2018-19

RESEARCH JOURNEY International Multidisciplinary E-Research Journal ISSN : 2348-7143
Impact Factor (SJIF) - 6.261 | *Special Issue 120 :* Recent Developments in Life Sciences for Human Welfare February 2019

Spectrophotometric determination of polyphenols in royal jelly

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Abstract :

Aim : This study was carried out to evaluate the spectrophotometric analysis of polyphenolic content in fresh and lyophilized royal jelly samples by Folin – Ciocalteu colorimetric technique. Royal jelly is asecretory productof worker honey bees (*Apis mellifera*) important for the growth and maintenance of queen honey bee.it is a glandular secretion recognized for complex composition, containing minerals, proteins, amino acids, steroids, phenols, carbohydrates, vitamins, lipids, acetylcholine and other unknown substances thus it is used in cosmetics for its alleged tonic and bio-stimulating effects, in various dietary products as a supplement and in medicines also. Royal jelly has a high nutritional values and functional properties.

Method : In this study polyphenolic contents were analyzed from fresh and lyophilized samples of royal jelly by spectrophotometrically using Folin – Ciocalteu colorimetric technique.

Results : results showing the highest range for protein present in fresh royal jelly is 13.15±0.60 mg% whilelyophilized royal jelly has a high content of total protein 36.87±0.56 mg%.so it is concluded that Significant high amount of total protein content is found in the lyophilized royal jelly instead of fresh royal jelly.

Keywords : Royal jelly,*Apis mellifera*, lyophilized,polyphenolic contents, spectrophotometer.

Introduction :

The royal jelly (RJ) a principal food of the queen honey bee, is produced by the hypopharingeal and mandibular glands of the worker honey bees (*Apis mellifera*). RJ is a natural material offers probably the greatest potential to find new substances with pharmaceutical potential. The physiological effects of RJ in humans are still not fully understood. However, RJ has been recognized in the world market as a dietary supplement. Biological activities of RJ are variable and have been correlated to their content of trace elements [1].

Some beneficial effects have been attributed to consumption of royal jelly, as elimination of physical and mentalfatigue, appetite normalization activation of brain function, improved vision, increased resistance against viral infections and skin rejuvenation. Literature shows some of the important components of Royal Jelly as: proteins, sugars, lipids, 10 HAD, polyphenols and flavonoids [2-4]. Royal Jelly has been accepted and often used as a substance that supports health.Research shows that Royal Jelly contents could have physiological functions,immune-stimulative suppression of allergic reactions, and antihypertensive andstimulate cell proliferation.

Spectrometric analyses exhibited that about 185 different organic compounds are found in RJ. It is consider that the royalactin is the compound in RJ most responsible for allowing a larva to morph into the queen bee [5].

These compounds are reported to exhibit anticarcinogenic, anti-inflammatory, anti-atherogenic, antithrombotic, immune modulating and analgesic activities, among others and exert these functions as antioxidants [6].

Phenolic compounds or polyphenols, are one of the most important groups of compounds occurring in plants, where they are widely distributed, comprising at least 8000 different known structures [7]. Polyphenols are the secondary metabolism of plants products. These compounds are reported to exhibit anticarcinogenic, anti-inflammatory, anti-atherogenic, antithrombotic, immune modulating and analgesic activities, among others and exert these functions as antioxidants [8].

In general, phenolic compounds can be divided into at least 10 types depending upon their basic structure: simple phenols, phenolic acids, coumarins and isocoumarins, naphthoquinones, xanthones, stilbenes, anthraquinones, flavonoids and lignins. Flavonoids constitute the most important polyphenolic class, with more than 5000 compounds already described [9].

Current study is proposed to investigate the

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concentration of polyphenols in fresh and lyophilized royal jelly samples.

Material and Method

Collection of royal jelly :

Fresh Royal jelly samples (n=10) were collected from the hives with colonies of the most common honeybee species *Apis mellifera* located in Himayat bagh of Aurangabad region. Lyophilizedroyal jelly samples (n=10) were purchased from the beekeeping farm of Hi-tech natural product Ltd. Fresh sample was kept in refrigerator at -4° C while lyophilized samples were kept at room temperature in the laboratory before analysis.

Experimental design :

Two groups were made. Group A& B. Group Acontains10 samples of fresh royal jelly while group B contains 10 samples oflyophilized royal jelly, respectively.

Spectrophotometric determination of phenolic compounds

The colorimetric assay based on the reaction of Folin- Ciocalteu reagent is a method widely used for the determination of total phenols [10-12]. The method consists of calibration with a pure phenolic compound, extraction of phenols from the sample and the measurement of absorbance after the color reaction.

Protocol is as follows. Each royal jelly sample is diluted with distilled water and filtered. This filtrate is then mixed with Folin-Ciocalteu reagent then after 5 min sodium carbonate is added. After incubation for 10 min at room temperature the absorbance of the reaction mixture is measured at 760 nm. Methanol is used as blank and Gallic acid is used as standard to produce the calibration curve. The mean of three readings is used and the total phenolic content is expressed in mg of gallic acid equivalents/100 g of royal jelly [13]. A modification of the Folin-Ciocalteu method has been carried out by Vinson et al. [14].

Statistical Analysis :

The analyses were carried out using ANOVA followed by Tukey's test by using graph pad prism Statistics version 6.0 for Windows. Data were expressed as means ± SEM. Level of statistical significance was defined as $p<0.05$.

Results :

All samples of fresh and lyophilized royal jelly sample were analyzedby using Folin- Ciocalteu reagent method. Table 1 shows total phenolic content as mg of Gallic acid equivalents/100 g of royal jelly.Comparison between fresh and lyophilized royal jelly revealed significant difference between the polyphenolic values in fresh and lyophilized royal jelly samples obtained by Folin – Ciocalteu method. Fresh royal jelly (A) contains a significant high ($p<0.05$) amount of polyphenol conten 21.81mg/100g of royal jelly. Normal polyphenolic conten in fresh royal jelly ranges between 11-36mg%.Similarly lyophilized RJ of (group B) exhibits a significant decrease level ($p<0.05$) of polyphenol 17.14mg/100 g royal jelly compared to fresh royal jelly sample of group A. normal polyphenolic content in lyophilized royal jelly ranges between 5-21mg%.

Discussion :

The phenolic compounds or polyphenols are one of the most important groups of compounds occurring in plants where they are widely distributed. The polyphenols are also products of the secondary metabolism of plants. These compounds are reported to exhibit anticarcinogenic, anti-inflammatory, anti-atherogenic, antithrombotic, immune modulating and analgesic activities, among others and exert these functions as antioxidants [6]. The phenolic compounds present in royal jelly are kno to possess antioxidant properties and these properties may play a key role in the pharmacological activities of RJ.

The spectrophotometric procedure with Folin – Ciocalteu reagent is widely used to determine total phenolic content in different bee products [15, 16]. Several studies indicates that the phenolics are one of the components responsible for the antioxidant behavior of RJ. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. [17-19].the total polyphenols in the samples increased in the following order: Gallic acid> fresh RJ >Lyophilized RJ. The differences for all analyzed parameters are significant. This result suggests that during lyophilization process the phenolic contents are destroyed thus fresh royal jelly possess a high antioxidant capacity than the lyophilized form.

Conclusion :

The important nutrients essential for our health i.e. carbohydrates, fats, proteins, vitamins, minerals, 10 HAD, polyphenols, flavonesetc. are present in both fresh and lyophilized forms of royal jelly samples.The Folin–Ciocalteu reagent method exhibits that fresh and lyophilizedroyal jelly offer values within the normal ranges for polyphenols.The spectrophotometric analysis of fresh as well as lyophilized royal jelly exhibits that all the samples of royal jelly contains a considered amount of polyphenols but a Significant high amount of polyphenolic content is found in the lyophilized royal jelly instead of fresh royal jelly, thus they could be useful in the formation of cosmetics, various pharmacological products, and as a dietary supplement instead of using

fresh royal jelly.

Acknowledgments :

Author is great fullyacknowledge University grant commission for the financial assistance.

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Table 1: total polyphenolic concentration in fresh and lyophilized royal jelly.

Sr. No.	Royal jelly	Mean poly-phenols	± Standard error of mean
1	Fresh royal jelly	21.81	1.85
2	Lyophilized royal jelly	17.14	2.05

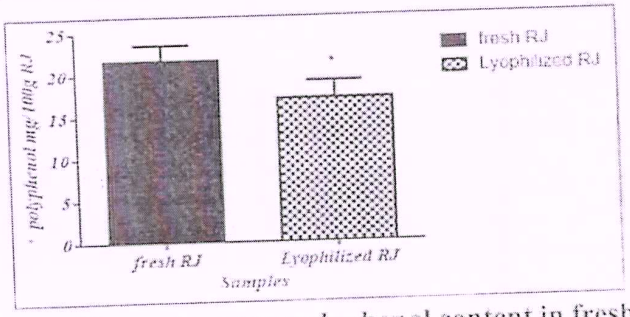

Figure 1 : mean polyphenol content in fresh as well as lyophilized royal jelly samples.