



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

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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.

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 spirallycoiled 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

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 Manickam's (2009) methodology. By this analysis, the presences of several phytochemicals were tested.

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.

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.

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.

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