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RESEARCH ARTICLE

PHYTOCHEMICAL ANALYSIS OF VARIOUS HONEY SAMPLES OBTAINED FROM THENI DISTRICT, SOUTH INDIA

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ABSTRACT

This study was undertaken to evaluate the phytochemicals of various pure honey samples. Three types of honey samples were collected from different area of Western ghats, Theni district, South India. The raw honey samples under study were marked as sample 1 (S1), sample 2 (S2) and sample 3 (S3). Various solvents such as aqueous, ethanol, ethyl acetate and methanol were used for extraction of honey samples. The phytochemicals such as alkaloids, carbohydrates, amino acids, proteins, flavonoids, glycosides, phenols, saponins, tannins, phlobtannins and terpenoids were analyzed by standard laboratory methods in honey sample extractions. The results showed that the Sample 1 (S1) methanol extractions are good sources of valuable phytochemicals as compared to other two samples.

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INTRODUCTION

Honey is a sweet liquid made by bees using nectar from flowers. Bees first convert the nectar into honey by a process of regurgitation and evaporation, and then store it as a primary food source in wax honeycombs inside the beehive. Honey can then be harvested from the hives for human consumption (Joseph Nordqvist, 2015). Traditionally honey is good sources of energy produced by honeybees (Sanz *et al*., 2004). It also has antioxidant and antimicrobial properties. It is a complex mixture of invert sugar, carbohydrates, aromatic substances, waxes, mineral, pollen grains, pigments, organic and amino acids (Gheldof *et al*., 2002). The actual composition of honey varies, depending on many factors such as the pollen source, climate, environmental conditions, and the processing it undergoes (Gunduz *et al*., 2008). All honey containing phytochemicals are important. The beneficial medicinal effects of honey typically result from the combinations of secondary products present in the honey. In honey, these compounds are mostly secondary metabolites such as alkaloids, carbohydrates, amino acids, proteins, flavonoids, glycosides, phenols, saponins, tannins, phlobtannins and terpenoids (Ferreres *et al*., 1994). In the present study, the various honey samples collected from Theni district are in the southern part of Tamilnadu. The district is surrounded by the Western Ghats, with its green stretches of cultivated lands, valuable herbals and tea gardens. Three types of honey samples were collected from various places of Theni district. Phytochemical properties of these honey samples were evaluated using Official Methods of Analysis (AOAC).

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MATERIALS AND METHODS

3 types of honey samples were used in this study were purchased from local bee keepers in various places of Theni District, Tamilnadu, South India in the month of October 2015. They were marked as sample 1 (S1), sample 2 (S2) and sample 3 (S3). The samples were stored in a dark place at room temperature. Several experiments were conducted to ascertain that the honey samples were pure and original. These include (Elijah *et al*., 2015):

I. Dropping some of the sample onto sand: if it is a pure honey, it will not sink immediately.
II. Pouring a small quantity into a cup of water: if pure, it will go down to the bottom of the cup without mixing up with the water except when stirred.

III. Dipping a finger into the honey sample, dropping one or two drops on the ground: if it is pure, it will go down like a thread without breaking.

Processing of honey samples (Olusolade and Akintayo, 2014)

Each sample was first filtered with a sterile mesh to remove debris; viscosity was reduced by heating honey at 30°C for 30 minutes. The samples were checked for purity by inoculating on blood agar plates and incubated overnight. Uncontaminated samples were stored at refrigeration temperature of about 4°C until used.

Preparation of honey extracts using various solvents (Abhishek Chauhan *et al.*, 2010)

Extraction of raw honey samples were performed by using various solvents such as aqueous, ethanol and ethyl acetate. For this 10 g of honey was taken in a centrifuge tube with 25 ml of solvent and then mixed well by vortexing and shaking with hands for about 30 minutes. This was centrifuged at 3000 rpm for 10 minutes at 25^0C. Supernatant was collected from each centrifuged tube in a round bottom flask by filtration. The resulting supernatant was dried under nitrogen gas with a temperature of 50^0C. All the extracts dissolved in DMSO were collected in stopper test tubes for phytochemical analysis.

Qualitative methods for phytochemical analysis (AOAC 1990)

The qualitative test for phytochemicals such as alkaloids, carbohydrate, aminoacids, proteins, flavonoids, glycosides, phenols, phlobatanin, sapanin, tannins and terpenoids of honey samples were carried out by Official Methods of Analysis (AOAC).

Detection of alkaloids

Mayer's test

50 mg of solvent free extracts was stirred with a few ml of diluted Hcl and filtered. To a 1ml of filtrate, few drops of Mayer's reagent are added by the side of the test tube. The white or creamy precipitated indicated as positive.

Wagner's test

5gm of honey sample was extracted by boiling in 50 ml of distilled water in a water bath for 30 mins. Samples were then filtered into a test tube and filtrate collected. To a 1 ml of filtrate, few drops of wagner's reagent were added by the side of the test tube. The color change was observed. A reddish brown precipitates confirms the test as positive.

Dragendorff's test

5gm of each honey sample was extracted by boiling in 50 ml of distilled water in a water bath for 30 mins. Samples were then filtered into a test tube and filtrate collected. To a 1ml of filtrate, 2ml of dragendorff's reagent are added and the result was observed carefully. A prominent yellow precipitate confirms the test as positive.

Detection of carbohydrate

Fehling's test

1ml of each honey extracts were boiled on water bath with 1ml each of fehling solutions A and B. the color change was observed. A red precipitates indicated the presence of sugar.

Barfoed's test

To 1ml of honey extracts, 1ml of barfoed's reagent was added and heated on boiling water bath for 2 mins. The color changes was noted and recorded. A red precipitated indicated the presence of sugar.

Benedict's test

To 0.5ml of honey extracts,0.5 ml of Benedict's regeant was added. The mixture is heated on a boiling water bath for 2 mins and the result was observed. A red precipitates indicated the presence of sugar.

Detection of Aminoacids and Proteins

Ninhydrin test

Two drops of ninhydrin solution (5 mg of ninhydrin in 200 ml of acetone) was added to two ml of aqueous filtrates. The color change was observed. A chararacteristic purple color indicated the presence of aminoacid.

Xanthoproteic test

The extracts were treated with a few drops of concentrated nitric acid. Formation of yellow colour indicates the presence of protein.

Biuret test

An aliquot of 2ml of filtrate was treated with drop of 2% copper sulphate solution. To this 1 ml of ethanol (95%) was added, followed by excess of potassium hydroxide pellets the pink colour in ethanol layer indicated the presence of proteins.

Detection of flavonoids

Alkaline reagent test

A few drops of diluted NaOH were added to the extracts. An intense yellow colour was produced and become colourless after of a few drops of diluted acid added indicating that the presence of flavonoids.

Lead acetate test

The extracts were treated with a few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Ferric chloride test

1 ml of the extract was treated with 1 ml of ferric chloride. The formation of brown color precipitates indicates the presence of flavonoids.

Table 1. Phytochemical analysis of various honey samples

S.No.	Phytochemical constituents	Aqueous extract			Ethanol extract			Ethyl acetate extract			Methanol extract		
		SI	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3
1	Alkaloids	-	-	-	-	-	+	+	-	+	+	-	+
2	Carbohydrate	+	+	+	+	+	+	+	+	-	+	+	+
3	Aminoacids and proteins	+	-	-	+	-	-	+	-	-	+	-	-
4	Flavonoids	+	-	+	+	+	-	+	-	+	+	+	+
5	Glycosides	-	-	-	-	+	-	+	-	-	+	+	-
6	Phenols	+	-	-	+	-	-	-	+	-	+	-	-
7	Phlobatannins	-	-	-	+	+	-	-	-	-	+	-	+
8	Saponins	+	-	-	-	-	-	+	+	-	+	-	+
9	Tannins	-	-	+	+	-	+	+	-	-	+	+	-
10	Terpenoids	+	-	-	+	-	-	-	-	+	+	-	+

Detection of Glycosides

Legals test

Chloroform (3ml) ammonia solution (10%) was added to 2ml of each honey extract.

Detection of Phenols

Lead acetate test

Raw honey extract (5mg) was dissolved in distilled water and 3 ml of 10% lead acetate solution was addded. A bulky white precipitates indicated the presence of phenols.

Ferric chloride test

50mg of each honey extracts were dissolved in 5 ml of distilled water. To this few drops of neutral 5% Ferric chloride solution was added. A dark green colour indicated the presence of phenolic compound.

Gelatin test

50mg of each honey extract was dissolved in 5 ml of distilled water, 2ml of 1% solution of gelatin containing 10% sodium chloride was added to it. White precipitate indicated the presence of phenolic compound.

Detection of Phlobatannins

Few drops of 2% Hcl were added to 0.5 ml of honey extracts. Appearance of pink colour precipitates indicated the presence of phlobatannins.

Detection of saponins

Distilled water 2ml was added to the each extract and shaken in a graduated cylinder for 15 mins lengthwise. Formation of 1cm foam indicates the presence of saponin.

Detection of Tannins

Ferric Chloride test

The 5mg of each extract was dissolved in 5 ml of distilled water and few drops of neutral 5% Ferric chloride solution was added. The formation of blue green color indicated the presence of tannins.

Detection of Terpenoids

Chloroform (2ml) and concentrated sulphuric acid was added carefully to 0.5ml of each extract. Formation of red brown color at the interface indicated the presence of terpenoids.

RESULTS

Several experiments were conducted to check the purity and originality of the honey samples. That experiments result showed that these honey samples were pure and original. The samples were checked for purity by inoculating on blood agar plates and incubated overnight. No growth was observed on blood agar plates after incubation. This study has revealed that the presences of phytochemicals are considered as active medicinal chemical constituents. Important medicinal phytochemicals such as alkaloids, carbohydrate, aminoacids and proteins, flavonoids, glycosides, phenols, phlobatannins, saponins, tannins and terpenoids were present in the samples (Table 1). The result of the phytochemical analysis shows that the three honey samples are rich in at least one of alkaloids, carbohydrate, aminoacids and proteins, flavonoids, glycosides, phenols, phlobatannins, saponins, tannins and terpenoids. Methanol extracts of S1 honey samples having all these phytochemicals. The phytochemical analysis of 3 honey samples studied showed that the methanol extract of S1 honey sample was rich in all of these phytochemicals.

DISCUSSION

The research work was carried out on the three selected honey samples which shows that phytochemical constituent's i.e., alkaloids, carbohydrate, aminoacids and proteins, flavonoids, glycosides, phenols, phlobatannins, saponins, tannins and terpenoids are either present or absent in these honeys and the results were summarized in Table 1. These classes of compounds had known to posses some therapeutic properties against several pathogens and are therefore supporting its traditional uses to cure diseases (Pietta, 2000). In previous studies phytochemicals are present in honey samples have been reported for its wound healing properties; these are anti-inflammatory and analgesic (Gill, 1992) and antioxidant (Harborne, 1992). In previous studies it was reported that the some phytochemicals were present in aqueous extract of the honey sample (Edeoga *et al*., 2005) which some of the phytochemicals were found to be absent in it. The recent research studies and previous research studies result were different, so it might be due to the change in location, geographical variation and climatic conditions. All these phytochemicals were found to be present in all extracts of honey samples according to the previous investigations (Turhan, 2007) while in the present investigation, all these phytochemicals were present in the methanol extract of the S1 honey sample was more concentration as compared to other phytochemicals in other extracts of honey samples. The presence of all ten phytochemical compounds in the methanol extract S1 honey sample showed that it possesses some therapeutic properties against several pathogens and are

therefore S3 honey sample supporting its traditional uses to cure diseases.

Conclusion

All these three varieties of honey samples are easily available in Western Ghats, Theni district and contains some of the phytochemicals especially as alkaloids, carbohydrate, aminoacids and proteins, flavonoids, glycosides, phenols, phlobatannins, saponins, tannins and terpenoids energy which having medicinal importance. The methanol extract of S1 honey sample containing all these ten phytochemicals than the other extracts of honey samples showed that it possesses bioactive compounds which are used for curing of various human diseases and also play an important role in healing

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