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Kshitij Navinprakash Shah
Associate Professor,
Department of Food Science,
A.S.C College, Chikhaldara,
Amravati, Maharashtra, India

Corresponding Author:
Kshitij Navinprakash Shah
Associate Professor,
Department of Food Science,
A.S.C College, Chikhaldara,
Amravati, Maharashtra, India

Studies on phytochemical composition of wild honey from Melghat province

Kshitij Navinprakash Shah

Abstract
Melghat is a dense forest region of central India with very rich flora and fauna. The wild honey found here is assumed to have tremendous therapeutic properties as it is used as a medicine by the tribal for years. Melghat honey is analyzed for the detection of phytochemicals. The standardized qualitative screening tests are applied for the detection of tannins, phenolic compounds, alkaloids, glycosides, saponins, steroids, carbohydrates, proteins, tri-terpenoids, and flavonoids. It is concluded from the results that tri-terpenoids are not found in any of these samples while most of the samples gave negative inference for alkaloids. All the other mentioned phytochemicals are detected in the Melghat honey samples.

Keywords: phytochemicals, Melghat, wide honey, extracts, amravati

1. Introduction
The Province of Melghat is located in a part of the Amravati district of central India. It is a hilly area comprising two sanctuaries and one tiger reserve (National park) in the Maharashtra state. The locality is predominantly of tribal people. The people are considerably dependent on forest products for their earnings. Honey is one of the most important forest produce collected and sold by the local people[1]. Due to the very prosperous biodiversity and availability of various kinds of flowers in all seasons of the year, the wild honey bees found a supportive atmosphere for their nourishment. Excellent quality honey with a prominent mixture of nectars of a variety of flowers is produced in the Melghat. It is found to have incredible medicinal properties [1-3].
Despite the other parameters, the therapeutic properties of honey are very much dependent on its phytochemical profile. A phytochemical analysis is a crucial aspect of the research of any plant-originated natural material.
Phytochemicals are also termed secondary metabolites, which are the chemical compounds formed during the various metabolic processes and they are essential for the growth of plants[4]. These are the bioactive compounds that possess human health-enhancing properties. This non-nutritive group of chemicals has the potential to protect us from a variety of health disorders[5]. In this study, the qualitative detection of phytochemicals in the Melghat honey samples is carried out.

2. Material and Methods
a. Collection of honey samples
Wild honey samples were collected from four diverse locations of the *Melghat* forest. It was assumed that the honey samples were produced by the wild species of honey bees i.e. *Apis dorsata* and *Apis cerana indica.* The honey samples were prepared for analysis [6].

b. Preparation of extracts
It was reported that most of the constituents of honey were extracted by using water as a solvent. The aqueous extract of honey was prepared by centrifuging the sample at the speed of 3000 rpm for ten minutes [5].

c. Phytochemical screening
The phytochemical screening of the wild honey samples was done by using the standard methods prescribed in the various articles published in reputed journals with slight modifications. The phytochemicals such as saponins, alkaloids, phenolic compounds, tannins, glycosides, tri-terpenoids, and flavonoids are detected to evaluate the functional and medicinal properties of the honey samples under investigation

I. Tests for glycosides

A. Keller-Kiliani test

1-2 drops of $FeCl_3$ solution were added to 4 ml glacial acetic acid solution taken in a test tube. Honey extract (10 ml) and concentrated sulphuric acid (2 ml) were added. Brown ring formation across the boundary of two immiscible solutions indicates the presence of glycosides [7].

B. Salkowski's Test

Two milliliters of H_2SO_4 solution were added to the 10 ml of extract and stirred well. The formation of reddish-brown color indicated the presence of glycosides [8].

II. Tests for alkaloids

A. Wagner's Test

To the five milliliters of the extract, 1 ml. of each Wagner's reagent and HCl were added. The formation of reddish-brown color shows the presence of alkaloids [7].

B. Dragendorff's Test

To five ml of honey extract, 2 ml of the Dragendorff's reagent and some drops of HCl were added. The formation of orange/ red precipitate shows the presence of alkaloids [9].

III. Tests for Saponins

A. Foam Test

1 ml of honey extract and 9 ml of distilled water were mixed with vigorous shaking and were allowed to stand for 10 minutes. The formation of stable foam shows the presence of saponins [9].

IV. Tests for tannins and phenolic compounds

A. Lead acetate test

2 ml each of the extract and 10% lead acetate solution were added to a test tube. The formation of white precipitate confirms the test for tannins and phenolic compounds [9].

B. Ferric chloride test

5 ml of the extract honey extract was mixed thoroughly with some drops of 2% $FeCl_2$ solution. Turning the solution to a blackish-blue or brownish-green color indicates the presence of the phenolic compounds and tannins [8].

V. Tests for steroids

A. Ring test

2 ml extract, 10 ml chloroform, and 1 ml acetic anhydride were mixed together in a test tube. Two ml of concentrated H_2SO_4 was poured slowly along the side of the test tube with slanting. The ring is formed on the boundary of solutions [9].

VI. Tests for tri-terpenoids

A. Methanol test

2 ml of extract was mixed with 1 ml of methanol. 4 to 5 drops of the blend were taken on a spot plate. It was then titrated with some drops of anhydrous acetate and H_2SO_4. The red color is formed [10].

VII. Tests for proteins

B. Ninhydrin test

The mixture of 5 ml of the honey extract and 2 ml of 0.2% solution of ninhydrin was boiled. The appearance of the violet color showed the presence of proteins [8].

VIII. Tests for flavonoids

A. Shinoda Test

5 ml of extract, 3 ml of concentrated HCl, and some pieces of magnesium ribbon were kept for some time taken in a test tube. The formation of reddish-pink color indicates the presence of flavonoids [8].

IX. Tests for carbohydrates

A. Fehling's test

5 ml of extract was hydrolyzed by HCl and then neutralized with some alkali. The contents were heated after the addition of an equal amount of Fehling's solutions A and B (2 ml). Red precipitate was formed as an indication of the presence of carbohydrates [8].

B. Benedict's test

Test solution i.e. extract was taken in a test tube. Few drops of Benedict's reagent were added to the extract and it was heated to boiling in a water bath. The reddish-brown precipitate was formed [11].

3. Results

There were four types of honey samples subjected to the phytochemical screening. The following results were obtained against the screening tests (Table 1).

Table 1: Phytochemical screening results of Melghat honey samples

S N	Phytochemical compounds	Test performed	Results obtained for the honey samples			
			H_1	H_2	H_3	H_4
1	Glycosides	Killer-Kiliani Test	+ ve	+ ve	+ ve	+ ve
2		Salkowski's test	+ ve	+ ve	+ ve	+ ve
3	Alkaloids	Wagner's Test	-ve	-ve	-ve	-ve
4		Test Dragendorff's Test	-ve	-ve	+ ve	-ve
5	Saponins	Foam Test	+ ve	+ ve	+ ve	+ ve
6	Tannins and Phenolic compounds	Lead acetate test	+ ve	+ ve	+ ve	+ ve
7		Ferric chloride test	-ve	+ ve	-ve	+ ve
8	Steroids	Ring test	+ ve	+ ve	+ ve	-ve
9	Tri-terpenoids	methanol test	-ve	-ve	-ve	-ve
10	Proteins	Ninhydrin test	+ ve	+ ve	+ ve	+ ve
11	Flavonoids	Shinoda test	+ ve	+ ve	+ ve	+ ve
12	Carbohydrates	Fehling's test	+ ve	+ ve	+ ve	+ ve
13		Benedict's test	+ ve	+ ve	+ ve	+ ve

4. Discussion

The tests for tri-terpenoids were found negative for all the honey samples while only one test out of the eight tests carried out for the detection of alkaloids was found positive. Thus it was revealed from the data that tri-terpenoids and alkaloids are not present in the *Melghat* honey samples.

The tests for all other secondary metabolites were found positive as an indication of the presence of glycosides,

saponins, steroids, tannins and phenols, carbohydrates, proteins, and flavonoids in the *Melghat* honey samples. Five results were positive for tannins and phenolic compounds out of the eight tests carried out thus there is the presence of tannins and phenolic compounds but might not be found in a significant amount.

There is a need for a detailed study of the *Melghat* honey samples for the phytochemical profile. The properties of the phytochemicals present in the *Melghat* honey make it an excellent natural forest produce that might be useful in the treatment of many clinical diseases and disorders.

Flavonoids observed to be present in these honey samples have tremendous clinical significance. These secondary metabolites of plants reported having antioxidant potential, the capacity of scavenging the free radicals, usefulness in the prevention of coronary heart disorders, anticancer, antiviral, and anti-inflammatory potential. They are reported as the essential components in pharmaceuticals, nutraceuticals, cosmetics, and medical applications.

Phenols and tannins are exhibited to be active in the uptake of nutrients, enzyme activation, and synthesis of proteins while working as an inhibitor of tumor genesis and pro-carcinogens. These are also found useful in the prevention of diseases caused due to free radicals. Tannins work fabulously against diarrhea, stomach tumors over and above as an inhibitor of breast and pancreatic cancer. They are also possessing anti-inflammatory, antiseptic, and antioxidantal properties.

Saponins are synthesized for the defense system in plants. They are useful in wound healing, as anti-hepatotonic, and anti-inflammatory agents. They have a cytotoxic effect against many cancer lines [12].

It was reported that some alkaloids may possess toxic properties [13]. Due to this nature of alkaloids, alkaloids containing honey may not be useful as medicines [14]. But it was said that a low amount of alkaloids did not show harmful effects [15]. It was revealed in some studies that alkaloids have protective properties that are useful in the inhibition of proliferation as well as it has an antimetastatic effect for cancers [12].

Alkaloids, flavonoids, and glycosides have properties that are beneficial for the good health of the heart favored by stimulating its activities. Alkaloids are effective in cold and cough as well as malaria. Flavonoids are useful in the healing of skin diseases, wounds, and skin ulcers as they reduce wound acidity and show anti-inflammatory effects [16].

Many researchers have reported the presence of various phytochemicals in the honey samples that are investigated. Most of them detected phenolic compounds and flavonoids. There was no data found regarding the investigation of the *Melghat* honey samples for the phytochemicals. The results are varied significantly. There are differences found in the phytochemical profile of honey samples from different origins. But it is clear from all the reports that the honey samples are rich in phytochemicals, some may be lacking some particular secondary metabolites.

The phytochemicals in honey are responsible mainly for its restorative properties against plenty of pathogens that are the cause of its traditional use in the remedies of many ailments. The differences in the use of honey in wound healing, anti-allergic, antioxidant, anti-inflammatory, and various therapeutic applications are due to the variations in the climatic conditions of the geographical area as well as the locations and other properties [10, 11, 17-19].

5. Conclusion

Detection of the phytochemicals was done qualitatively in the current study by keeping in mind the importance of phytochemicals in the detection of the therapeutic as well as medicinal properties of any natural food material.

The secondary metabolites produced during the plant's metabolism that are essential for their growth are known as phytochemicals. Phytochemicals are a member of a large group of compounds such as gums, Saponins, polysaccharides, terpenoids and triterpenoids, flavonoids, phenolic compounds & tannins, glycosides, alkaloids, etc.

During the current work, the *Melghat* honey samples were examined qualitatively for the screening of phytochemicals. Some of the important phytochemicals such as tannins & phenolic compounds, alkaloids, glycosides, saponins, steroids, carbohydrates, proteins, tri-terpenoids, and flavonoids were detected qualitatively by applying the standard screening test methods that are published earlier.

It was observed from the results that tri-terpenoids did not present in any of the honey samples since all the honey samples gave a negative response to their screening tests. The same conclusion could be drawn for alkaloids also as only one screening test was found positive for alkaloids while a total of eight tests were done. Thus alkaloids were also absent in the *Melghat* honey samples under examination.

Other than the above-discussed phytochemicals i.e. alkaloids and tri-terpenoids, other remaining compounds were found to be present in the honey samples. Flavonoids, glycosides, steroids, saponins, tannins and phenols, proteins, carbohydrates, and proteins got positive inference by all the honey hence said to be present in the *Melghat* honey samples. For phenolic compounds and tannins, five tests out of eight were positive thus the amount of these phytochemicals might be very less in the samples.

Most of the honeys showed good results in the screening tests of the remaining compounds. It can be concluded from the results that the *Melghat* honey samples are having a good profile of phytochemicals which make them natural curative agents against plenty of diseases.

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