



Experimental Studies on Alcoholic Extraction of Azadirachtin from the Neem Seed Kernels

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EXPERIMENTAL STUDIES ON ALCOHOLIC EXTRACTION OF AZADIRACTIN FROM THE NEEM SEED KERNELS

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Abstract:

Azadirachtin is a compound found in the neem tree which is responsible protection of trees from insects and pests. This is so effective component if we consider other insecticides and pesticides. This is eco-friendly and has high efficiency for the protection of plants (herbs and Serbs especially). But processes of extraction which exist are not economical and not applicable in industries.

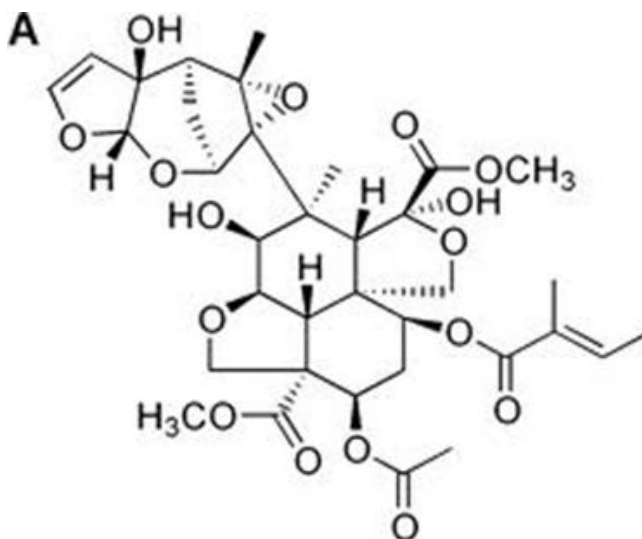
Azadirachtin is complex in molecular structure and highly polarized in nature. Alcohol also has the same nature and polarity. We have studied the alcoholic extraction of Azadirachtin.

Keywords:-Azadirachtin, ethanol,

Introduction:

Neem (*Azadirachta indica*) tree has medicinal importance throughout the era. It has various medicinal properties, and it well known by the people through all section. Especially in India it has such a medicinal status which is given by 'Ayurveda', each part of plant have medicinal uses, from root to leaves. The seeds are specially having rich amount of oil. Oil is used by the people as the insecticides, which protect them from the insects e.g. mosquitoes.

Neem seed kernels have the composition of the various aromatic compounds, which are the responsible for the bitter taste and also for the medicinal purpose. It contains Nimbin, Nimbinin, Nimbidin, oleic stearic and palmitic acids, queritins and other limnoids. Especially it contains Azadirachtin which is the most active compound and currently considered as neem's main agent for controlling insects. It has the molecular weight 720 and its melting point 160⁰ C. its influence on insect feeding behavior and insect developments. Azadirachtin is used to manufacturing Eco-friendly insecticides.



Structure of Azadirachtin

India has large sector in which the neem trees planted and developed, but there is no collection of fruits they are going on waste. 14 million trees are present in India. (Per sector)

Still researchers have been studies on the extraction process of Azadirachtin, but up till economical and easy methods not in exist. We have studied the extraction of Azadirachtin by using alcohols.

Materials and Instruments:

Seeds: Ripped and totally dried seeds are available in the local market. Especially from herb shops (Ayurvedik). Only the seed kernels are used. Local market were from seeds are purchased, Akola, Maharashtra.

Chemicals: The chemicals were used in experiment, Ethanol (99 %), Dichloromethane (99%,), Petroleum ether, boiling point 60⁰-80⁰ C (99%,) was obtained from the Jyoti Chemporium, Akola. 1 litre saturated solution of sodium chloride was prepared in lab.

Instruments: Pulveriser used to grind the seed kernels (grade and specification). The three necked flaks with stirrer motor and mental heater was used in the preparation of blending mixture of seed powder and solvent. This set up is shown in the picture as well as in diagram. Simple distillation column was used to obtained the residue contain the Azadirachtin.

Experiment:

We had done the small scale experiment first. We were studied on composition of need seed kernels. The composition is given below.

Content	Oil	Azadirac htin	Protein s	cake	Total
Percenta ge	25	1	14	60	100

We were prepared the solution of neem seed kernels and ethanol as the solvent in the proportion of 1:4 kept it for some time left. Then it was kept at magnetic stirrer with initially at low speed of revolution and at 35⁰ C. After the 15 minute temperature increased by us up to 60⁰ C, throughout the temperature kept constant. This is just like the blending process, miscibility was starting to found after the 40 minutes, with the increased and viscosity had been increased. We were increased the speed of revolution with in viscosity. After the 15 minutes of starting of miscibility powder and ethanol is totally miscible, then after it we were added the one unit of petroleum ether which the non-polar solvent for stabilizing the neem oil. The blending mixture was filter by filter paper. In this way two samples were prepared. The two layers were found in test tube after some time as shown in image. Upper layer has the composition of azadirachtin and ethanol, lower layer has composition of oil and other impurities. Separate both the layer and analyze under the UV spectrophotometer. Samples are stealing testing under the HPLC.



We had performed the same experiment as describe above, but made some quite changes in set up. We have a set up, in which the three necked flask with motor stirrer and have condenser, mental heater for the heating purpose as shown in figure. We had taken the reactant and reagents in some more quantity. The neem seed kernels powder had taken in quantity of 50 gm, with ethanol as the solvent in 250 ml in a beaker

and then it was poured in to the flask and starts to heating the mixture of powder and solvent.

Throughout the process temperature of solution kept constant and observed mixture up to miscibility. Miscibility was appeared after 1 hour and

S. N.	OBSERVATIONS	Experiment no.1	Experiment no.2	Experiment no.3
1	Feed Ethanol Powder	250 ml 50 gm	250ml 50 gm	250m 50 gm
2	Oil and Petroleum ether	43 ml	44.1 ml	43.55 ml
3	Recovery Ethanol Petroleum ether	196 ml 32.9 ml	194 ml 34 ml	195 ml 33.45 ml
4	Oil extract	10.1 ml	10 ml	10.05ml
5	%Recovery Ethanol Petroleum ether	78.4 % 65.8 %	77.6 % 68 %	78% 66.9 %
6	Azadirachtin yeild	0.464 gm	0.47 gm	0.467 gm
7	%yeild Azadirachtin	92.8	94	93.4

30 minutes. Heating was stop, when the mixture totally miscible. Stirring process was continues for some time during that petroleum ether was added in the quantity of 50 ml at 50⁰ C temperature of mixture i.e. below the boiling point of petroleum ether keep the stirring continue.

The partial vaporization of ethanol was take place. The vaporized ethanol condensed by means of condenser and collect in beaker. Throughout the process 43 ml of ethanol collect.

The mixture was filter with help of separating funnel. The oil comes out with the non-polar solvent means petroleum ether. The ethanolic extract and oil were separated. The petroleum ether was recovered 33 ml. it was done by soxhlet apparatus.

The ethanolic extract was distillate by simple distillation column. The residue was weighing the 0.98 gm, might ne 0.5 gm of azadirachtin present. Then it saturated with 10 ml of dichloromethane for 3 hours. That solution was again distillate and residue was get weigh of 0.61 gm of azadirachtin get. This experiment was performing two times.

Steal we studying the water solubility and its solubility in ethanol under UV-spectroscopy and gas chromatography.

Analysis:

We have studies the azadirachtin under the UV spectroscopy by preparing solutions of dichloromethane. We had taken the reference of standard studies and select the wavelength of 220 nm. Under this wavelength azadirachtin shows the maximum absorbance. The calibration curve obtain linear when concentration is 30 µg/ml. The calibration curve is shown in figure below.

To analyses the sample of project, we used the photometric method of UV-spectroscopy. We had prepared the sample to analyses the AZA in sample before some months, that samples didn't shows show the proper result. We had separate applied to purification. Then we were tested on UV-Spectrometer with water as a reference solvent had taken. It shown the three absorbance points which given in below which various solution composition.

The calibration of UV-spectroscopy is found with three various solution, the wavelength set at range of 217-220 nm. We had primarily calibrated in time vs. absorbance.

- The calibration of UV-spectroscopy is found with 3 various solutions. The wavelength 220nm.the UV calibration data.

Solution proportion	Concentration (mg)	Absorbance
1:20	60	0.558
1:50	80	0.921
1:60	100	1.378

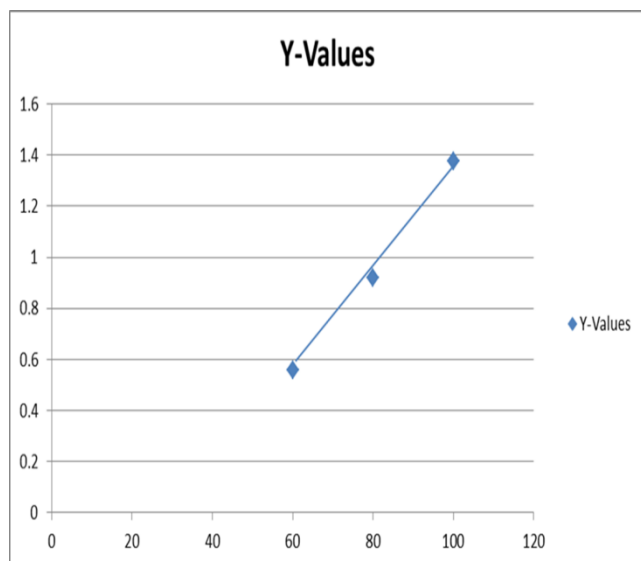


Fig.3: Calibration curve

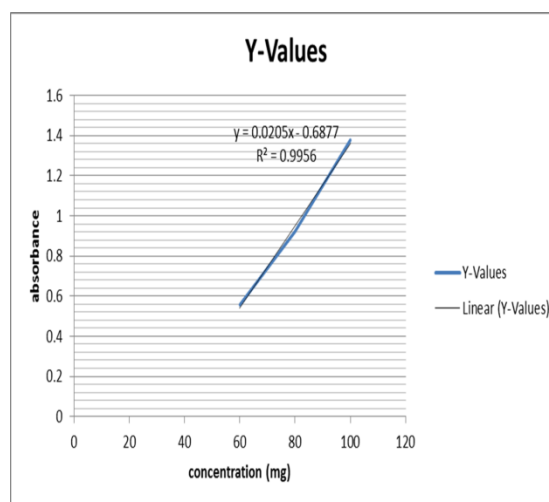


Fig. 4: Computational calibration data curve

This way we have studied calibration data of AZA, which is found from this graph. We have compared our data with the standard study data and with the computational calibration data we have near about the standard studies. The value R^2 is near to the standard study data which shows the purity and occurrence of compound.

The standard study value of R^2 is 0.9981 and we have got the value of R^2 is 0.9956. This ways complete our study. Where the R^2 is correlation coefficient.

We have been studies solubility, peak value and concentration of azadirachtin in water and ethanol under the UV-spectroscopy and HPLC.

Conclusion: We have been studies alcoholic extraction of azadirachtin from the neem seed kernels which is feasible and may be economic with good yield and good recovery of solvent.

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