

## ABSTRACT

Honey, a natural substance has immense health benefits due to its associated nutraceutical properties. The health benefits of honey accompanying its consumption have emerged as one of the most important functional food and therapeutic agent in the field of food and medicine. Due to the potential to treat diseases, honey is considered a functional food and can be an alternative to chemotherapeutic agent in near future. The present study is one such attempt that deals with studies on honey and its biocomponents imparting bioactivities. Several monofloral honey namely, *Litchi chinensis*, *Brassica napus*, *Eucalyptus globulus*, *Syzygium cumini*, *Azadirachta indica*, *Acacia nilotica* were collected and studied to fit in the best biomolecular composition.

Firstly, melissopalynology was carried out to confirm the botanical honey origin followed by selection of the honey for further studies based on its characterization. Pearson's correlation coefficient ( $r$ ) in bivariate linear correlation-based studies were conducted to probe the correlation between biochemical composition and antioxidant activity. Melissopalynology analysis confirmed the honey samples as monofloral whereas, physico-chemical and biochemical characterization suggested *Litchi chinensis* honey to be a darker in color and rich in phenolics (88.04 mgGAE/100g), flavonoids (16.67 mgQE/100g), protein (0.42 g/100g) exhibiting strong antioxidant activity viz. DPPH (70.15 %), FRAP [1405.6  $\mu\text{M Fe (II)}$ ], ABTS (89.46 %) respectively. Correlation studies further suggested strong correlation between biomolecular components and honey antioxidant activity.

Further work to gain an insight into the proteomic studies was carried out using *Litchi chinensis* honey. Protein was isolated from honey by physical method of ultrafiltration devoid of chemical agents. Further purification of protein from honey was achieved using ion-exchange chromatography with protein concentration 0.102 mg/mL. The protein showing a single band in SDS-PAGE confirmed the homogeneity of purification having a molecular weight of 55 kDa which was further confirmed by MALDI-TOF/MS analysis. The protein was further characterized and found to exhibit an isoelectric point of 5.5 and a N-terminal sequence N-I-L-R-G-E-S-L-N-K-S-L-P-I-L-H-E-W-K-F showing high similarity with other bee species [*Apis cerana* (96 %), *Apis dorsata* (79 %), *Apis florea* (94 %) and *Drosophila melanogaster* (51 %) respectively]. The protein

was identified as Major Royal Jelly Protein 1 (MRJP1) having a Mascot score of 77 and a sequence coverage of 25 %.

To evaluate biological activity, honey and the protein was assessed for cytotoxic effect on cervical cancer cell (HeLa). MTT assay revealed both honey and protein to inhibit cancer cell growth in a dose dependent manner showing IC<sub>50</sub> value 3.54 mg/mL (honey) and 4.14 ng/mL (protein) respectively. Morphological alterations (cell shrinkage, cytoplasm condensation and cell distortion) in the HeLa cells upon honey and protein treatment was depicted via Phase Contrast Microscopy. Further alteration of mitochondrial membrane potential (MMP) was assessed by JC-1 dye via MMP assay, which showed a reduction in the mitochondrial membrane potential of HeLa cells due to disintegration of the mitochondrial membrane integrity indicating apoptosis. To observe DNA fragmentation and other morphological changes in cells undergoing apoptosis, TUNEL assay counterstained with DAPI was carried out. Fluorescence microscopic observations revealed honey and protein treated cells to have irregular nuclear fragmentation which stained further with DAPI confirmed DNA damage in honey and protein treated cells.

Lastly, interaction studies of protein with phenolic compounds through molecular docking was explored which showed successful interaction between the honey protein (MRJP1) and phenolic compounds (gallic acid, ellagic acid, caffeic acid and catechin). The binding affinity of the biomolecules and their active binding site was revealed which showed ellagic acid to have the strongest affinity for the protein molecule having a G-score of -7.57, and the active site of binding of the phenolic molecule to the protein was hydrophobic in nature (Leu188, Val311, Ileu359). The docking study explains the protein-phenolic interaction for better understanding of drug research and development.