STUDY OF RESIDUAL ANTIBIOTICS AND THEIR METABOLITES IN HONEY

ABSTRACT

Honey is a natural product havingnutritional and medicinal properties. The evaluation of honey for the detection and quantification of antibiotic residues such as oxytetracycline, streptomycin, gentamycin, penicillin, sulfonamide, chloramphenicol,nitrofuran and their metabolites were performed. The extracts of branded, unbranded and natural honey samples were tested for antioxidant activity, phenolic acids, antimicrobial activities, chemicalcomposition, phytochemicals,carbohydrates, Hydroxy Methyl Furfural (HMF) content,aflatoxins (B1, B2, G1, G2) and heavy metals (cadmium, manganese, lead, nickel cobalt). Samples of honey (n=100) were collected from local market of Khyber Pakhtunkhwa, Pakistan and categorized as branded, unbranded and natural for comparative study. The samples under study were Marhaba, Qarshi, Versatile, Al-hayat, Young's, Pak-salman, Langnese, Big bees honey, Small bees honey, Beera, Palosa, Sperkay, Bekerr and Granda.

The detection of antibiotics such as tetracycline, streptomycin, gentamycin and penicillin residues was carried out by thin layer chromatography (TLC), while the positive samples were quantified by an optimized HPLC-UV method. The sulfonamide residues such as sulfamethazine, sulfacetamide, sulfathiazole and chloramphenicol were analyzed by HPLC, whereas nitrofuran and their metabolites were determined by LC-MS-MS. The antioxidant activity of all the extracts were determined against 1, 1 -diphenyl-2-picryl hydroxyl (DPPH)using different concentrations (100, 200, 300, 500 and 600 µg/ml), by spectrophotometer. Phenolic acids (chloroganic, gallic, vanallic, benzoic and syringic) were identified and quantified by HPLC using UV-VIS Detector.The antimicrobial activities were evaluated by disc diffusion methodagainst *Candida albicans*,*Aspergillusniger*, *Escherichia coli* and *Bacillus cereus*.The chemical composition (total ash, pH, moisture, total acidity, electrical conductivity and total sugars) were estimated by standard methods of AOAC. The phytochemicals(tannins, phlobatanins, flavonoids, terpenoids, glycosides, saponins, alkaloids and fluorides) were screened by UV-VIS Spectrophotometer.

Carbohydrates (lactose, maltose, glucose, xylose, fructose, ribose, mannose, arabinose, glactose and sucrose) were quantifiedby HPLC using UV-VIS Detector. The Hydroxy methyl furfural (HMF) content was determined by Winkler's methodusing UV-spectrophotometer. Heavy metals concentration was detected using atomic absorption spectrophotometer. Antibiotics residues of penicillin (10.05mg/kg), streptomycin (12.02mg/kg) and oxytetracycline (15.31mg/kg) were found maximum in unbranded honey,while negative in all natural honey samples.The sulfonamides, chloramphenicol and nitrofuran residues and their metabolites were not detected in any sample.

In case of branded,Al-hayat honey showed maximum antioxidant activity (81.26±1.44) at the concentration 600 µg/ml among all honey samples, whereas the lowest activity (20.22±1.19) was observed at the concentration 100 µg/ml in Marhaba honey. In Unbranded, Small bees honey showed maximum antioxidant activity (84.33±1.23) at the concentration 600 µg/ml, whereas the lowest activity (24.12±1.17) was observed at the concentration 100 µg/ml in Beera honey. In natural comb honey,Big bees honey showed maximum antioxidant activity (85.22±1.23) at the concentration 600 µg/ml, whereas the lowest activity (10.11±1.34) was observed at the concentration 100 µg/ml in Beera honey.

The phenolic acids were found higher in all natural honey samples as compared to branded and unbranded honey. Among the natural honey samples, the maximum concentration (4.26mg/100g) of phenolic acids was found in Palosa honey while minimum (1.93mg/100g) in Bekerr honey sample. Similarly the maximum concentration (2.78mg/100g) was found in Langnese honey, while minimum (0.71mg/100g) in Versatile honey sample. In unbranded honey maximum concentration (2.46mg/100g) was found in Beera honey, while minimum (0.62mg/100g) in Palosa honey sample. Maximum antifungal activities (16mm) have been shown by natural (Big bees honey), while minimum activity (1mm) by branded (Young's) and unbranded (Bekerr) honey against *Aspergillusniger*. Maximum antibacterial activities (44mm) and (37mm)was observed in natural comb honey, while minimum (1mm) inbranded (Versatile) and unbranded (Sperkay) respectively, against *Escherichia coli*and *Bacillus cereus*.

In branded honey, maximum chemical composition (crude fiber, crude ash, crude fats, reducing sugar, vitamin C, pH and electrical conductivity)was observed in versatile honey, minimum in Qarshi honey. In unbranded honey, maximum content was observed in Palosa honey, minimum in Small bees honey. In natural comb honey, maximum content was observed in Beera honey, minimum in Small bees honey. Among the branded honey sample, maximum concentration (78.00g/100g)of carbohydrates was found in Langnesehoney, while minimum (54.25g/100g) in Al-hayathoney sample. In unbranded honey maximum concentration (76.10g/100g) was found in Beera honey, while minimum (54.84g/100g) in Sperkay honey sample.Similarly in natural honey samples,maximum concentration (78.22g/100g) of carbohydrates was found in Beera honey while minimum (70.18g/100g) in Sperkay honeysample.

Higher concentration of heavy metals(µg/kg) was found in branded honey as compared to unbranded and natural honey. In Marhaba, Ni concentration (0.49±0.03) found maximum, while Co (0.15±0.02) was lowest. Pb concentration (0.85±0.03) was maximum, whereas Cd (0.16±0.03) found lowest in Qarshi. Versatile contains maximum Pb (1.34±0.02), while lowest Cd (0.12±0.02). In Al-hayat Cu concentration (1.23±0.03) was maximum, while Pb (0.11±0.03) was lowest. Young's honey contains maximum Ni (2.41±0.01), while lowest Mercury (0.16±0.03). Ni (1.25±0.02) was found maximum and Mn (0.14±0.03) lowest in Pak-salman, whereas in Langnese Hg concentration (0.71±0.03) found maximum while Cd (0.13±0.02) was lowest. The contamination level of aflatoxins (B1, B2, G1,G2) was also evaluated in both types of honey. Minimumaflatoxins were detected in branded and unbranded honey sample are B1and B2 such as (2.14 and 1.25) and maximum concentration are (2.33 and 2.15).

It is concluded that the unbranded honey had more contamination of antibiotic residues as compared with branded and natural honey. All the branded, unbranded and natural honey samples showed antioxidant activity. Natural honey samples presented better antioxidant activity as compared to branded and unbranded samples. Honey showed effective antimicrobial activity against different microbial strains (*Candida albicans, Aspergillus niger, Escherichia coli, Bacillus cereus*). The unbranded honey samples are also good source of nutrients and valuable phytochemicals as compared to branded samples. Carbohydrates were found in all natural and farm honeysamples. It is also concluded that contaminants are less as compare to the reported values so mostly the honey produces in Khyber Pakhtunkhwa are good for user.So these available honeys can be utilized in various food products as well as in herbal formulations.