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Antitumour and antioxidant potential of some selected Pakistani honeys

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ABSTRACT

Antitumour potential of honey is attributed to its excellent antioxidant activity which in turn depends on the geographical origin. The present study focuses on exploration of antioxidant and antitumour potential as well as total phenolic contents (TPC) of 58 Pakistani honeys involving spectrochemical techniques and potato disk assay. *Agrobacterium tumefaciens* was used to induce tumours in potato disks. All analysed honey samples exhibited 1.33 ± 0.00–155.16 ± 0.98 mg/100 g of TPC, 50% 2,2-diphenyl picryl hydrazyl (DPPH) inhibition, ⩾7.36 ± 0.43–39.86 ± 2.34 mg/100 g qurecitin equivalent antioxidant contents, ⩾13.69 ± 0.91–65.50 ± 1.37 mg/100 g ascorbic acid equivalent antioxidant contents, 64.65 ± 0.43–1780.74 ± 11.79 mM ferric reducing antioxidant power and 60% peroxide inhibition. Antitumour activity observed for 43 natural and 10 commercial samples was ⩾20%. Two samples from Faisalabad region showed 87.50 ± 5.50% and 79.00 ± 5.56% antitumour activity which were reference standard. It was concluded that Pakistani honeys possessed excellent antioxidant and antitumour potential overall.

1. Introduction

Antioxidants play a vital role against the deteriorating action of free radicals in the organisms. Deficiency of antioxidants in living organisms leads to oxidative stress. The natural products rich in antioxidants are of great importance for scientists (Amarowicz et al., 2010; Chan, Khong, Iqbal, Mansor and Ismail, 2013; Craft, Kosinska, Amarowicz, & Pegg, 2010). Honey a sweet natural product produced by honey bees form nectar and other plant juices is also a good source of antioxidants. Antioxidant and antimicrobial properties of honey are due to presence of variety of compounds like phenolics, ascorbic acid, α-tocopherol, proline, vitamins, catalase and glucose oxidase (Al et al., 2009; Lachman, Orsak, Hejtmankova, & Kovarova, 2010; Liu, Ye, Lin, Wang, & Peng, 2012). Several studies evidenced that different types of honeys from various countries have shown antioxidant capacity; dependent on the concentration of different bioactive compounds (Mariod, Ibrahim, & Ismail, 2009; Vit et al., 2009). Honey is important not only due to its unique composition but also due to its therapeutic properties (Blasa, Candiracci, Accorsi, Piacentini & Piatti 2007; Silici, Uluozlu, Tuzen, & Soylak 2008; Tuzen, Silici, Mendil, & Soylak, 2007). Therefore it is largely produced and characterised by many countries of world. Natural antioxidants are effective against inflammation, cancer, coronary diseases, burns, aging, wound healing, gastrointestinal and heart diseases. Anticancer potential of honey from different origins have been characterised by cell line assays (Jaganathan & Mandal, 2009). To the best of our knowledge, honey has never been characterised for its antitumour activities induced by *Agrobacterium tumefaciens* in potato previously. Potato disk assay is a simple, rapid, robust, easily available and sophisticated method to primarily evaluate the antitumour activity of any natural product. There was a need to analyse the antitumour activities of Pakistani honeys.

Extensive data is available for antioxidant properties of honeys from different origins of world which evidenced that bioactivities of honeys vary from each other due to botanical and geographical variations (Al et al., 2009; AL-Waili et al., 2013). Very limited data about antioxidant properties and total penolic contents of honey indigenous to Pakistan is available, therefore; it became imperative to characterise antioxidant properties and total phenolic contents of honey samples from different origins of Pakistan. Hence, the aim of present study was to evaluate the total phenolic contents, antitumour and antioxidant properties of Pakistani honeys as well as to find a correlation of total phenolic contents and antioxidant properties with antitumour activities parameters. The second objective of our investigation was to compare antitumour activities, total phenolic contents and antioxidant potential of local natural honey samples of different localities and commercial honey samples.

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2. Materials and methods

2.1. Materials

All the reagents and chemicals used were of analytical grade. Nutrient broth, agar powder were supplied by OXOID (Hamsphire, UK), 2,2-diphenyl picryl hydrazyl (DPPH), ascorbic acid, qurecitin, gallic acid, trichloroacetic acid, hydrogen peroxide, ferric chloride and pottasium ferricynaide were supplied by Sigma (ALDRICH, Germany). Sodium carbonate, HPLC grade methanol and ethanol, Folin Ciocalteus reagent, monopotassium phosphate, dipotassium phosphate, potassium iodide, iodine and mercuric chloride from Applichem (Darmstadt, Germany), vinracine from UNITED PHARM. INC., KOREA.

A.tumefaciens was obtained from Nuclear Institute of Biotechnology and Genetic Engineering, Faisalabad, Pakistan. Red skinned fresh healthy potatoes were purchased from local market of Faisalabad, Pakistan.

2.2. Samples

Total forty five natural honey samples from seven localities of the Central and Southern Punjab, Pakistan were extracted directly from beehives and filtered with cheese cloth. Beekeeper's honey samples were purchased from different localities of Pakistan. Whilst commercial honey samples of different brands were purchased from local markets of Faisalabad, Pakistan. Samples were stored at room temperature in plastic bottles.

2.3. Antioxidant activities

2.3.1. Total phenolic content (TPC)

Standard Folin–Ciocaltue method followed by Al et al. (2009) & Saxena, Gautam, and Sharma (2010) was used with some modifications to determine the total phenolic contents. Honey solution 1 mL (10% w/v in methanol) was added to 5 mL of 0.2 N Folin Ciocalteu reagent and mixed well for 10 min by using rotary shaker. Sodium carbonate 4 mL (75 g/L) was added and incubated for 2 h. Absorbance of reaction mixture was measured at 760 nm. Methanol was used as blank. Gallic acid (0–200 mg/L) was used for standard curve (R^2 = 0.99). The collected data was presented as Gallic acid equivalent mg TPC per 100 g of honey.

2.3.2. Total antioxidant contents

Total antioxidant contents were determined by following the method of Saxena et al. (2010) with some modifications. Methanolic honey solution 3 mL (4 g/10 mL) was mixed with 1.5 mL methanolic DPPH solution (.02 mg/mL). Reaction mixture was incubated for 15 min at room temperature. Absorbance was determined at 517 nm against methanol blank. Ascorbic acid (R^2 = 0.98) 0–16 μg/mL and Qurecitin (R^2 = 0.99) 0–8 μg/mL were used as standards. Results are expressed as ascorbic acid equivalent (AEAC) and qurecetin equivalent antioxidant contents (QEAC). Experiment was performed in triplicate.

2.3.3. DPPH radical scavenging activity (%RSA)

DPPH % RSA was measured by method of Meda, Lamien, Romito, Millogo, and Nacoulma (2005) with slight modifications. Honey solutions and DPPH solution used in above experiment were mixed in 2:1 proportion respectively. Reaction mixture was incubated at room temperature for 15 min followed by measurement of absorbance at 517 nm against methanol as blank. 5 mL DPPH solution mixed with 2.5 mL methanol was used as negative control (A_c) whilst ascorbic acid was used as standard. Each experiment was performed in triplicates. % inhibition of DPPH radical was determined by following formula

$$\% \text{ inhibition} = [A_c - \text{absorbance of sample or standard}/A_c] \times 100$$

2.3.4. Ferric reducing antioxidant power assay

Reducing power of honey was determined by following the method of saxena et al. (2010) with some modifications. Honey solution 5 mL (10%W/V) in ethanol was mixed with 5 mL of 0.2 m phosphate buffer (pH 6.6). Above reaction mixture was mixed with 5 mL of potassium ferricyanide (1%) and incubated for 20 min at 50 °C. Trichloroacetic acid (10%) 5 mL was added followed by thorough mixing via vortex mixture. Reaction mixture thus obtained was centrifuged for 10 min at 3000 rpm. Supernatant 2.5 mL was mixed with 5 mL of double distiled water and 0.5 mL of $FeCl_3$ (0.1%) solution. Absorbance was measured at 700 nm. Ascorbic acid (0–10 mg/mL) was used as reference standard (R^2 = 0.95).

2.3.5. Peroxide scavenging activity

Peroxide radical scavenging activity was determined by method of Olayinka & Okoh (2010) with some modifications. Honey solution 8 mL (0.05 g/mL) in deionized water mixed with 1.2 mL mM H_2O_2 in 0.1 M (pH 7.4) phosphate buffer. Samples were incubated for 10 min and measured absorbance at 230 nm against phosphate buffer as blank. Whilst H_2O_2 in 0.1 M (pH 7.4) phosphate buffer was used as negative control and ascorbic acid was used as positive control. % inhibition of peroxide was determined by following formula

$$\% \text{ inhibition} = [\text{Abs. of} - \text{ve control} - \text{absorbance of sample or standard}/\text{Abs. of} - \text{ve control}] \times 100$$

2.4. Potato disc assay

Antitumour activity was evaluated by procedure followed by (Hussain, Zia, & Mirza, 2007; Lellau & Liebezeit 2003) with slight modifications. Fresh culture of *A.tumefaciens* was prepared by inoculation of 100 mL (1.3%) autoclaved nutrient broth pH 7.4 with 10 μL of stock culture. This media was left at 28 °C for 48 h to get 5×10^9 cells per mL. Potatoes were surface sterilized for 20 min with 20% $HgCl_2$ solution and cut (5 × 5) mm by sterilized cork borer. Seven potato disks along with positive and negative controls in the center were placed in autoclaved petri dish containing 1.5% agar medium. Honey samples 300 μL of each was mixed with 50 μL of cultured *A.tumefaciens* and poured 50 μL of each on the potato disks. The whole experimental work was done in laminar air flow. Petri plates were incubated at 28 °C for 21 days and sprayed with Lugol's solution (potassium iodide 10% and Iodine 5% in distiled water). tumours were counted under microscope after 20 min. Each sample was replicated for five times. % inhibition was determined by following formula

$$\% \text{ Inhibition} = [1 - \text{no. of tumours in sample}/\text{no of tumours } \textit{negative control}] \times 100$$

3. Results and discussion

In this study, we explored and compared antioxidant potential of 45 natural honeys native to Pakistan, all the samples exhibited excellent antioxidant activity as compared to available data. Total