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STUDY OF ANTIOXIDANT AND PHYTOCHEMICAL PROPERTIES OF COMPOUNDS FROM *Trigonella foenum-graceum* and *Trigonella Corniculata*

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ABSTRACT

Bioactive compounds are gaining immense attention in the commercial sector owing to their enormous applications in myriad of industries. These include alkaloids, steroids, flavonoids, terpenoids, tannins etc. Numerous plants have been explored so far to obtain different types of phytochemicals. These compounds are used by the human beings as antioxidant, anti-microbial, anti-cancer, anti-hypersensitive. In the present study, Trigonella foenum-graceum (methi) and Trigonella corniculata (kasuri-methi) was used to extract different value added compounds to establish its nutraceutical potential. Its ethanolic extract was found to contain sugars, flavonoids, tannins, alkaloids, steroids but free amino acids were absent.

KEY WORDS

Alkaloids, Bioactive compounds, Flavonoids, Steroids, Trigonella foenum-graceum, Trigonella corniculata

INTRODUCTION

Bioactive compounds are natural occurring molecules and these molecules are synthesized by the plants, animals, marine organisms etc [1]. Most of the fermented products of marine (fish, microalgae and seaweeds), dairy, fruits and vegetable; are also known as the bioactive compounds. For example, milk and colostrum of bovine are considered as a most important source of bioactive compounds [2]. These compounds are used by the human beings as antioxidant, anti-microbial, anti-cancer, anti-hypersensitive compounds. Many of plant material used in traditional medicines are readily available in rural areas. The use of plant extract and phytochemical can be of great significance in therapeutic treatments and would help to cure the problems of these multidrug resistant microorganisms [3]. A worldwide increase in the incidence of bacterial infections as well their resistance to different antibiotics has been observed. Thus, there is need for numerous new antimicrobial agents to be used in these situations [4]. Antibacterial and antifungal effect of the aqueous, methanol and chloroform extracts of *Nigella sativa* seeds against the various strains like *Candida albicans, Staphylococcus aureus* were studied. These extracts were compared against various standard drugs [5]. Numerous plants with bioactive compounds have been reported so far like *Digitalispurpurea, Seneciojacobaea, Cicutavirosa, Cicutavirosa, Narthecium ossifragum,*

Aconitum septentrionale, Pteridiumaquilinium, Taxusbaccata, Quercus spp. *etc*. Ethanolic extract of stem bark, root bark and leaves of *Terminalia avicennoides* were assayed on the strains like *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* for their antimicrobial activity. The important bioactive agents like phenols, steroids, glycosides, flavonoids, tannins were detected in bark, leaves and roots of *T. avicennoides*. Thus, there is a need of constant search for more potent and cheaper raw material to feed the drug industry. Extensive research and development efforts may add medicinal plants to provide raw material to the indigenous and herbalists [6]. Therefore, the main objective of Present study was to analyse the phytochemical, antioxidant and antimicrobial nature of *Trigonella foenum-graceum* and *Trigonella corniculata*. *Trigonella foenum-graceum* and *Trigonella corniculata* is an annual legume crop mainly grown for use as spice in many parts of the world [7]. *Trigonella foenum-graceum* is known as one of the oldest medicinal plant [8]. Leaves and seeds of *Trigonella foenum-graceum* have been used to prepare extracts for medicinal use [9]. *Trigonella foenum-graceum* have anti-diabetic, anti-fertility, anti-cancer [10, 11], antimicrobial [12], antiparasitic and hypocholestrolaemic effects [13]. The seeds of *Trigonella foenum-graceum* herb possess toxic oils, volatile oils and alkaloids. The potential use of these propagated plants as a source for newer drugs is still under investigation [14].

MATERIALS AND METHODOLOGY

Collection of Plant extract

Methi seeds (50.0 gm) and kasuri-methi (25.0 gm) (*Trigonella foenum-graecum* and *Trigonella corniculata*) were purchased from the main market, Ropar, Punjab. 25.0 gm of the methi seeds were soaked in water for 24 h and remaining 25.0 gm of seeds and 25.0 g, of kasuri-methi was homogenized with blender to make powder.

Preparation of extract

The soaked methi seeds, homogenized dried methi seeds and homogenized kasuri-methi were added separately to the flasks, each containing 100ml of solvent. Temperature was maintained at the boiling point of the solvent (ethanol-78.5°C). These flasks were incubated in the rotating shaker incubator for 24-48 h. The content of the flask was filtered using Whatman filter paper. The dried extracts were stored at 4°C in a refrigerator [15].

Phytochemical analysis

The extracts were tested for the presence of active compounds such as diterpenes, sugars, flavonoids, alkaloids, proteins, free amino acids, glycosides, carbohydrates, steroids, saponins, phenols, tannins [16].

Test for sugars

Fehling's test: Fehling solution A was prepared by using 7gm of $CuSo_4.5H_2O$ in 50ml of distilled water containing 2 drops of H_2SO_4. Fehling solution B was prepared by using 35 gm of potassium tartrate and 12 gm of NaOH in 100ml of distilled water. The two solutions were mixed in 1:1 ratio. The mixture (2 ml) was added in the test tube followed by addition of 3 drops of extract. The test tubes were placed in a water bath at 60°C. A positive test was indicated by the green suspension and red precipitates.

Molisch test: Molisch reagent was prepared by 10 gm of Napthol in 100ml of 95% ethanol. 1ml of extract was taken in a test tube. After that, 2-3 drops of Molisch reagent was added followed by addition of 2 ml of conc. H_2SO_4. A positive test was indicated by the formation of red or brown ring in a test tube.

Tests for phenolic compounds

Test for flavonoids (Aluminium test): 1ml of extract was taken in a test tube. Added dilute NaOH drop wise into the test tubes. An intense yellow color was formed and then added few drops of dil. H_2SO_4. A positive test was indicated by the formation of colorless liquid after the addition of dilute acid.

Test for tannins (ferric chloride test): 5% solution of ferric chloride was prepared in 90% of alcohol. 1ml of extract was taken in test tubes and drops of ferric chloride solution were added to it. A positive test was indicated by the formation of dark green or deep blue color.

Tests for alkaloids

Potassium dichromate test: One ml of extract was taken in a test tube. Added potassium dichromate solution drop wise in it. A positive test was indicated by the formation of dark color.

Dragendorff's test: One ml of extract was taken in a test tube. Added Dragendorff's reagent drop wise in the test tube. A positive result was indicated by the formation of orange red precipitate or suspension.

Mayer's test: One ml of extract was taken in a test tube. 5ml of dilute HCl was added in the test tube. The test tubes were incubated in a water bath at 60°C for 15-30

mints. After that added Mayer's reagent drop wise in the test tube. A positive test was indicated by the formation of turbidity in the test tubes.

Test for steroids

One ml of extract was taken in a test tube. The extract was dissolved in 10 ml of chloroform followed by addition of 10 ml of conc. H_2SO_4. A positive test is indicated by the formation of red color ring in a test tube with the yellow or green fluorescence.

Test for free amino acids (Ninhydrin test) Ninhydrin reagent was prepared by the addition of 0.1 gm of ninhydrin reagent in 100ml butanol. In this test 1ml of extract was taken in a test tube. The ninhydrin reagent was added drop wise in test tube. A positive test was indicated by the formation of violet purple suspension.

Test for saponins (foam test) 1ml of extract was taken in a test tube. The little amount of sodium bicarbonate and water was added in this test tube and mixed properly. A positive test was indicated by the formation of honey comb like froth.

Test for proteins: The protein concentration was calculated by Lowry method.

Test for carbohydrates: The carbohydrate concentration was calculated by Anthrone method [17].

Free Radical Assay: DPPH (2,2-diphenyl-1-1picrylhydrazyl) method was used for free radical assay in order to determine the antioxidant properties [18].

RESULTS AND DISCUSSION

Bioactive compounds such as flavonoids, alkaloids, terpenoids, proteins, tannins, sugars, steroids, amino acids and carbohydrates are known to be involved in various defense mechanisms against various microorganisms, herbivore and insects. The presence of pharmacological compounds in the extracts of *Trigonella foenum-graceum* (methi) was reported in the literature [19]. In the present study, different bioactive compounds like flavonoids, alkaloids, terpenoids, saponins, free amino acids, proteins, carbohydrates and tannins was studied. For this purpose, ethanolic extracts were prepared and phytochemical analysis was performed.

Test for sugars

Fehling's test: No red precipitates were formed after the addition of Fehling reagent in the extracts of kasuri-methi and unsoaked methi seeds, therefore, sugars are absent in these extracts. But there were formation of red precipitates after the addition of Fehling's reagent in the extract of soaked methi seeds confirming the presence of reducing sugars in the extract (figure 1).

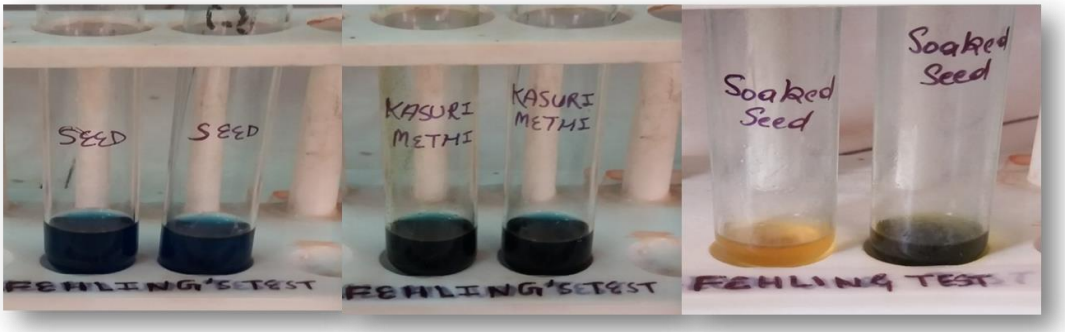

Figure 1: Fehling test

Molisch test: No color change was appeared in the extract of kasuri-methi after the addition of Molisch reagent, therefore, sugars are absent in this extract. But there was a change in color in the extracts of both unsoaked and soaked methi seeds after the addition of Molisch reagent (figure 2). Therefore, sugars are present in extracts of unsoaked and soaked seeds.

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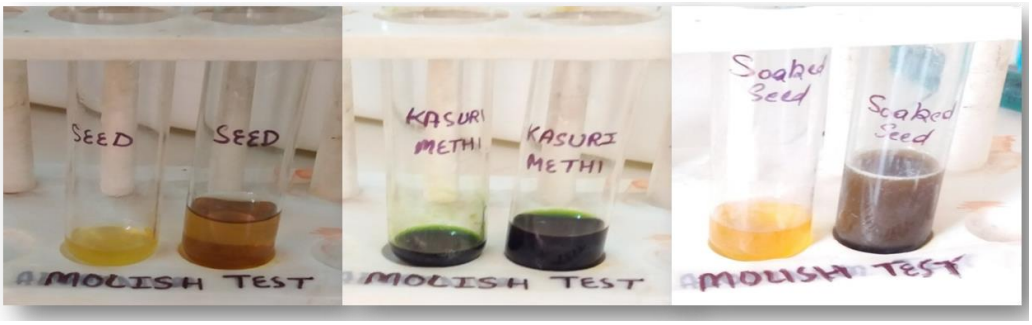

Figure 2: Molish test

The sugars were found in the ethanolic extracts of unsoaked and soaked seeds but in extracts from kasuri-methi sugars were absent The similar results with the extracts of fenugreek seeds was also reported in literature [20].

Test for phenolic compounds

Test for Flavonoids (aluminium test): Yellow color was turned into the colorless liquid after the addition of dilute H_2SO_4 in all extracts of unsoaked seeds, soaked seeds and kasuri-methi. Therefore, it confirms the presence of flavonoids in these extracts (figure 3).

Figure 3: Aluminium test

Test for tannins (ferric chloride test): Deep green or deep blue color was obtained after the addition of ferric chloride in all extracts of unsoaked seeds, soaked seeds and kasuri-methi. It showed that tannins were present in these extracts (figure 4).

Figure 4: Ferric chloride test

Tannins are the naturally occurring compounds that are mostly used in food and leather industries. Our present

study showed the presence of tannins in the extracts of unsoaked, soaked seeds and kasuri-methi similar to the study reported in literature [15].

Test for alkaloids

Potassium dichromate test: Dark color was obtained after the addition of potassium dichromate solution in all the extracts of unsoaked seeds, soaked seeds and kasuri-methi. Therefore, it showed the presence of alkaloids in all these extracts (figure 5).

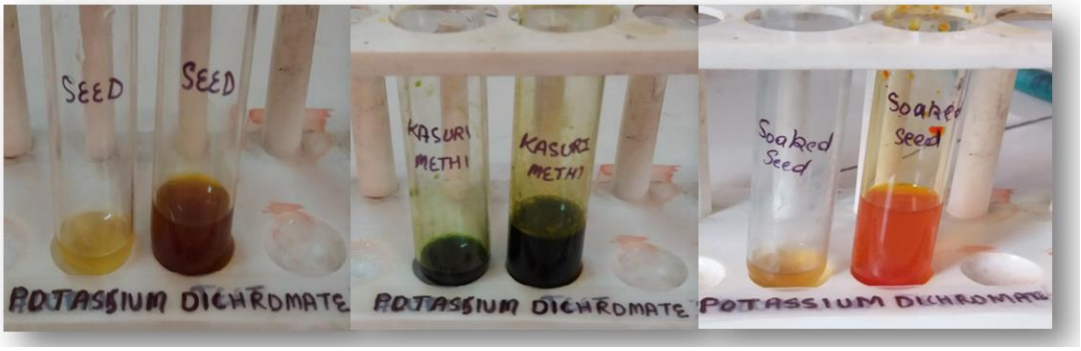

Figure 5: Potassium dichromate test

Dragendorff's test: Orange color was formed after the addition of dragendorff's reagent in all the extracts of unsoaked seeds, soaked seeds and kasuri-methi; confirming the presence of alkaloids in all the extracts (figure 6).

Mayer's test: After the addition of Mayer's reagent in all the extracts of unsoaked seeds, soaked seeds and kasuri-methi turbidity was formed showing the presence of alkaloids in all these extracts (figure 7).

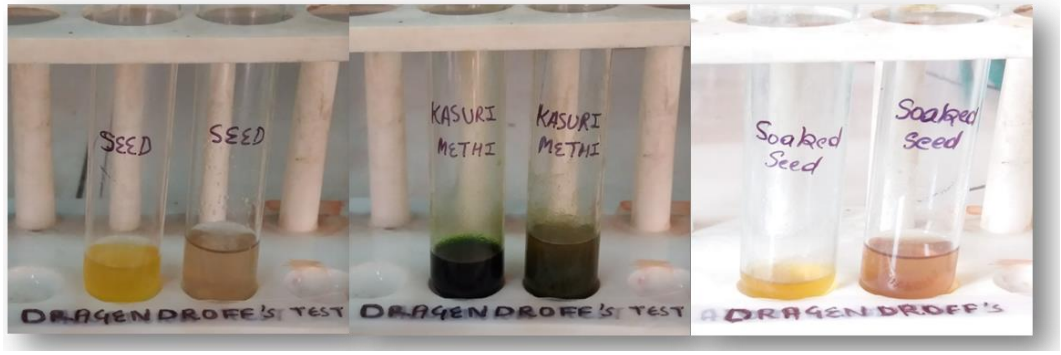

Figure 6: Dragendorff's test

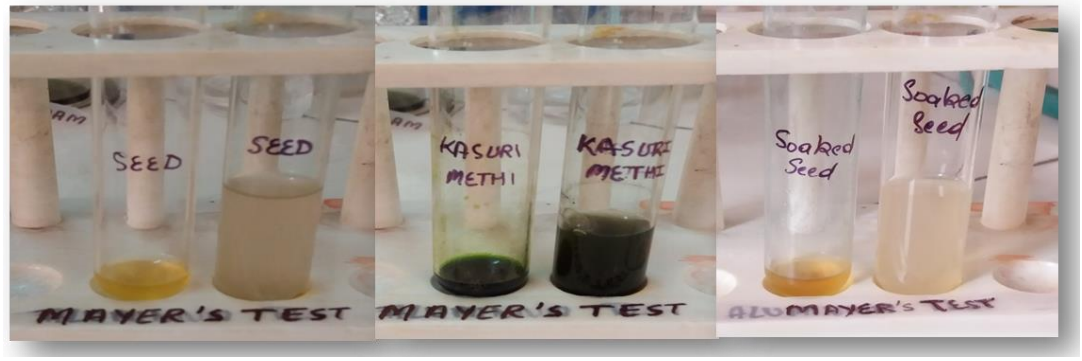

Figure 7: Mayer's test

Flavonoids were found in the ethanolic extract of unsoaked seeds, soaked seeds and kasuri-methi. Alkaloids are the naturally occurring compounds from plants. Diet that contains alkaloids is very helpful for healing the wounds, ulcers, hemorrhoids and burns [21]. Our present study showed the presence of alkaloids in the ethanolic extracts of all the samples *i.e.* unsoaked methi seeds, soaked methi seeds and kasuri-methi. Numerous reports are in accordance with our study [15,21].

Test for Steroids

After the addition of concentrated H_2SO_4 in all the extracts of unsoaked seeds, soaked seeds and kasuri-methi a red color ring with yellow and green fluorescence was formed suggesting the presence of steroids in all these extracts (figure 8).

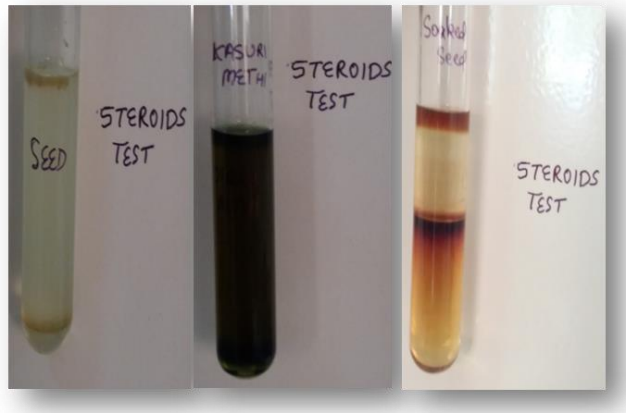

Figure 8: Test for steroids

The steroids were detected in the extracts of unsoaked, soaked seeds and kasuri methi. Amino acids are the essential nutrients that are present in various food products. But in the present study, the amino acids were absent in the extracts of unsoaked, soaked seeds and kasuri methi but in contrast some showed the presence of amino acid 4-hydroxyisoleucine in the extract of fenugreek seeds.

Test for free amino acid (Ninhydrin test)

No violet color was formed in all the extracts of unsoaked seeds, soaked seeds and kasuri-methi. Therefore, free amino acids were absent in these extracts (figure 9).

Figure 9: Ninhydrin test

Test for Terpenoids (Foam test)

Honey comb like froth was obtained after vigorous shaking in the extract of kasuri-methi and soaked seeds. Therefore, terpenoids were present in these extracts. But no honey comb like froth was obtained in unsoaked seeds suggesting the absence of terpenoids in the same (figure 10).

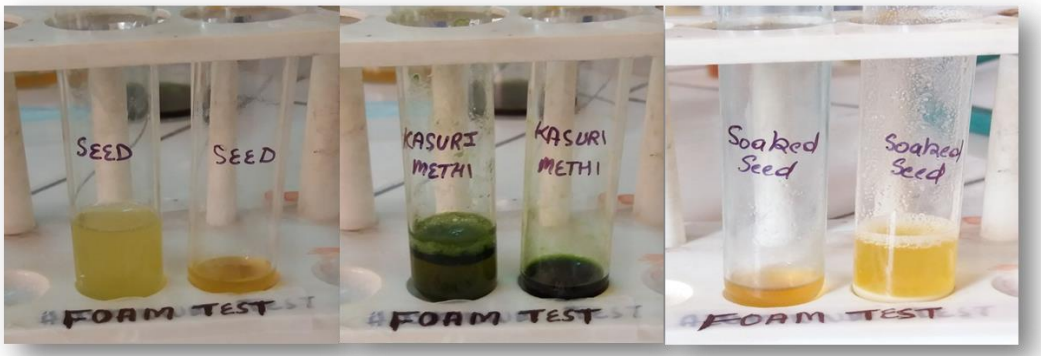

Figure 10: Foam test

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Terpenoids are the constituent of essential oil of both plant and flowers. Our study showed the presence of terpenoids in the extracts of soaked methi seeds and kasuri-methi but there were no terpenoids present in unsoaked seeds extract. The literature showed the presence of terpenoids in the extract of leaves and shoot tips of methi.

Test for Proteins

The concentration of protein in unsoaked seeds, soaked seeds and kasuri-methi was calculated by lowry method (table 1).

Table 1: Concentration of proteins

Source	Protein concentration (mg/ml)
unsoaked seeds	0.908
soaked seeds	0.426
kasuri-methi	0.664

Proteins are very essential component of our diet that are needed for the survival of all animals and human beings. The basic function of protein is to supply the adequate amount of required amino acids. Diet deficient in protein causes the growth retardation, muscle wasting, abnormal swelling of belly etc. In the present study protein content in unsoaked seeds was 0.908mg/ml, soaked seeds was 0.426mg/ml and in kasuri methi was 0.664mg/ml. Protein content in the present study was lower. These differences are may be due to the climatic conditions, temperature *etc*.

Test for Carbohydrates (Anthrone method)

The concentration of carbohydrates in unsoaked seeds, soaked seeds and kasuri-methi was calculated as shown in table 2.

Table 2: Concentration of carbohydrates

Source	Carbohydrate concentration (mg/ml)
unsoaked seeds	0.373
soaked seeds	0.072
kasuri-methi	0.049

Carbohydrates are the used as an energy source, flavoring agent, dietary fiber. These are also used to prevent ketosis. In the present study the carbohydrates content was 0.373mg/ml in the unsoaked seeds, 0.072mg/ml in soaked seeds and 0.049mg/ml in kasuri-methi**.** The literature showed the favorable amount of carbohydrate content in fenugreek extract.

Free radical assay (DPPH method)

The concentration of free radicals in unsoaked seeds, soaked seeds and kasuri-methi was calculated as shown in table 3.

Table 3: Concentration of free radicals

Source	Free radical concentration (mg/ml)
unsoaked seeds	0.787
soaked seeds	0.092
kasuri-methi	0.606

The DPPH radicals are widely used to test the potential of the compounds as free radical scavengers of hydrogen donors. These are also used to investigate the antioxidant activity of plant extracts. The result of DPPH assay in the present study indicates that soaked seeds, unsoaked seeds and kasuri were active against free radical scavenging. This suggests that the seed extract contain compounds that are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity. The

quantitative analysis of the extracts showed the presence of high amount of total phenols (0.787mg/ml in unsoaked seeds, 0.092 mg/ml in soaked seeds and 0.606mg/ml in kasuri-methi).

Thus, the present study was carried out to establish methi as a potential candidate for bioactive compounds extraction. The extraction of bioactive compounds was carried from both dry and water-soaked seeds to get an overview of changes in concentration of bioactive compounds after imbibition of water by the methi seeds. Kasuri-methi was also subjected to extraction of bioactive compounds to visualize differences in the concentration and presence or absence of different bioactive compounds in comparison to methi seeds.

CONCLUSION

The presence of bioactive compounds in *Trigonella foenum-graec*um (methi) and *Trigonella corniculata* (kasuri-methi) showed that these can be potential sources of nutraceuticals and flavoring agents. The flour from the leaves can be incorporated into processed foods and thus provide health along with taste. Stems also have very good antioxidant properties and good amount of phytochemicals. Furthermore, the high correlation observed between the various assays employed and presence of phenolics indicated that (total, free and flavonoids) are among the predominant sources of antioxidant activity in both the species of *Trigonella*. It can therefore be concluded that apart from flavor and fragrance, kasurimethi and methi leaves have a lot to offer in terms of health improvement. Phytochemical analysis showed the presence of terpenoids, sugars, alkaloids, flavonoids, proteins, carbohydrates, saponins, phenols, steroids and tannins. In conclusion *Trigonella foenum-graceum* and *Trigonella corniculata* contain considerable amount of bioactive compounds and are good source of bioactive compounds. So, these can be potentially exploited for the development of various value-added products and other food applications to provide enhanced nutrition and improved health.

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