



IJPPR

INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals

ISSN 2349-7203




Human Journals

Research Article

September 2018 Vol.:13, Issue:2

© All rights are reserved by P. Senthilkumaran et al.

Antioxidant and Antimicrobial Activity of Honey from Mangrove Forest

 **IJPPR**
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals

P. Senthilkumaran^{*1}, J. Mallika², A. Nelson³

¹Assistant professor, and Head, Department of Biochemistry, Sengunthar Arts and Science College, Tiruchengode, Tamilnadu, India..

²Assistant professor, Department of Biochemistry, Sengunthar Arts and Science College, Tiruchengode, Tamilnadu, India.

³Assistant professor, Department of Biochemistry, Sengunthar Arts and Science College, Tiruchengode, Tamilnadu, India

Submission: 20 August 2018
Accepted: 27 August 2018
Published: 30 September 2018

Keywords: Honey, crude protein, hemolytic assay, antioxidant assay, antibacterial activity

ABSTRACT

Honey was subjected to extraction and further fractionation to obtain antioxidant-rich fraction. The crude as isolated from aqueous extract and PBS extraction of honey by DEAE Cellulose Anion Exchange chromatography Different concentrations aqueous extract of honey was used as assay the antioxidant activity by DPPH method and Illeal loop assay. The crude of aqueous and PBS extract at a different concentration of 5mg/ml, 10mg/ml and 15mg/ml were tested against a species of bacteria viz. *Pseudomonas* sp., *Streptococcus aureus*, *Vibrio cholerae*, *Vibrio parahaemolyticus*. SDS – PAGE on 12% gel, the crude protein toxins yielded 5 bands in aqueous extract and 8 bands in PBS extract of honey. ranging from 7.8 to 116 KDa with three well-defined bands at 129, 97.8 and 7.2 KD a defined bands at in both extract. In the first set with the retention time of 4.37min, the percentage intention was found to be 20 for aqueous extract and 25 for PBS extract.



HUMAN JOURNALS

www.ijppr.humanjournals.com

1. INTRODUCTION:

Honey is a natural product made by honeybees (*Apis mellifera*) which have a highly variable sensorial and physicochemical characteristics due to climatic and environmental conditions and diverse origin of plants from which it is harvested. Having that in mind, different honey types have diverse phenolic content and consequently different antioxidant activity [Gheldof *et al.*, 2002].

Honey contains a variety of phytochemicals (as well as other substances such as organic acids, vitamins, and enzymes) that may serve as sources of dietary antioxidants [Engeseth 2002]. The amount and type of these antioxidant compounds depend largely upon the floral source/variety of the honey [Gheldof *et al.*,2002].The main characteristics of an antioxidant is to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acid, protein and lipids can initiate degenerative diseases [P.Senthilkumaran *et al.*,2015] Honey was prescribed for a variety of uses including baldness, contraception and as a wound treatment. Frequently, honey was mixed with herbs, grains and other botanical from the geographic area summarizes some of the ways honey has been used through the ages. Uses that have continued into modern folk medicine include treatment for coughs and sore throats, lotus honey for eye diseases in India, infected leg ulcers in Ghana, earaches in Nigeria, topical treatment of measles in the eyes to prevent corneal scarring, gastric ulcers and constipation. [Saul, R.L.D. *et.al.*, 1987].

Besides carbohydrates, honey contains small amounts of protein, (including enzymes), vitamins and minerals. Honey contains a number of enzymes including glucose oxidase, invertase, diastase (amylase), catalase and acid phosphatase (Crane, E.1976). The glucose oxidase reaction produces glutamic acid and hydrogen peroxide from glucose. It also produces gluconolactone on that equilibrates with gluconic acid. The hydrogen peroxide contributes to the antimicrobial properties of honey. (Floyd, R.A.*et.al.*, 2002) Oxidative damage is implicated in the etiology of cancer, cardiovascular disease, and other degenerative disorders. Free radical-mediated lipid peroxidation has been proposed to be critically involved in several disease states including cancer, rheumatoid arthritis, drug-associated toxicity, and postischemic oxygenation injury, as well as in the degenerative processes associated with aging (Horton, A.A.& Fair Hurst, S.,1987).

2. MATERIALS AND METHODS:

2.1 Collection of sample

The Honey samples were collected from Mangrove forest, Parangipettai, Tamilnadu.

2.2 Extraction of Crude Toxin

2.2.1 Aqueous extraction

The aqueous extract of Honey was prepared dialyzed by using Sigma dialysis membrane-500 (Av Flat width-24.26 mm, Av. Diameter -14.3 mm and capacity approx-1.61ml/cm) against D-glucose to remove the excess water. The supernatant so obtained was lyophilized (Labcono Freeze Dry System) and stored at 4°C in a refrigerator for the further use as crude aqueous the extract.

2.2.2 Aqueous extraction

The aqueous extract of Honey was prepared dialyzed by using Sigma dialysis membrane-500 (Av Flat width-24.26 mm, Av. Diameter -14.3 mm and capacity approx-1.61ml/cm) against D-glucose to remove the excess water. The supernatant so obtained was lyophilized (Labcono Freeze Dry System) and stored at 4°C in a refrigerator for the further use as a crude aqueous extract [Bakus, (1981)].

3.1 PARTIAL PURIFICATION OF CRUDE PROTEIN

Partial purification of the crude extract was carried out using DEAE Cellulose Anion Exchange chromatography according to the procedure of [Stempion *et al.*, (1970)].

3.1.1 Preparation of DEAE Cellulose Column

26 gm. of DEAE Cellulose was taken. and swelled in 650ml of Distilled water and incubated for 3 hrs. Then the supernatant was discarded. The sediment was mixed with half a liter of 1M NaOH. This mixture was incubated for 30 minutes and again the supernatant was discarded. The collected sediment was mixed with half a liter of 1M HCl and it was incubated for 30 minutes and the supernatant was discarded. Then this was washed with distilled water and again with PBS till it reaches pH 7.4.

3.2 Protein Estimation

Protein estimation was done by using Bovine Serum Albumin at the rate of 1mg/ml as the standard. Different concentrations of the standard ranging from 0.1 to 1mg/ml were taken and made up to 1 mg/ml. Then 5ml of alkaline copper reagent was added, mixed well and allowed to stand for 10 minutes at room temperature. Then 0.5ml of diluted Folin's phenol reagent was added and mixed well. The mixture was incubated for 30 minutes at room temperature. The absorbance at 650nm was read spectrophotometrically. The protein concentrations of Honey extracts were estimated [Lowry *et al.*, (1946)].

3.4 Hemolytic Activity

Hemolytic activity of crude toxin on chick, goat and human RBC was tested by Micro Hemolytic Method [Venkateshvaran, 2001].

3.4.1 Preparation of Erythrocyte Suspension

The blood sample was obtained from a nearby slaughter house Aravakurichi, Karur Dt. Using EDTA solution (2.7g in 100 ml of distilled water) as an anticoagulant at 5% of the volume of blood and brought to the laboratory. The blood was centrifuged at 5000 rpm for 5 minutes. The supernatant was discarded and the pellet was suspended in normal saline (pH-7.4). This process was repeated thrice. 1% erythrocyte suspension was prepared by adding 99 ml normal saline to 1 ml of packed RBC.

3.4.2 Hemolytic Assay

The micro hemolytic test was performed in 96 well 'V' bottom micro titer plates. Serial two-fold dilutions of the crude toxin were made in 100ml of Normal saline. This process was repeated up to the last well. Then 100 μ l of RBC was added to all the wells. Appropriate controls were included in the test. To the 1%, RBC suspension 100 μ l was added normal saline, that served as negative control. The plate was gently shaken and then allowed to stand for two hours at room temperature and the results were recorded. Uniform red color suspension in the wells was considered as positive hemolysis and a button formation in the bottom of these wells was considered as lack of hemolysis. Reciprocal of the highest dilution of the crude toxin showing pattern was taken as 1 Hemolytic Unit (HU).

3.5 Antioxidant Assay (DPPH assay)

Concentrate extract was prepared with 0.1 mg/ml of crude extract with and 50 µl of each extract was loaded into 'U' bottomed 96 well plates to which 50µl of 0.1% DPPH was added and then ascorbic acid (0.1 mg/ml) was used as the positive control and sterile water was used as negative control. The plate was incubated at dark for 30 minutes for the color change. Development of Purple color to a yellow color within 30 minutes indicates a positive result.

3.6 Illegal loop Assay

The effect of the drug at lower and higher doses in the intestine of the chick was found by injecting 25µl/ml and 100 µl/ml to its intestinal wall. This was then stored in phosphate buffer saline. The extract would attach to the acetylcholine receptor and the sodium-potassium pump will be blocked as a result it brings out the fluid secretion. This proves that the presence of a toxin and the intensity of low dose can be used as a therapeutic protein.

3.7 Antimicrobial Activity

3.7.1 Antibacterial Activity

Petri dishes with nutrient agar were inoculated with four different species of bacteria. Honey extracts were sterilized by passing each through a 0.22 µm Millipore GV filter (Millipore, U.S.A) Round paper discs with a radius of 0.8 cm were dipped into each honey extract and placed in the center on inoculated Petri dishes. Bacterial colonies were allowed to grow overnight at 37 °C, then the inhibition zone around the disc was measured.

4. RESULTS AND DISCUSSION:

Honey is a natural product made by honeybees *apis mellifera* which have a highly variable sensorial and physicochemical characteristics due to climatic and environmental conditions and diverse origin of plants from which it is harvested. Having that's in mind, different honey types have diverse phenolic content and consequently different antioxidant activity [Gheldof et al., 2002].

The natural acidity of honey will inhibit many pathogens. The minimum pH value for some species that commonly infect wounds ranges from 4.0-4.5. Dilution of honey especially with

body fluid, will raise the pH and lessen the antibacterial effect that results from its acidity [Crane, E.1976].

The present investigation is aimed to evaluate the antioxidant and antimicrobial activity of the honey samples collected from mangrove forest, parangipettai, Tamilnadu.

The results obtained are discussed below.

Table 1: Preparation of Crude Extract

Sr. No.	Sample	Amount of Crude Extract (g)
1	PBS extract	5.2
2	Aqueous extract	4.9

The total amount of crude extract obtained from PBS extract is more than the aqueous extract. The partial purification of the crude protein from the extract prepared was done by the column chromatography.

Protein Estimation

The protein content in crude extracts of honey was found to be 1.9 mg/ml in case of aqueous extract and 0.98mg/ml in case of PBS extract. The results are presented in Table 2.

Table 2: Protein Estimation

Sr. No.	Sample	Absorbance at 650nm	Protein Content(mg/ml)
1	PBS	0.980	0.98
2	Aqueous extract	1.920	1.9

The protein content in aqueous extract was found to be more than in PBS extract.

Hemolytic Assay

The results of the hemolytic assay on erythrocyte using crude aqueous and PBS honey. The crude methanolic extract induced hemolysis on chicken blood. The hemolytic titer in case of PBS extract found to be 14 and its specific hemolytic activity was estimated to be 11.09 HT/mg of protein. The hemolytic titer of aqueous extract of honey was found to be 10 and its hemolytic activity was found to be 9.8 HT/mg of protein.

Table 3: Hemolytic Assay

Sr. No.	Extract	H/assay	HTV (HT/mg)
1	Aqueous extract	10	9.8
2	PBS extracts	14	11.09

HTV = Haemolysis Titre Value

HT = Haem Titer.

H/assay = Haemolytic Assay

The hemolytic activity of aqueous extract of honey was found to be more than the hemolytic activity of PBS extract of honey.

Antioxidant Assay

The DPPH reaction was carried out with the aqueous and PBS extract of honey. The yellow coloration was noted. Figure 1 shows the antioxidant assay.

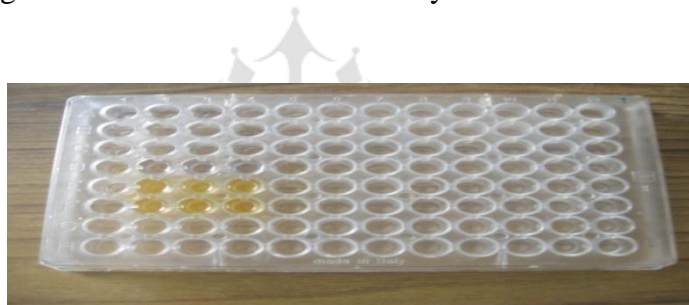


Figure 1: Antioxidant Assay

The DPPH reaction on both the extracts changed the color which indicated the presence of antioxidant molecule supporting the thin layer chromatography result. DPPH is a useful reagent for investigating the free radical-scavenging activities of compounds. In the DPPH test, the extracts were able to reduce the stable radical DPPH to the yellow colored 1-1diphenyl 2picrylhydrazine. This method is based on the reduction of the alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H by the reaction. Thus shows the antioxidant activity.

Illeal Loop Assays

The minimal and maximal dose of aqueous and PBS extract(25µg /ml and 100µg/ml) were taken for illeal loop assay.

The minimal and maximal dose of both extracts developed inflammation and accumulation of fluid. But the intensity of inflammation was less in 25µg/ml of both the extracts and high fluid accumulation was observed in the higher dose of 100µg/ml. The intestinal loop assay was to find the toxicity in mammalian tissues by any tissue damage or any inflammation in the intestine. This was found by the accumulation of fluid in the intestinal loop. The more fluid in the loop leads to more toxicity to the tissues. The fluid secretion was due to the toxic protein in the extract. If toxic substances present in the extract would be attached to the acetylcholine receptor and the sodium-potassium pump will be blocked as a result it brings out the fluid secretion. This proves that the presence of a toxin and the intensity of low dose can be used as a therapeutic protein.

ANTIMICROBIAL ACTIVITY

Antibacterial activity

The crude of Aqueous and PBS extract at the different concentration of 5mg/ml, 10mg/ml and 15mg/ml were tested against the four species of bacteria viz. *Pseudomonas* sp., *Streptococcus aureus*, *Vibrio cholerae*, and *Vibrio parahaemolyticus*.

Table 4: Antibacterial Activity

S. No.	Bacterial Culture	Aqueous Extract	PBS Extract
1	<i>Pseudomonas Sp</i>	4mm	5.5mm
2	<i>Streptococcus Aureus</i>	6.5mm	4mm
3	<i>Vibrio cholerae</i>	7mm	4.5mm
4	<i>Vibrio parahaemolyticus</i>	5mm	7mm

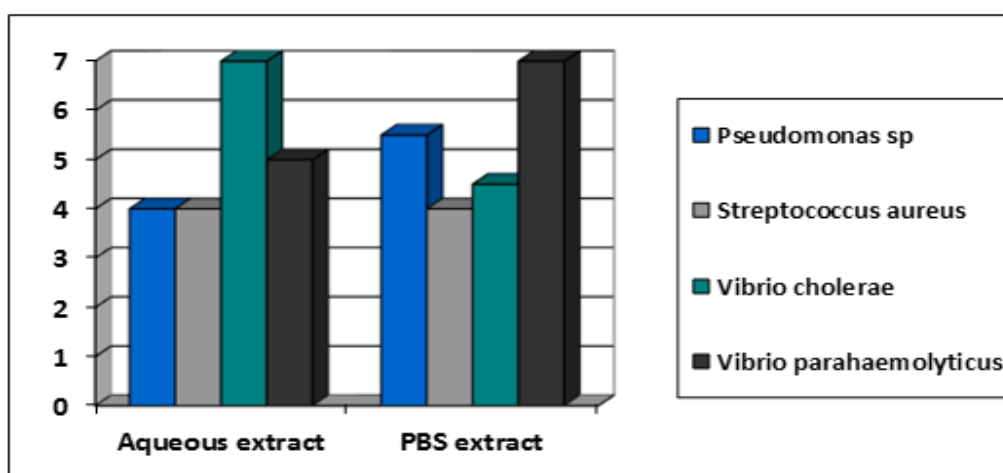


Figure 2: Antibacterial Activity

SUMMARY AND CONCLUSION:

The Honey sample was collected from Mangroove forest aqueous extract of Honey yielded a total amount of 4.9g of crude extract from 500 ml of honey. Similarly, PBS extracts a total amount of 5.20g of crude extract, the protein content in crude extracts of Honey, was found to be 1.9mg/ml in case of aqueous extract and 0.98mg/ml in case of PBS extract. The results of the hemolytic assay on erythrocyte using crude aqueous and PBS honey. The DPPH reaction on both the extracts changed the color which indicated the presence of antioxidant result. The crude of Aqueous and PBS extract at a different concentration of 5mg/ml, 10mg/ml and 15mg/ml were tested against 4 species of bacteria viz. *Pseudomonas* sp., *Streptococcus aureus*, *Vibrio cholerae*, and *Vibrio parahaemolyticus*. SDS–PAGE on 12% gel, the crude protein toxins yielded 5 bands in aqueous extract and 8 bands in PBS extract of honey. ranging from 7.8 to 116 KDa with three well-defined bands at 129,97.8 and 7.2 KDa defined bands at in both extracts. In the first set with the retention time of 4.37min, the percentage intention was found to be 20 for aqueous extract and 25 for PBS extract.

Many studies have carried out assays of food items to determine their total antioxidant capacity (TAC), but there are problems with trying to correlate the numbers obtained from these assays with nutritional value. Uptake in the gastrointestinal tract, metabolism and excretion, and biokinetics modify the impact of food items, which may have health effects independent of the antioxidant component. Even if the compound is active in vitro, it may not be taken up in the gastrointestinal tract or metabolized before or after uptake to products which are not redox (reduction and oxidation) active, therefore those in vitro data do not apply. Whether pro-oxidant, antioxidant or any other biological effects potentially exerted by polyphenols account for the health benefits of certain food and drinks is uncertain.

6. REFERENCES:

1. Cheung LM, Cheung PCK, Ooi VEC (2003). Antioxidant activity and total phenolics of edible mushroom extracts. *Food Chem.* 81: 249- 255
2. Crane, E., 1990. Bees and beekeeping: science, practice, and world resources. Ithaca, New York, Cornell University Press, 109-111.
3. Floyd, R.A.&Hensley.(2002) Therapeutics of Neurodegenerative Diseases. *Neurobiol. Aging* 23, 95-807.
4. Gheldof.N& Engeseth, N.J.(2002).*Journal of Agricultural and Food Chemistry*, 50(10), 3050–3055.
5. Ishikawa Y, Morimoto K, Hamasaki T, 1984.*Journal American Oil Chemists Society* 61:1864–1868.
6. Jiratanan, T., & Liu, R. H. (2004). *Agricultural and Food Chemistry*, 52(9), 2659–2670
7. Lowery, O.H. and J.A. Lopez, 1946. *J. Biol. Chem.*, 162: 421-428
8. Nzeako BC, Hamdi J (2000).Antimicrobial potential of honey on some microbial isolates. *Medical Sciences* 2: 75-79

9. Oka H, Ihai Y, Kawamura N, Una K, Yamada M, Harada K, Suzuki M (1987). Improvement of chemical analysis of antibiotics: Simultaneous analysis of seven tetracyclines in honey. *J. Chromotogr.* 400: 253-261.
10. Shahidi, F., Janitha, P.K., & Wanasundara, P.D. (1992). Phenolic antioxidants. *Critical Reviews of Food Science & Nutrition*, 32(1), 67–103.
11. Venkateshvaran, K., 2001. *Pharmacol. Bioresources.*, pp: 14-29.
12. Vanitha, Kalaiselvi, P. Senthilkumaran., et al. (2015). *European journal of pharmaceutical and medical research* 2(7), 194-198.
13. Wahdan HA (1988). Causes of the antimicrobial activity of honey. *Infection* 26: 26-31.
14. Wang-H., & Engeseth, N.H. (2002). *Journal of Agricultural and Food Chemistry*, 50(21), 5870–5877.
15. White JW., Subers M.H., Schepartz A.L. (1963) The identification of inhibine, the antibacterial factor in honey, as hydrogen peroxide and its origin in a honey glucose-oxidase system, *Biochem. Biophys. Acta* 73, 57–70.
16. Yao, L., Jiang, Y., Singanusong, R., D'Arcy, B., Datta, N., Caffin, N., et al. (2004). *Food Research International*, 37(2), 166–174.
17. Yao, L., Jiang, Y., Singanusong, R., Datta, N., & Raymont, K. (2003b). Phenolic acids and abscissic acid in Australian Eucalyptus honeys and their potential for floral authentication. *Food Chemistry*, 86(2), 169–177.

