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Determination of Antioxidant content in prepared *Syzigiumaromaticum* and *Bacopa monnieri* mixed honey

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Abstract
Development of new innovative food product, rich in major and minor nutrients are required for adults. The aim of this research was to formulate a nutritious flavoured honey using different components and to study its nutritional, functional profile. Samples were taken in four different compositions with addition of Brahmi, Clove and Honey in different concentrations. Results showed that out of four samples with different concentrations S1 demonstrated the highest percentage of antioxidant activity (57.4±0.4) followed by samples S2, S3 and S4 which reported 56.4±0.2%, 55.6±0.2% and 53.2±0.3% antioxidant activity respectively. Cloves which include eugenol increase self-life of new food product. Consumption of this flavoured honey also helps to strengthen an individual's immune system.

Keywords: Antioxidant, *Syzigiumaromaticum*, *Bacopa monnieri*

1. Introduction
The immune system is considered as a host defence system comprising of several living structures and tasks in a body that safeguards against disease. For an immune system to work efficiently, it must diagnose a broad range of pathogens including viruses and parasitic worms, and discriminate them from the healthy tissues that an organism possess.
In Jawed vertebrates, including living beings these are much increasingly modern protection components, (Beck and Habicht, 1996) [4] including the capacity to adjust after some time to recognize explicit pathogens all the more practically. Autoimmune diseases, inflammatory diseases and cancer can be caused by immune system disorders. (O'Byrne and Dalgleish, 2001) [15] Immunodeficiency happen when the immune system is less imperative than usual, leading to repetitive and deadly infections.
When the immune system over responds to the liability of antigens present in the environment, allergic disorders occur. These substances that evoke such assaults are known as allergens. These invulnerable reactions can cause side effects, for example swelling, common cold, cough, sore throat, watery eyes, and even a life-risking reactions called anaphylaxis. Thus, immunity is the reasonable condition of several celled organisms having adequate natural defences to fight infection, disease, and other unwanted biological invasion while having requisite tolerance to avert allergy, and autoimmune diseases.
Nowadays, undesirable way of life is pursued by many individuals because of which they experience sickness, incapacity and demise. Issues like metabolic sicknesses, joint and skeletal agony, hypertension, overweight, savagery, etc., can be brought about by undesirable way of life. Unhealthy diet, improper sleep, pollution, alcohol consumption, drug abuse, stress etc. causes damage to the immune system. Long-term infections cause variation among individual immune systems. Individuals without these diseases, are not influenced even with the incidental cold or fever and their immune system remains generally stable after some time. These are some common natural ingredients which can be used to strength an individual's immune system.
Since earlier times honey has both food and medical usage. Honey is a natural substance obtained from honeybees, *Apismillifera*, from the nectar of flowers or from transudates of trees and plants producing nectar honeys or honeydews, respectively. Honey was an important food for humans since ancient times as it was the only available natural source of sweetness. Moreover, the relationship between honey bees and Homo sapiens was established in the early Stone Age. (Crane, 1983) [8].

Honey is indispensably a extremely rigorous water solution comprising of two sugars, dextrose and levulose, with little measures of least 22 other progressively complex sugars. The shade of honey ranges from light yellow through ambers to a darkish red golden to about black.

In extended human folklore, honey has been used both as a nutrient and as a medicine. (Jones, 2001) [21]. In several cultures, nectar has been utilized for its therapeutic properties, for example, in treatment of burns, ulcers and healing of wounds as honey gives a calming impact when at first administered to open injuries (Coulston, 2000) [7]. The physical properties of honey provides a defensive hindrance, because of its high osmolarity and creates an injury mending condition as an answer which does not stick to the wound tissues and averts bacterial colonization. Thus, honey decreases and lower the formation of exudates more promptly than do standard treatments (Coulston, 2000) [7]. In many cases, honey is being used successfully as infections which are not cured by standard antibiotics and antiseptic therapy.

Clove (*Syzigiumaromaticum*) are fragrant dried flower buds which belong to the family Myrtaceae. Clove comprises of 10% fixed oil (non-volatile and saponifiable), 15-20% fundamental oil (volatile and unsaponifiable), 6-7% non essesntial ether extract (containing ethyl caproate, methyl caprylate and methyl stearate, and so on) and 13% tannins, aside from glycosides, (for example, acetophenoneglucosides) and flavonols (Pathak *et al.,* 2004) [17].

The primary compound present in clove is eugenol that is utilized as an antiseptic, antibacterial, pain relieving agent in conventional therapeutic practices. Nowdays, it is applicable in pharmaceuticals and nourishment items and in refreshment items like beverages as a flavouring agent. Eugenol has popular medical advantages. Clove is one of the chief spices in Indian kitchen. It has been accounted for as a strong chemopreventive specialist, which is utilized by conventional Ayurvedic healers of India since many years for curing respiratory and stomach related infirmities (Banerjee *et al.,* 2006) [3]. Because of the presence of eugenyl acetate, clove possess excellent antihistamine and antispasmodic properties.

Clove has a powerful impact against oral bacteria which is associated with dental caries and also periodontal diseases (Ramadan *et al.,* 1996) and furthermore efficient against other bacteria counting *Listeria monocytogenes*, *Escherichia coli*, *Salmonella enteric* (Friedma *et al.,* 2002) [10] etc. Antiallergic, anticarcinogenic and antimutagenic activities of clove have been reported in many studies. Studies have likewise revealed antifungal, anticarcinogenic and antimutagenic tasks of clove. A few discoveries have additionally unveiled the antiviral properties of clove and also the inhibitory impact of clove on viral infections like Herpes Simplex Virus (HSV) and Hepatitis C Virus (HCV) (Montes-Belmont and Carvajal, 1998; Shiraki, 1998) [13, 23]. Subsequently clove can give resistance against microorganisms, like bacteria, fungi and virusess and have insecticidal, antioxidant and anti-inflammatory activities. (Scott *et al.,* 2009) [22].

*Bacopa monnieri*is a perennial crawling herb having a place with family Scrophulariaceae, local towards the westlands of Southern and Western India, Australia, Europe, Africa, Asia and North & South America (Wiersema, 1994) [24]. B. monnieri is an herb which is utilized in Ayurveda, where it is recognized as "Brahmi", named after Brahma. It is a non-aromatic herb and can develop in water which makes it a well known aquarium plant. It can likewise develop in marginally harsh conditions. Bacopa monnieri has been utilized in conventional Ayurvedic medication for different purposes including memory decrease, swelling, torment, pyrexia, epilepsy and furthermore as a narcotic (Russo and Borreli, 2005) [20]. Extract of B. monnieri contains Bacoside A and Bacoside B which are steroidal saponins and are assumed to be important for the clinical strength of the item.

Although Brahmi has many uses, but its effect in enhancing memory has bewitched most attention. It also has anti-inflammatory (Jain, 1994) [11], anxiolytic and antidepressant actions, (Bhattacharya and Gtioshal, 1998) [6], relaxant properties in the blood vessels (Dar & Channa, 1999) [9] and adaptogenic activity (Rai, *et al.,* 2003) [18].

There are many active constitutents found in Brahmi, of which saponins are the major constituent which are incharge for most of its pharmacological actions (Russo & Borreli, 2005) [20].

Brahmi is an excellent cognitive enhancer and also offers protection from neusodegenerative disorders. With regards to improvement of memory function, Brahmi has more effect on reducing forgetfulness rather than increasing learning. (Pase *et al.,* 2012) [16].

If these natural ingredients honey, clove and brahmi are mixed together to form a paste like compound, the resulting preparation can be used to strengthen a person's immune system.

2. Material and Methods

2.1 Procurement of raw materials

Fresh clove buds, honey and *Bacopamonnieri* powder were procured from the local Premnagar market, Dehradun.

2.2 Preparation of sample (clove and brahmi mixed honey)

The collected raw materials are kept under sterilized conditions. Fresh clove buds are ground into a fine powder using grinder or mixer. Honey is warmed slightly so that clove and brahmi can easily mix in it. Finally, clove, honey and brahmi are mixed together in appropriate concentrations and the samples are stored in suitable containers at room temperature in a dry place.

Collection of honey, clove and brahmi
↓
Keeping under sterilized conditions
↓
Grinding of fresh clove buds
↓
Mixing of clove powder in honey
↓
Mixing of brahmi powder
↓
Storage at room temperature in a dry place

Sample	Concentration of ingredients		
	Honey	Clove *(Syzigiumaromaticum)*	Brahmi *(Bacopamonnieri)*
S1	10ml	2g	1g
S2	8ml	1g	0.5g
S3	6ml	0.5g	0.2g
S4	4ml	0.1g	0.1g

2.3 Methods

2.3.1 Moisture Content

Moisture content was estimated by the standard procedure of

Rangnna, 1986. The moisture content can be described as the amount of water present in food sample. For determining the moisture content in the sample, dry empty petri dish is weighed and then 2g of sample is added to it and it is kept in hot air oven at 110°C for 2-3 hours. After the given time the petri dish are kept in the desiccators to cool down and the weight is taken using weighing balance. Calculation is done by the formula:

$$\text{Moisture content (\%)} = \frac{W2-W3}{W} \times 100$$

Where, W = weight of sample (g);W2 = weight of empty petri dish (g) + sample (g);W3 = weight of the petri dish after dying (g)

2.3.2 pH

The pH was estimated by the method given by Bogdanov, 2009. The digital pH meter was used to calculate the pH value of the sample. Before analysis standard buffer solution of pH 4.0 and 9.1 and of double distilled water solution of pH 7.0 was used for the standardization of pH meter. Aqueous solutions of honey were made by dissolving 1g of honey in 10 ml distilled water (10% w/v).

2.3.3 Reducing Sugar

The total reducing sugar was estimated by the method given by Wang, 1999. The absolute reducing sugar was resolved utilizing 3,5-dinitrosalicylic acid(DNSA).In guideline the reducing sugar decreases DNSA to 3-amino-5-nitrosalicylic acid bringing about the development of reddish- orangish colouration which is estimated spectrophotometrically at 540nm.

2.3.3.1 Procedure

3ml of DNS reagent (prepared by adding dinitrosalicylic acid, 10g; phenol, 10g;sodium sulphite, 0.5g) was added to 3ml of glucose sample in a test tube. The mixture was heated at 90ºC for 5-15 minutes in order to develop the red brown colour. Then 1ml of 40% potassium sodium tartrate was added to stabilize the colour. The mixture was cooled to room temperature by putting in a cold water bath and thereafter absorbance was recoreded at 575nm using a spectrophotometer.

2.3.4 Total Phenolic Content (mg GAE/100g)

Total phenolic content was measured by the method of Makkar, H.P.S., *et.al*, 2007.

2.3.4.1 Procedure

0.57 gm of sample was mixed in 11.5 ml of distilled water. From this solution 1 ml was taken and subsequently 0.5 ml Folin-ciocalteu and 2.5 ml Na_2CO_3(20%) was added in test tubes after vortexing the mixture with the help of vortex shaker and thereafter placing the test tubes for 40-45 minutes in the dark and taking the absorbance at 760 nm with UV-VIS spectrophotometer.

2.3.4.2 Preparation of standard curve

A standard curve of phenolic content was plotted in the range of 50-500 mg GAE/L by taking 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 mg/ml in test tube and making up the volume to 1 ml using distilled water. Thereafter adding 0.5 ml of folin-ciocalteu reagent and 2.5 ml of 20% sodium carbonate in each test tube and keeping for 40-45 minutes in a dark place and taking the absorbance at 725 nm.

2.3.5 Antioxidant activity by DPPH

The DPPH test, which estimates the capacity of compounds to exchange apt H-atoms to radicals, is the simplest technique to evaluate the antioxidant activity (Brand-Williams *et al*., 1995). The reflection of hydrogen by this steady free radical causes bleaching with a greatest absorbtion band around 515-528 nm and can be simply observed spectrophotometrically.
DPPH (2,2-diphenyl-1-picryl hydrazyl) radical scavenging action was estimated by an altered version of Blois (1958).

2.3.5.1 DPPH Reagent

0.004% DPPH was made by measuring 10 mg (0.01 gm) of DPPH reagent in 250 ml of methanol (90%). Its absorbance was taken in UV- Visible spectrophotometer at 517 nm, absorbance should be 0.98±0.02.

2.3.5.2 Procedure

Sample solution was prepared by measuring 0.57 gm of extract in 11.5 methanol (80%). From the stock solution, aliquot was made by blending 10µl aliquot from the stock solution with 990µl of methanol in the test tube separately. After this 4 ml of 0.004% DPPH was incorporated to every sample concentration in the test cylinders and thereafter blended by vortexing. Then the sample was incubated in the dark for 30 mins at room temperature. After incubating for 30 minutes, the absorbance was estimated utilizing an UV Visible Spectrophotometer (UV-7804C) at 517 nm. Free radical scavenging activity or inhibition activity of free radical DPPH in percentage (IA%) was calculated with the help of the following formula:

$$\text{IA\%} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

3. Results and Discussion

Parameters	S1	S2	S3	S4
Moisture Content (%)	15.3±0.5	15.8±0.4	16.1±0.5	16.9±0.6
pH	4.1±0.1	4±0.2	3.8±0.1	3.5±0.2
Reducing Sugars (%)	64.8±0.6	60.3±0.5	57.9±0.2	53.2±0.2
Total Phenolic Content (mgGAE/g)	87.8±0.3	46.4±0.4	25.1±0.1	8.8±0.1
DPPH Scavenging Activity (%)	57.4±0.4	56.4±0.2	55.6±0.2	53.2±0.3

3.1 Moisture Content

The moisture content (%) in the given samples was in the range of 15.3 to 16.9.As per Codex standards, the maximum prescribed limit for honey should be below 20% (Codex Alimentarius, 2001). Due to this range of moisture content of flavoured honey, the samples are prone to unwanted fermentation during storage that occurs due to the activity of osmotolerant yeasts leading to the development of ethyl alcohol and carbon dioxide. The alcohol can be further oxidized leading to the formation of acetic acid and water as a result of which honey becomes sour in taste. It is therefore important to store the flavoured honey in airtight containers.

The results of the analyzed samples were parallel to the previously calculated results by (Ahmed *et al.,* 2014) [2] where the moisture content ranged from 15.87 to 17.4.

3.2 *pH*

The pH of the given samples was found to be 4.13.4, 3.8, 3.5 respectively. This acidity is because of the acidic substances present in nectar, fundamentally amino acids and organic acids that are accountable for the peculiar flavour of honey. The average acidity of honey is 3.9 but its range is3.4 to 6.1.Since the sample also contains clove power, which has a pH of 3.8, the samples showed a considerable increase in the acidity. The results obtained were similar to the previously determined values for some Indian honeys whose resultant values ranged from 3.7 to 4.4 (Saxena *et al.,* 2010) [21].

3.3 Reducing Sugars

The percentage of reducing sugars in flavoured honey samples ranged from 56.9 to 64.8 respectively. Since the amount of reducing sugars found in clove and brahmi is neligible, honey is the major component which contributed to these values of reducing sugars in flavoured honey samples. Glucose and fructose are the major reducing sugars found in honey. Quantitatively fructose is always the most significant sugar after glucose. The percentage of reducing sugars in sample 1 was found to be the highest as it had maximum amount of honey. The results obtained were comparable with the previously calculated values for some Indian honeys whose resultant values ranged from 57 to 65.5% (Saxena *et al.,* 2010) [21].

3.4 Total Phenolic Content

The total phenolic content of the samples was in the range 8.86 to 87.8 mg GAE/g respectively. It was seen that the phenolic content of S1 was highest as it had the highest quantity of clove compared to the rest of the samples. Clove is entitled to be a chief vegetative source containing phenolic compounds like flavonoids, hidroxibenzoic acids, hidroxicinamic acids and hidroxiphenylpropens. The use of honey as a base material also contributes to high polyphenolic composition of these flavoured honey samples as honey contains considerable amount of benzoic acids, cinnamic acids, flavonoids (quercetin, luteolin, kaempferol, chrysin, galangin) and phenolic acids (caffeic acid, gallic acid, vallinic acid, chlorogenic acid). Bacoside A present in brahmi also contributed to the polyphenolic composition of these flavoured honey samples. There exists a critical connection between antioxidant properties and the total phenolic content which shows that the phenolic compounds are the key donor of antioxidant properties of the plant extracts. Hence, higher is the antioxidant capacity higher will be the phenolic acid composition. The similar results of honey, clove and brahmi were reported individually by Gautam *et al.,* 2010 [21]; Nisha and Arulmozhi, 2103 [14] and Bhardwaj *et al*., 2016 [5] respectively.

3.5 Antioxidant activity by DPPH

DPPH has been generally employed toassess the free radical scavenging effectiveness of different antioxidant rich substances. With this technique it is feasible to predict the antiradical intensity of an antioxidant by estimating the decline in the absorbance of DPPH at 517 nm bringing about a colour change from purple to yellow. It was found that the percentage of DPPH scavenging activity ofthe flavoured honey samples ranged 53.26 to 57.45 respectively. It can be seen that the DPPH free radical scavenging activity of every sample depends on the concentration of honey clove and brahmi used. Hence, this paste provides a good source of antioxidants to the body. The antioxidant capacity of flavoured honey is because of the existence of natural compounds such as flavonoids, phenolic acids and some enzymes, caroteniod like substances, organic acids. Since the sample contains cloves which is rich in phenolic compounds like phenolic acids (gallic acid), flavonolsglucosides, phenolic volatile oils (eugenol, acetyl eugenol) and tannins, it proved to be a mercenary source of polyphenols. *Bacopamonniera* present in flavoured honey also contributed to its antioxidant activity as itb contains a good quantity of bacoside A and bacoside B. The results obtained were consistent with the values obtained for the DPPH scavenging activity of honey, clove and brahmi by Gautam *et al.,* 2010 [21]; Adaramola and Onigbinde, 2016 [1] and Bhardwaj *et al*., 2016 [5] respectively.

4. Conclusion

Clove is entitled to be a chief vegetative sources containing phenolic compounds like flavonoids, hidroxibenzoic acids, hidroxicinamic acids and hidroxiphenylpropens. The antioxidant capacity is high in all samples due to the presence of natural compounds available in clove and brahmi. There exists a critical connection between antioxidant properties and the total phenolic content which shows that the phenolic compounds are the key donor of antioxidant properties of the plant extracts. Hence, higher is the phenolic acid composition higher is the antioxidant activity. Sample S1 demonstrated highest percentage of antioxidant activity which was found to be 57.4±0.4.The moisture content of the flavoured honey samples was found to be in the range of 15.5% to 16.9% respectively. Due to this reduced moisture content, the flavoured honey samples exhibit longer shelf-life. The pH level of samples represent the average acidity of honey. of Cultivated Plants 413 (pp. 109-116).

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