



Phytochemical Analysis of Alfalfa (*Medicago sativa*) Seed Extract by Soxhlet Extraction Using Different Solvents

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Date of Receipt- 03/02/2014
Date of Revision- 11/02/2014
Date of Acceptance- 14/02/2014

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ABSTRACT

According to a recent report nearly 42 per cent Indian kids are malnourished and stunted. The aim of our project was to develop nutraceutical products using alfalfa as one of the components to overcome undernourishment particularly among women and children. The seeds of alfalfa (*Medicago sativa*) contain more nutritional property when compared with other leguminous seeds. It contains 18.9% of protein when compared with egg (13.1%), milk (3.3%) and beef (16.5%). This paper reports the investigation results of phytochemical analysis of alfalfa seed extracts using the procedure of Sadasivam and Manickam (2009). The presence of proteins, carbohydrates, saponins, phenolic compounds, alkaloids, flavonoids etc. was observed in sprouted alfalfa seeds extracted by solvents such as ethanol, petroleum and chloroform. The presences of proteins, carbohydrates, saponins, alkaloids etc were confirmed by the present investigation. Thus the cost effective nutraceutical product containing alfalfa along with other cereals and pulses would serve the humanity to fight undernourishment in an easy and economical way.

Keywords: Phytochemical, Alfalfa, Petroleum, Chloroform, Ethanol.

INTRODUCTION

As a social concern oriented and need of the hour development this project aims to strengthen the population of under nourished children in India who account for nearly 42 % as per the recent survey by the Naandi Foundation in their Hunger and Malnutrition (HUNGAMA) report¹. Under nutrition is a consequence of consuming too

few essential nutrients or excreting them more rapidly than they can be replaced. Alfalfa is a perennial plant which belongs to the legume family-Leguminosae. It grows up to 2-3 feet tall and has smooth and erect stem. Leaves are pinnately trifoliate, Flowers are purple-violet in colour, found in racemes form and its flowering season is

June to August, and the seeds are spirally-coiled in form. It is also called as “Father of all Plants”. Until now alfalfa seeds are not used widely for human consumption for increasing the nutritional level because of the presence of canavanine sulfate. It is most commonly used for cattle and horse feed. By reducing the presence of canavanine sulfate by proper laboratory methods, we can use *Medicago sativa* (alfalfa) seeds for daily consumption as a good provider of nutrition.

Health benefits of alfalfa sprouts

Sprouted alfalfa seeds contain saponins, which are reported to be toxic to red blood cells *in vitro*. However, it is harmless to human consumption and has many health beneficial properties like anti-inflammatory, immune-stimulating activity, anti-tumor activity etc. Alfalfa sprouts have about 8% saponins content according to commercial sprout growers. When compared with alfalfa seeds sprouting increases the saponin content to 450%. Saponins bind with bile acids. Some large intestinal bacteria converts bile into highly carcinogenic substance where, bile that binds with saponins prevents the formation of toxin³. Saponins appear to be beneficial, being responsible for major part of cholesterol lowering effect of legumes. Saponins have a direct stimulatory effect on the immune system². Saponins inhibit cancer cells in many ways. Saponins will fight against the fungal, microbial, viral infections. Presence of saponins in alfalfa sprouts will destroy tumor causing cells particularly in lung and blood cancer it is more effective. It gives more immunity for stomach.

MATERIALS AND METHODS

The laboratory methods include sprouting, autoclaving, roasting and powdering. Sprouting is a method to reduce

the canavanine sulfate from the seeds. After sprouting the seeds were autoclaved at 121°C, roasted and powdered and processed for further use. After powdering of sprouted alfalfa seeds the soxhlet extraction was done in order to get a concentrated extract. The extraction was done with the help of powder of alfalfa sprouts by using three successive solvents namely Chloroform, Petroleum Ether and Ethanol. The recovery % of Petroleum ether, Ethanol and Chloroform extracts were 12%, 8% and 14% respectively. With the help of extract the phytochemical studies are done. Phytochemical analysis of seed extracts was conducted using the procedure of Sadasivam and Manikam's (2009) methodology. By this analysis, the presences of several phytochemicals were tested.

Iodine test

The iodine solution was prepared by adding iodine to 2% potassium iodide solution till the colour becomes deep yellow. 0.5 g of the concentrated extracts of *Medicago sativa* from 3 successive solvents was added with a few drops of the prepared iodine solution².

Fehling's test

Fehling's reagent A: 34.65g copper sulphate was dissolved in 500ml distilled water. Fehling's reagent B: 125g potassium hydroxide and 173g Rochelle salt (potassium sodium tartarate) was dissolved in 500ml distilled water. To 1ml of Fehling's A, add 1ml of Fehling's B and a few mg of the extract which is called as the test sample. Boiled for few minutes and noted the colour change².

Test for flavonoids

0.5mg of the extract or 0.5 ml of the extract in solvent was taken. 1ml of ethyl acetate was added to the extract to observe the formation of yellow organic layer. The

formation of yellow organic layer indicates the presence of flavonoids².

Test for tannins

To 1ml of the extract or few mg of the concentrated test sample, 1% HCl was added. The mixture was boiled in hot water bath for few minutes. For preparing 1% HCl, 0.1 ml of concentrated HCl was added to 10ml of distilled water. The formation of red precipitate shows the presence of tannins².

Test for saponins

0.5ml of the extract or 0.5mg of the test sample was added to 5ml of distilled water. The content was shaken well for few minutes. The froth formation was observed after 20 minutes for the conformation of saponins².

Test for alkaloids

0.5mg of the test sample was dissolved in 1% HCl and filtered using Whatmann No.1 filter paper. To the filtrate few drops of Dragendorff reagent was added. The formation of prominent yellow precipitate indicates the presence of alkaloids².

Test for phenolic compounds

To 0.5mg of the extract, 3 drops of 1% ferric chloride solution was added and mixed with 1ml of potassium ferrocyanide. The formation of bluish green colour indicates the presence of phenolic compounds².

Test for phytosterols

0.05mg of the extract was dissolved in 0.5ml of chloroform. From the sides of test tube concentrated sulphuric acid was added. The formation of red colour indicates the presence of Phytosterols².

Spot test

The presence of oily substance in the extracts was determined by spot test. Few milligrams of the extract was taken on a filter paper. The filter paper was folded and pressed against the extract kept within. The appearance of oily layer was observed².

Test for carbohydrates

100mg of the extract was dissolved in 5ml of water and filtered. The filtrate obtained was subjected to Fehling's test as described earlier. 0.5 ml of the filtrate obtained from above step was boiled with 0.5ml of Fehling's reagents A and B².

Ninhydrin test

This test was done to detect the presence of proteins. The aqueous filtrate if the powdered seed sample was taken. 2 to 3 drops of Ninhydrin solution was added to 2 ml of the aqueous filtrate of the sample².

RESULTS AND DISCUSSION

Test for flavonoids

0.5mg of the extract was taken. 1ml of ethyl acetate was added to the extract to observe the formation of yellow organic layer. In this plant extract from ethanol solvent shows the formation of yellow organic layer [Plate 1.3].

Test for tannins

To 1ml of the extract .1% HCL was added. The mixture was boiled in hot water bath for few minutes. The formation of red precipitate shows the presence of tannins. In this plant extract, there was no formation of red precipitate, which indicates the absence of tannins [Plate 1.3].

Test for phenolic compounds

To the few mg of extract, 3drops of 1% ferric chloride solution was added and mixed with 1ml of potassium ferrocyanide.

The formation of bluish green colour in the ethanol extract of the plant indicates the presence of phenolic compounds [Plate 1.4].

Test for saponins

0.5ml of the extract was added to 5ml of distilled water. The content was shaken well for few minutes. The froth formation was observed after 20minutes which indicates the presence of saponins. Ethanol and chloroform extracts showed the frothing formation which indicates the presence of saponins [Plate 1.5].

Test for alkaloids

Few milligrams of the sample was dissolved in 1% HCL and filtered using Whatmann filter paper. To the filtrate few drops of Dragendroff reagent was added. The formation of prominent yellow precipitate indicates the presence of alkaloids. In this chloroform extract shows the formation of prominent yellow precipitate which indicates the presence of alkaloids [Plate 1.2].

Test for Phytosterols

0.05mg of the extract was dissolved in 0.5ml of chloroform. Then to this mixture of concentrated sulphuric acid was added along the sides of the test tubes. Red colour indicates the presence of phytosterols.

Spot test

The presence of oily substance in the extract was determined by spot test. Few mg of extract was taken in the filter paper. The filter paper was folded and pressed against the extract kept within. The appearance of oily layer was observed in ethanol, chloroform and petroleum extract which indicates the presence of oily substance in the plant.

Test for carbohydrates

100mg of the extract was dissolved in 5ml of water, it was filtered. The filtrate obtained was subjected to Fehling's test as describe earlier. 0.5ml of the filtrate obtained

from above was boiled with 0.5ml of Fehling's A and B. the formation of red precipitate indicates the presence of carbohydrates. In this ethanol extract shows the formation of red precipitate and it indicates the presence of carbohydrates [Plate 1.1].

Ninhydrin test

This test is done to detect the presence of protein. The aqueous filtrate of the powdered seed sample was taken. 2 to 3 drops of ninhydrin solution was added to 2ml of aqueous filtrate of the sample. There is no formation of purple colour indicates the presence of protein [Table 1].

SUMMARY AND CONCLUSION

The toxic substance canavanine sulfate from alfalfa seeds are removed by applying some laboratory process like sprouting, air drying, roasting and it is made as powder by grinding for human consumption for increasing the nutritional level. Alfalfa seeds are not to be taken by pregnant women. Its use is restricted to them even though in the absence of canavanine sulfate. In the present investigation the presence of proteins, carbohydrates, saponins, alkaloids etc were confirmed by different extraction methods Thus our cost effective nutraceutical products containing alfalfa along with other cereals and pulses would serve the humanity to fight undernourishment in an easy and economical way. Our future studies are to check the effectiveness of the nutraceutical products in animal models and human volunteers.

ACKNOWLEDGEMENTS

The authors are grateful to the Chancellor of Karunya University, Coimbatore, India Dr. Paul Dhinakaran, Vice Chancellor Dr. E. J. James, Registrar Dr C.

Joseph Kennady, Director School of Biotechnology and Health Sciences, Dr. M. Patrick Gomez for their encouragement and support.

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Table 1. Preliminary phytochemical analysis

Compounds	Ethanol	Petroleum ether	Chloroform
Saponins	+	-	+
Phenolic compounds	+	-	-
Carbohydrates	+	-	-
Spot test	+	+	+
Tannins	-	-	-
Alkaloids	-	-	+
Phytosterols	-	-	-
Flavonoids	+	-	-

Plate 1. Phytochemical analysis

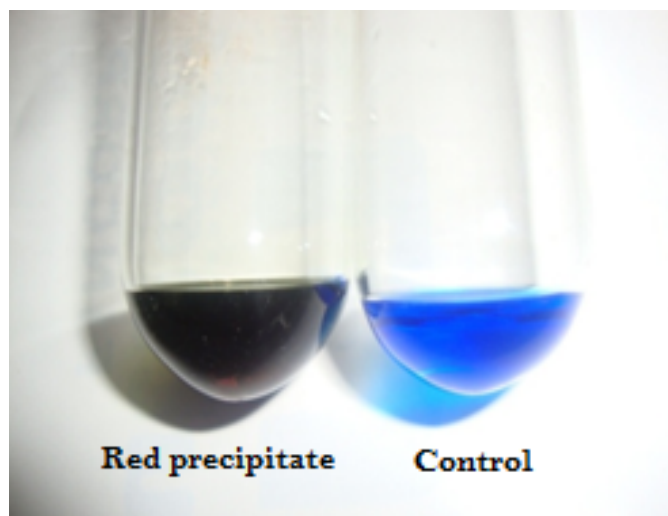


Plate 1.1. Presence of carbohydrate Plate

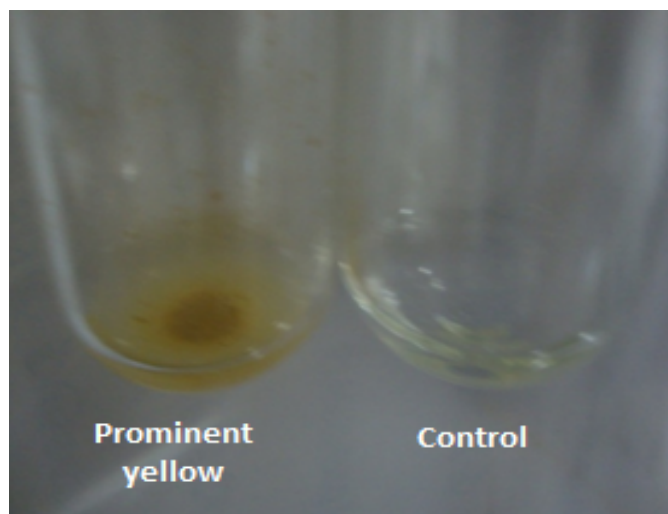


Plate 1.2. Presence of alkaloids

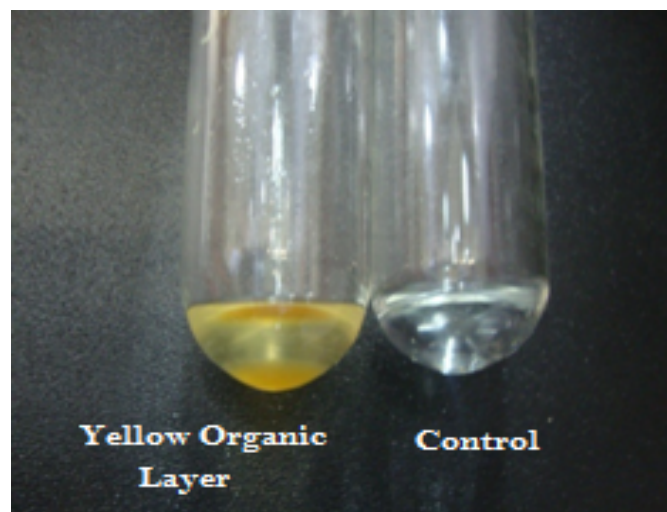


Plate 1.3. Presence of flavonoids Plate

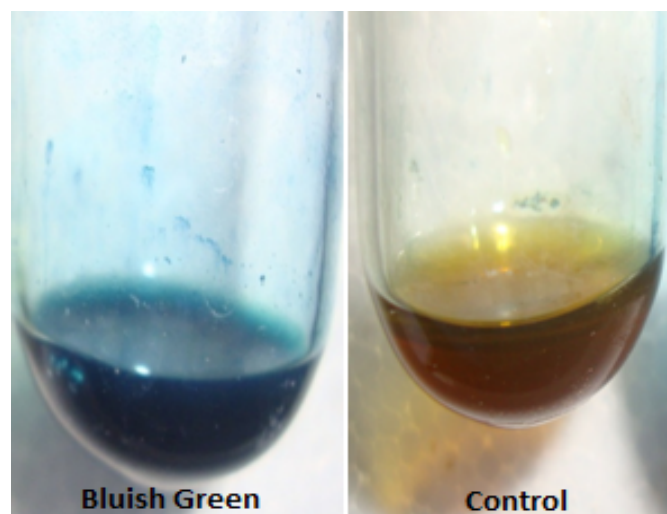


Plate 1.4. Presence of phenolic compound

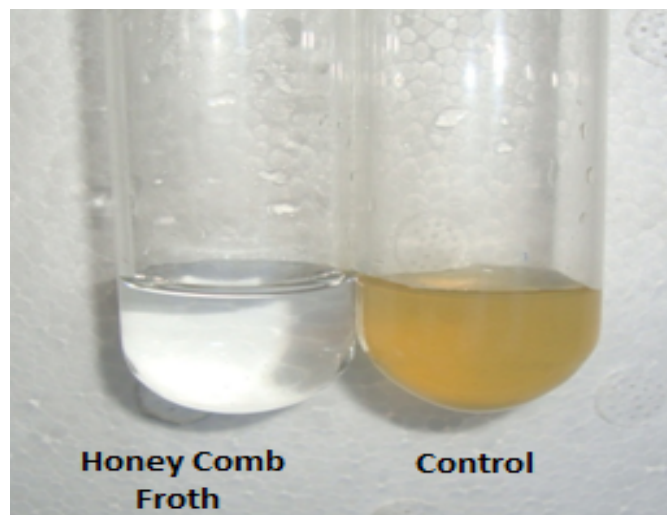


Plate 1.5. Presence of Saponins