 1) measuring 6 mm in size which were incubated under laminar air flow chamber. The extract was tested in triplicate and the trays were incubated at 37^{0}C for 24 hrs to record the inhibition zone and compared with the control (Amphicillin) at four different concentrations of extracts (50 mg/ml, 100mg/ml, 150mg/ml, 200mg/ml) that were tested.

RESULTS

Phytochemical analysis

The phytochemical screening of methanolic extractives of propolis *Terminaliacatappa* contains bioactive compounds such as alkaloids, flavonoids, saponins, phenols, essential oils, tannins, terpenoids as shown in Table 1.

GC – MS analysis

The methanolic extractive of propolis contains maximum number of bioactive compounds determined through GC – MS. The figure – 1 showed full scan gas chromatography of the compound 3 which conforms the presence of

various bioactive compounds with different retention time, peak area and height. The peaks of each compound were obtained from mass spectra as shown in the Table – 2 along with their molecular formula and weight, percentage peak areas. Total 9 compounds were detected in the methanolic extract of *Terminalia catappa* the propolis. The pre dominant compounds were S – Methyl –L- cysteine with retention time (1.472) and peak area (62.36%) followed by cis – 2,3 – Epoxyoctane ranging from retention time (1.383) and peak area (31.78%) respectively. Retention time followed by the other compounds having 28.486 to 1.047 and peak area percentage of 5.33 to 0.01 % respectively.

Table 1: Phytochemical constituents of methanolic extracts of propolis

Sl. No.	Phytochemical constituents	Methanolic extracts of propolis
1	Alkaloids	+
2	Phenols	+
3	Flavonoids	+
4	Terpenoids	+
5	Tannins	+
6	Saponins	+
7	Steroids	+
8	Essential oils	+

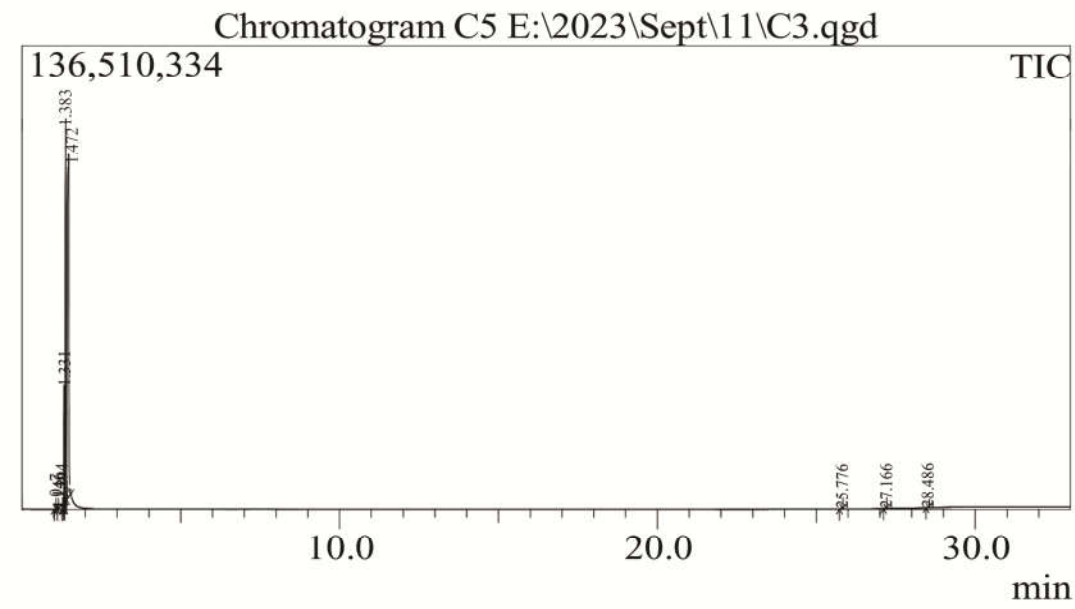

Figure 2: Gas chromatography - Mass spectrometry (GC-MS) chromatogram of significant Propolis compounds

Table 2: GC- MS analysis used to identify bioactive compounds in propolis

Pea	R. Time	Area	Area%	Height	A/H	Base m/z	Name
1	1.047	59753	0.01	73087	0.82	44.05	2-Butynoicacid
2	1.146	85443	0.01	157501	0.54	43.10	Butane,2-methyl-
3	1.294	2238207	0.32	3499241	0.64	43.10	Pentane,2-methyl-
4	1.331	36719060	5.33	43005734	0.85	57.15	Pentane,3-methyl-
5	1.383	218997016	31.78	134555820	1.63	44.85	cis-2,3-Epoxyoctane
6	1.472	429690669	62.36	120259478	3.57	88.95	S-Methyl-l-cysteine
7	25.776	461597	0.07	241419	1.91	57.10	Tetratriacontane
8	27.166	507605	0.07	221293	2.29	57.15	Nonacosane
9	28.486	314678	0.05	157854	1.99	57.10	Docosane
		689074028	100.00	302171427			

Antibacterial activity:-Table – 3 and Figure – 3 shows the antibacterial activity of different concentrations of methanolic extracts of propolis against *B. cereus*

Table 3: Different concentrations of methanolic propolis extracts against Bacillus cereus

Treatments	Concentration (mg/ml)	Inhibition zone (mm)
T_1	50.0	6.5
T_2	100.0	7.0
T_3	150.0	8.5
T_4	200.0	10.5
Control	Amphicillin	5.5

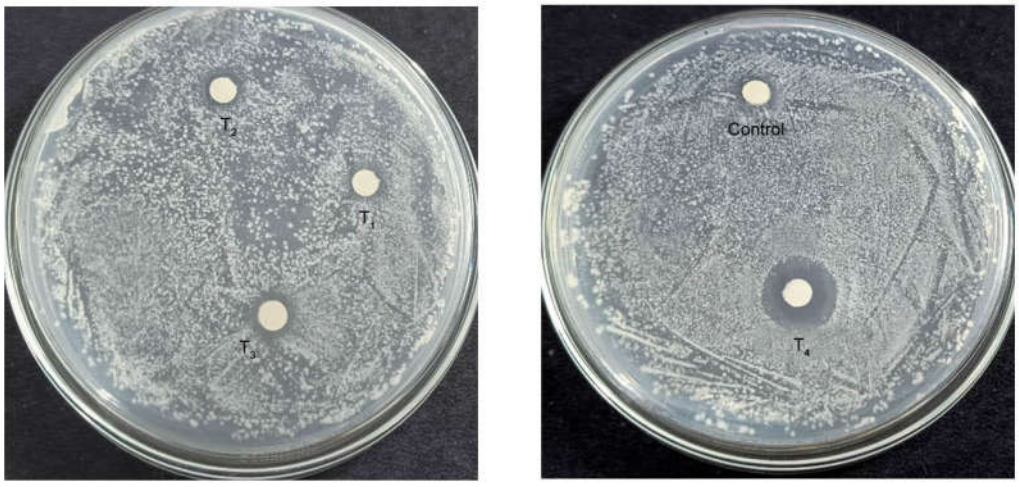

Figure 3: The zone of inhibition (mm) of different concentrations of methanolic extract of Propolis against Bacillus cereus

DISCUSSION

The several active compounds are unique to this extract includes 2 – Butynoic acid, Butane – 2 – methyl pentane – 2 – methyl, Pentane – 3-methyl, Cis – 2,3 – Epoxyoctane, S – Methyl – L – cysteine, Tetra triacontane, Nonacasane, Docosane. Considering the use of propolis extracts in the current study we found the existence of range of bioactive compounds that includes flavonoids, alkaloids, terpenoides, saponins, phenols, essential oils, tannins and carbohydrates which belongs to different groups. Propolis is commercialized globally and it is considered as a significant source of phytochemicals that have many pharmalogical effects (Miyataka *et al*., 1997).

The presence of 9 compounds from various groups was revealed by GC – MS analysis which included many bioactive compounds. In the current study, the antibacterial activity of propolis was studied against *B, cereus*. The methanolic extract of propolis showed highest antibacterial activity due to the presence of flavonoids and phenolics which may have inhibited the microorganisms (Mercan *et al*., 2006).

The results of the present study are consistent with the finding reported for solvent extract of propolis that showed highest antimicrobial activity against *S, aureus* at lower doses (Georgieva *et al*., 2019). The obtained results of the investigated propolis extracts revealed that different concentrations of propolis showed antibacterial activity against the micro organism.

Moreover, EI. Sayed (1999) stated that a mixture of honey and black seeds not only showed anti bacterial activity but also increased silk and egg production.

CONCLUSION

The present study was the first approach to identify the numerous bioactive compounds in propolis found in Warangal area, Telangana by GC – MS analysis and also to assess the antibacterial potency of propolis extract which revealed the presence of 9 bioactive compounds that had wide range of pharmacological potential. Hence can be used as disinfectant for infected cadavers.

REFERENCES

1. Brown R, (1989). Hive products: pollen, propolis and royal jelly. *Bee world* 70, 109 – 117.
2. De Figueiredo, S.M; Binda, N.S.; Almeida, B.D.M.; Abreu, S.R.L.; De Abreu, J.A.S.; Pastore, G.M.; Sato, H.H; Toreti, V.C; Tapia, A.V.; Park, Y.K.; et al, (2015). Green propolis: Thirteen constituents of polar extract and total flavonoids evaluated during six years through RP-HPLC. *Curr. Drug Discov. Technol,* 12, 229 – 239.
3. Wagh, V.D, (2013). Propolis: A wonder bees products and its pharmacological potentials. *Adv Pharmacol. Sci*. 308249 [Cross Ref] [Pub Med].
4. Toreti, V.C; Satu, H.H. Pastore, G.M.; Park, Y.K, (2013). Recent progress of propolis for its biological and chemical compositions and its botanical origin. *Evid Based complement Alternat. Med*. 697390. [Cross Ref].
5. G. Negri, M.L.F. Saltino and A.S Altino, (2003). Green Propolis: unreported constituents and a novel compound from chloroform extracts, *Journal & Apicultural Research,* 42(3), 39 – 41.
6. C. Medana, F. Carbone, R. Aigotti, G. Appendino and C. Baiocchi, (2008). Selective analysis of phenolic compounds in propolis by HPLC – MS / MS *phytochemical analysis*, 19(1), 32 – 39.
7. A. Salatino, E.W Teixeira, G. Negri and D. Message, (2005). Origin and Chemical variation of Brazilian Propolis, *Evidence Based complementary and alternative medicine,* 2(1), 33 – 38.
8. Velikova, M; Bankova, V.; Tsvetkova, I.; Kujumgiev, A.: Marcucci, M.C (2000). Antibacterial ent – Kaurene from Brazilian propolis of native stingless bees. *Fitoterapia,* 71, 693 – 696 [Cross Ref].
9. Barrientos, L.; Herrera, C.L.; Montenegro, G.; Ortega, X.; Veloz, J.; Alvear, M.; Cuevas, A.; Saavedra, N.; Salazar, L.A, (2013). Chemical and botanical characterization of

Chilean propolis and biological activity on carcinogenic bacteria Streptococcus mutans and streptococcus sobrinus. Braz. *J. Microbiol*, 44, 577 – 585 [Cross Ref].

10. Barbaric, M.; Miskovic, K.; Bojic, M.; Loncar, M.B.; Smolcic – Bubalo, A.; Debeljak, Z.; Medic – Saric, M. (2011). Chemical composition of the ethanolicpropolis extracts and its effect on HeLa cells. J. *Ethnopharmacol.* 135, 772 – 778 [Cross Ref].

11. Cavendish, R.L.; De Souza Santos, J.; Neto, R.B.; Paixao, A.O.; Oliveira, J.V.; De Araujo, E.D.; Silva, A.E.B.E.; Thomazzi, S.M.; Cardoso, J.C.; Gomes, M.Z, (2015). Antinociceptive and anti – inflammatory effects of Brazilian red propolis extract and formononetin in rodents. *J. Ethanopharmacol, 173*, 127 – 133 [Cross Ref].

12. Sawaya, A.C.H.F.; Calado, J. C.P.; Santos, L.D.; Marcucci, M.C.; Akatsu, I.P.; Soares, A.E.E.; Adbelnur, P.V; Cunha, I.B.D.S.; Eberlin, M.N, (2009). Composition and anti oxidant activity of propolis from three species of Scaptotrigona stingless bees. *J. Apiprod, Api Med. Sci,* 1, 37 – 42 [Cross Ref].

13. Guimaraes, N.S.; Mello, J.C.; Paiva, J.S.; Bueno, P.C.; Barretta, A.A.; Torquato, R.J.; Nantes, I.L.; Rodrigues. T, (2012). Baccharid-sdracunculifolia, the main source of green propolis, exhibits potent antioxidant activity and prevents oxidative mitochondrial damage. *Food chem. Toxicol,* 50, 1091 – 1097 [Cross Ref].

14. Campos, J.F.; Dos Santos, U.P.; Macorini, L.F.B.; De Melo, A.M.M.F.; Balestieri, J.B.P.; Paredes – Gamero, E.J.; Cardoso, C.A.L.; Souza, K.D.P.; Dos Santos, E.L, (2014). Antimicrobial, antioxidant and cytotoxic activities of propolis from Meliponaorbignyi (Hymenoptera, Apidae). *Food chem. Toxicol,* 65, 347 – 380 [Cross Ref] [Pub Med].

15. Viuda – Martos, M.; Ruiz – Navajas, Y; Fernandez – Lopez, J.; Perez – Alvarez, J.A, (2008). Functional properties of honey, propolis and royal jelly. *J. Food Sci*, 73, 117 – 124 [Cross Ref] [Pub Med].

16. Pinheiro, K.S. Ribeiro, D.R.; Alves, A.V.; Pereira Filho; R.N.; Oliveira, C.R.; Lima, S.O; Reis, F.P.; Cardose, J.C.; Albuquerque – Junior, R.L, (2014). Modulatory activity of Brazilian red propolis on chemically induced dermal carcinogenesis. *Acta Cir Bras,* 29, PP 111 – 117.

17. Ribeiro, D.R, Alves, A.V; des Santas, E.P.; Padilha, F.F.; Gomes, M.Z.; Rabela, A.S.; Cardoso, J.C.; Massarioli, A.P.; de Alencar, S.M; de Albuquerque – Junior, R.L, (2015). Inhibition of DMBA – induced oral Squamos Cells Carcinoma Growth by Brazilian Red propolis in Rodent model. *Basic clin pharm, Toxical,* 117, PP 85 – 95.

18. Lucarini, M; Durazzo, A.; Kiefer, J.; Santini, A.; Lombardi – Baccia, G.; Souta, E.B, Romani, A.; Lampe, A.; Ferrari Nicoli, S.; Gabrielli P; (2019). Grape seeds: Chimatographic profile of fatty acids and phenolic compounds and qualitative analysis by FTIR – ATR spectroscopy. *Foods (Basel, Switzerland)*, *9*(1), 10.

19. A. Kujumgie, I. Tsvetkova, Y. Serkedjieva, V. Bankova, R. Christov and S. Popov, (1999). Antibacterial, antifungal and antiviral activity of propolis of different geographic origin, *Journal of Ethnopharmacology,* 64(3), 235 – 240.

20. A.H. BAnkota, Y. Tezukaand S. Kadota, (2001). Recent progress in pharmacological research of propolis, *phytotherapy Research*, 15(7), 561 – 571.

21. C. Chen, C. Wu, H. Shy and J. Lin, (2003). Cytotoxic Prenyl Flavonones from Taiwanese Propolis, *Journal of Natural products*, 66(4), 503 – 506.

22. K. Salamao, P.R.S. Pereira, L.C Campos, (2008). Brazilian Propolis: Correlation between chemical composition and antimicrobial activity, *Evidence _ Based complementary and Alternative Medicine*, 5(3), 317 – 324.

23. Harborne, A.J, (1998). Phytochemical methods aguide to modern techniques of plant analysis, 3rd ed.; Chapman and Hall: London, UK, Volume XIV, 302 p.

24. Misra, C.S.; Pratyush, K.; Sagadevan, L.D.M.; James, J.; Veettil, A.K.T., Thankamani, V.A, (2011). Comparative study on phytochemical screening and antibacterial activity of roots of alstoniascholaris with the roots, leaves and stem bark. *Int. J. Pharm, Pharm Res*. 1, 77 – 82.

25. Gul, R.; Jan, S.U.; Faridullah, S.; Sherani, S.; Jahan, N, (2017). Preliminary phytochemical

screening, quantitative analysis of alkaloids and antioxidant activity of crude plant extracts from Ephedra intermedia indigenous to Balochistan. *Sci. World J,* 5873648 [Cross Ref].

26. Mohan B, Nayak JB, R Sunil kumar, L P Shiva kumara, Mohan Ch and Rajini B, (2016). Phytochemical screening, GC – MS analysis of Decalepsishamiltonii Wight & Arn. *An endangered medicinal plant,* 5(5), 10 – 16.

27. Prakash P, Manivasagaperumal R, Thiyagarajan G, (2015). Phytochemical screening and GC – MS analysis of methanolic root extract of decalepsishamiltonii. *International journal of pharmaceutics and drug analysis.* 3(1), 1 – 5.

28. Aneja KR, (2003). Experiments in microbiology, plant pathology and biotechnology, new age pvt. Ltd publishers 4thedn, pp. 376.

29. Miyataka, H; Nishiki, M.; Matsumoto, H.; Eujimoto, T.; Matsuka, M.; Satoh, T., (1997). Evaluation of propolis. I. Evaluation of Brazilian and Chinese propolis by enzymatic and physic – chemical methods. *Biol. Pharm. Bull.* 20, 496 – 501.

30. Mercan, N.; Kivrak, I.; Duru, M.E.; Katircioglu, H.; Gulcan, S.; Malci, S.; Acar, G.; Salih, B, (2006). Chemical composition effects on to antimicrobial and antioxidant activities of propolis collected from different regions of Turkey. *Ann. Microbial,* 56, 373 – 378.

31. Georgieva, K.; Popova, M.; Dimitrova, L.; Trusheva, B.; Thanh, L.N.; Phuong, D.T.L.; Lien, N.T.P.; Najdemski, H.; Bankova, V, (2019). Phytochemical analysis of Vietnamese propolis produced by the stingless bee Lisotrigonacacciae. *Plos ONE*, 14, e0216074. [Cross Ref].

32. EI. Sayed NAA (1999) evaluation of six mixtures of food additives on some biophysiological and reproductivety parameters of the mulberry silkworm. *Agri Res,* 24(6), 1971-1986.

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