Published by :
http://www.ijert.org

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 7 Issue 12, December-2018

Extraction of Effective Phytochemical- Saponin From Herbal Plant Tribulus Terristris

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***Abstract*:-The Phyto-chemicals present in the various herbs have adequate medicinal effects in different parts of the plants. Various Phyto-chemicals include glycosides, alkaloids, flavanoids, tannins, resins and volatile oils. The presence of different Phyto-chemicals results in various treatments like cancer, diabetics, inflammations, ureic infections etc. The saponin is one of the glycosides division consist of high wide of pharmacological activity. Various herbs consist of saponin at a high rate, in which Tribulus terrstris is one of the herbs which have high content of saponin in it. Due to which T. terristris is used widely in the Ayurvedicand homeopathic formulations as a main source. In the present study, Extraction of Saponin is performed with different solvents to identify the majority of saponin content to be extracted from the plant. The major contents of saponin are found to be with ethanol as solvent. The highest saponin content is found when 1 gm of seed powder is extracted with 70% ethanol is used as a solvent for 240 min at 70°C with a yield of 79.26%.**

INTRODUCTION:

Herbalism[1] is use of various herbal plants for the treatment of medicinal diseases Almost 80% of world's population relies on traditional medicines for primary health care, most of which engross the use of plant extracts.. *Tribulus terrestris* L.*[2]* is also known as *mitha* (sweet) *gokhru* as differentiated from *kadva* or *moto gokhru* (Pedalium murex). *T.terristris is one of the herbs which have a plenty pharmocological activity due to the presence of various phytochemical[3] like* steroids, saponins, flavonoids, alkaloids, oils, vitamins, tannins, resins, nitrate potassium, aspartic acid and glutamic acid. Saponin is one of the important phytochemicals which is used in the treatment of various disease like eye infections,odeam,inflammations,cancer etc[4,5]. In the present study,extra tion of saponin are performed with various solvents like thanol,methanol and etc which is furthur used as various herbal extraction for the treatment of diseases.

MATERIALS AND METHOD:

Collection of plant:
Tribulus terristris fruit powder is collected from the nearby ayurvedic shop.

Fig 1 Gokhru Seed Powder

CHARACTERIZATION OF THE SAMPLE:

Scanning Electron Microscope Analysis (SEM):
To evaluate the particle size of Tribulus powder, surface topography is to be studied. SEM[6] analysis of the seed powder is performed using S-3400n SEM-Hitachi at a range of 12X. The powder was coated on specimen stub, then placed into the microscope's vacuum column evaporator and a beam of electrons are then passed through powder, inturn travelled through a series of magnetic lenses. The electrons beams are then counted by the detector fixed inside microscope and final signals are received by the recorder as images. The number of electrons detected from each spot of the sample createsfurther resultant images. The micrographs decide sufficient data for the topography of the sample.

Fourier Transform Infrared Spectrophotometer (FTIR):
Fourier Transform Infrared Spectrophotometer (FTIR) [6] is one of the high qualified tools for identifying different chemical bonds (functional groups) present among tribulus powder sample. The wavelength of electrons obtained by

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the powder are used to identify the various chemical bonds present in the sample which can be detected in the annotated spectrum .By IR absorption spectrum, the chemical bonds in a molecule can be observed. Dry powder of ethanolic extracts of tribulus is determined by FTIR analysis. 1 mg of the dried Tribulus seed powder is capsulated in sufficient amount of KBr pellet (100 mg), as translucent sample discs. The Tribulus seed powdered is then loaded in FTIR spectroscope, at aScanning range of 400to 4000 cm 1 with a resolution of 4 cm [1].

OPTIMIZATION OF THE DIFFERENT PROCESS PARAMETERS FOR EXTRACTION:

The initial process parameters are optimized to obtain the high yield of saponin content from the T.terristris seed powder. For the optimization, effect of different parameter are studied as different solvents like ethanol, methanol and distilled water, different solvents concentrations from 50-100%, Different extraction time from 0-270 min, different molar concentrations of hydrochloric acid from 1- 5M, different dosage concentrations like 0.25-1.25 % w/v, different temperatures from 50-70°C.[7,8]

SAPONIN CRUDE EXTRACTION:

Soxhlet Extraction:

The soxhlet apparatus is the best and highly accurate extraction method mainly for the alcoholic extraction. For the ethanolic/methanolic extraction soxhlet apparatus is used.8 gm of T.terrestris [9,10] powder is weighed and poured in an soxhletapparatus and 250 ml ethanol is measured and taken in round bottom flask. The extraction is performed for 6-8 hrs and the sample is then collected.
Depending on the optimized conditions the extracted sample is then collected. The sample collected is pure saponin extract.

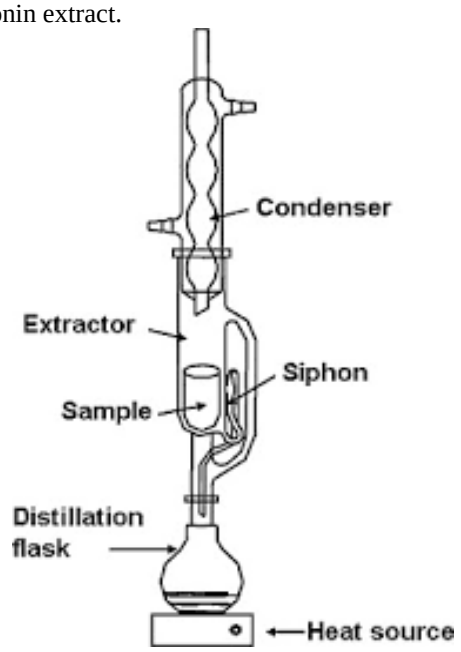

Fig 3.3 Soxhlet Ethanolic Extraction

RESULTS AND DISCUSSION

Optimization of parameters like pH, Contact time, Initial concentration, Dosage, Temperature plays a key role after the extraction of saponin from tribulus terristris to determine the level of rate efficiency by soxhlet apparatus[11,12]

CHARACTERISATION OF THE TRIBULUS SAMPLE:

Scanning electron microscope: To evaluate the size of the particle, SEM analysis is carried out using S-3400n SEM-Hitachi at 12X. The size of the particle and constituent of the sample are distinguished clearly as shown in the figure. The size of the particle varies depends on the saponin extraction from the T.terristris sample.
The size of the T.terristris seed powder is found to be 5µm and the clear structural visibility is observed at a higher magnification of 2700. The size and shape of the Tribulus powder is clearly observed at various magnifications of 1900, 2700, 1400 using sem and results are observed as shown in fig.4. The increase in its particle size at 5,10 µm in reference to magnification is clearly differentiated.

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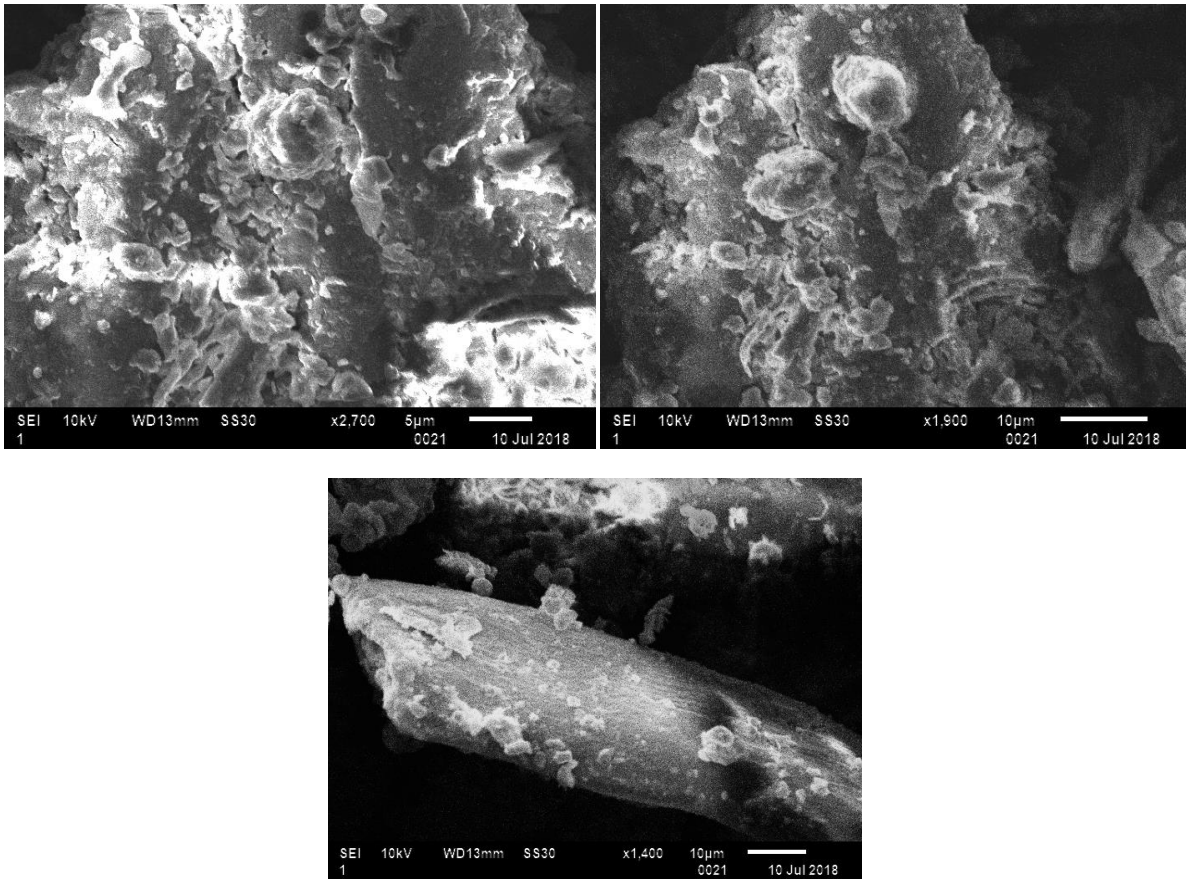

Fig 4.1 Scanning Electron Microscope Images for the Crude Sample Before Extraction

After the extraction, the size and the shape of the powder is disturbed and the extraction of various components are performed. Once after the extraction is completed. The sample is dried and the precipitate is further characterized to identifythe variation. The difference is clearly identified as shown in fig 5.The evident of the size varied a lot because of the extraction separated the compound formations.

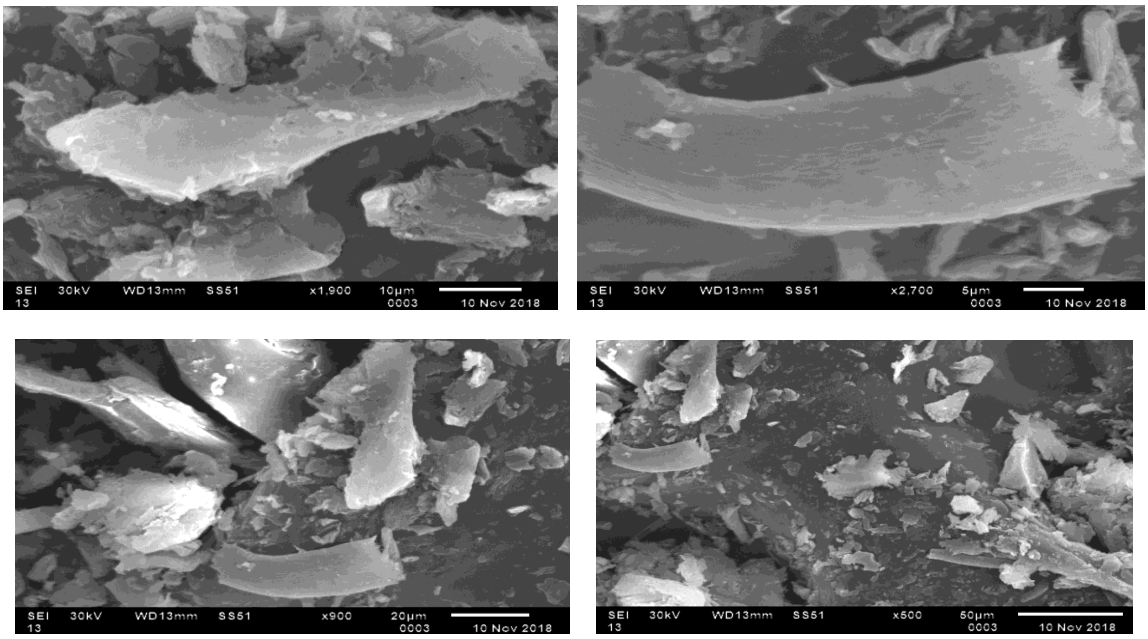

Fig 4.2 Scanning Electron Microscope Images for the Crude Sample after Extraction

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4.1.2 FOURIER TRANSFORM INFRARED SPECTROPHOTOMETER

Fourier Transform Infrared Spectrophotometer (FTIR) is intended for identifying different types of chemical bonds (functional groups) present in molecular compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. After the extraction, the size and the shape of the powder is disturbed and the extraction of various components are performed. Once after the extraction is completed. The sample is dried and the precipitate is characterized to identify the variation. The difference is clearly identified as shown in fig 5.The gradient of the size varied a lot because of the extraction separated the compound formations.In accordance with the spectra reading the denaturation of the bond and the formation of new bonds are clearly identified.
The T.terristris sample is collected and sieved and carried out with FT-IR to identify the presence of the chemical constituents by its bonding between them. The identification of compound is a key criterion for the identification and for further extraction, purification of the compound. As shown in Fig 4.3, the compound bonds are clearly observed at a certain range of extent. The wavelength of the compound varies for each and every composition. In the crude sample the compound are found to be equally at a particular level of wavelength. Once after the extraction is performed the chemical bonding is denatured. Variation of the wavelength ranges indicates the arrangement of the chemical constituents in the sample powder.

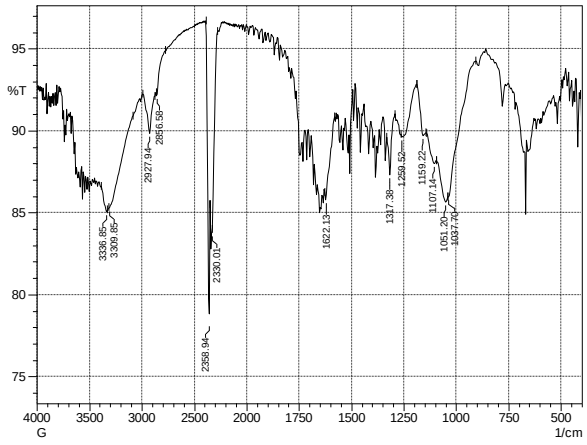

Fig 4.3 FTIR for the Crude Sample before Extraction

After the extraction, the sample is filtered and the dried precipitate is performed for after sample of FT-IR to identify the separation of compound from the crude mixture.
In fig.4.4, it is clearly identified the variation of the wavelengths of different samples at wide range indicate the extraction of the solvents. The clear separation of saponins re visible at the higher range of wavelengths separating from other constituents.

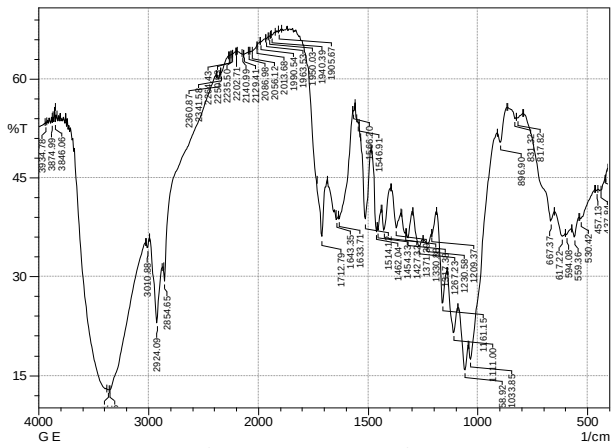

Fig 4.4 FTIR for the Crude Sample after Extraction

OPTIMIZATION FOR THE EXTRACTION OF CRUDE SAMPLE:

4.3.1 Effect of different Solvent for the extraction of saponin from crude sample:

It is critical to optimize the solvent parameter to attain maximum extraction efficiency. The effect of extracting solvents for Soxhlet extraction are investigated .The saponin being polar compounds; polar solvents like ethanol, methanol, water, and their combinations were generally employed for their extraction. In Present study, it was reported that the polar solvents are better for the extraction of steroidal saponin than non polar ones.

S.no	Types of solvents	Saponin concentration (gm/ml)	% extraction of the saponins
1	Ethanol	1.95	51.75
2	methanol	2.72	51
3	Water	3.95	37.625

Table 4.2 Effect of % yield of saponin with different solvents

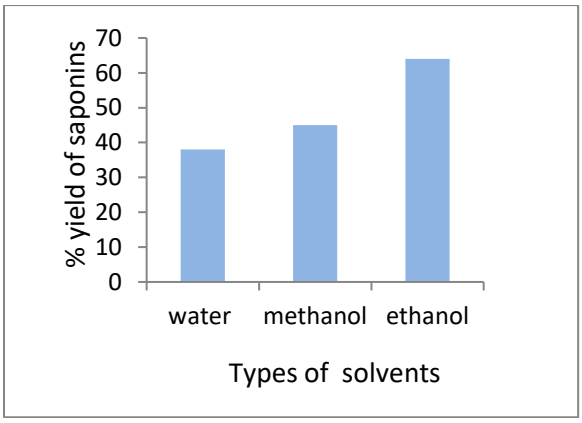

Fig 4.5 Effect of % yield of saponin with different solvents

4.3.2 Effect of Solvent at different concentrations for the extraction of saponins from crude sample:

The extractions of saponin are performed at different solvents and accordingly, ethanol is observed as more

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extraction concentration. Further, To obtain the optimum concentration of ethanol, different concentration are carried on soxhlet to get accurate optimum concentration values as 30, 50, 70, and 100% (v/v) were investigated in the present study. An extract with 70%(v/v) ethanol was found to be the optimum extracting solvent as shown in Figure 4.6. On adding small amount of water in the extracting solvent can significantly increase the extraction efficiency due to high polarity of the water.

Table no 4.3 Effect of % yield of saponin with ethanol at different concentration

S.no	Dosage (% w/v)	Saponin concentration (gm/ml)	% Yield of saponins
1	0.25	2.88	45
2	0.5	4.14	62.28
3	0.75	5.15	76.41
4	1.0	6.58	84.12
5	1.25	5.7	75.76

Water enables the solvent to absorbs deeper into plant matrix easily and facilitate greater mass transfer rate of saponin into the extracting solvent.

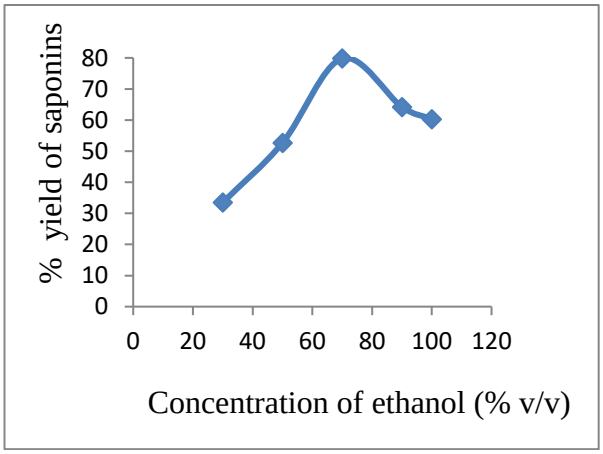

Fig4.6 Effect of % yield of saponin with ethanol at different concentrations

4.3.3 Effect of Dosage for the extraction of saponins from crude sample:

The effect of dosage plays an important role in determination of T.terristris concentration for the optimum extraction yield. Various concentrations of T.terristris powder as 0.25,0.5,0.75, 1.0 and 1.25 (% w/v) are collected, dissolved in 70% ethanol. The solution is further proceeded for extraction for 4 hr. The final samples are collected and its % Yield of extraction of is obtained at 1 gm/100ml dosage.

Table no 4.4 Effect of % yield of saponin with dosage

s.no	Ethanol Concentrations (% v/v)	Extracted concentration (gm/ml)	% extraction of saponins
1	30	4.09	33.5
2	50	3.15	52.68
3	70	1.92	79.84
4	90	2.95	64.38
5	100	3.19	60.23

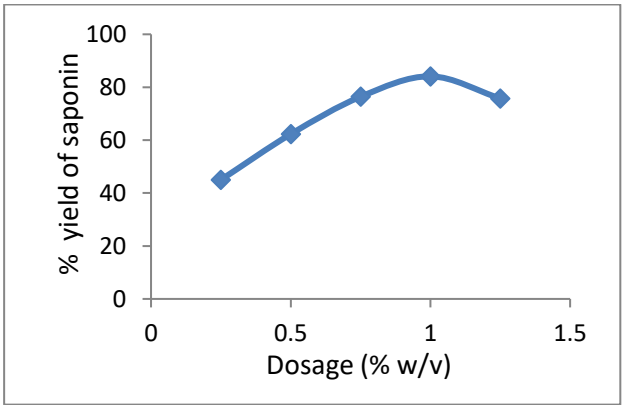

Fig no 4.6 Effect of % yield of saponin with dosage

4.3.4 Effect of time for the extraction of saponins from crude sample:

1 gm of T.terristris powder is weighed and mixed in 100 ml of the 70 % ethanol. The solution is then extracted with soxhlet extraction for 4-5 hr. The samples at standard interval are collected i.e. 60, 90,120,150,180,210, 240 and 270 min. The samples are filtered and further extracted concentration is observed. In the present study, it is reported that optimum extraction of saponin from tribulus powder is found to be at 4 hr.

s. no	Extraction time(min)	Saponin concentration (gm/ml)	% extraction of saponin
1	30	2.85	35.64
2	60	3.25	40.7
3	90	4.08	51
4	120	4.48	59.4
5	150	5.7	71.25
6	180	6.38	79.75
7	210	6.65	83.13
8	240	6.65	83.13
9	270	6.65	83.13

Table 4.4 Effect of % yield of saponin at different time intervals

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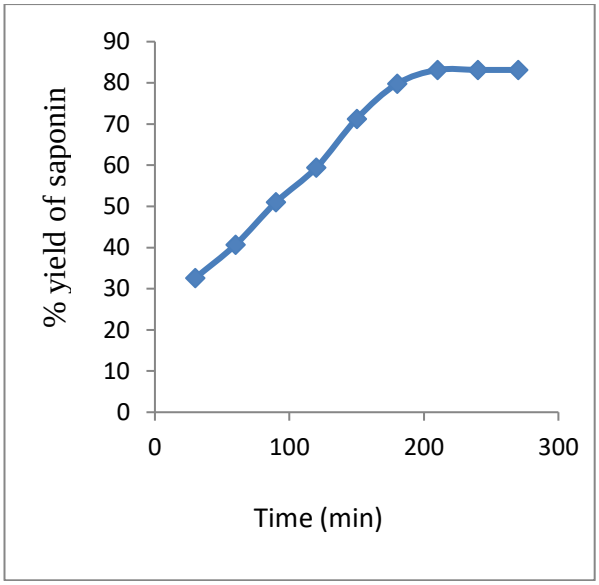

Fig 4.7 Effect of % yield of saponin at different time intervals

4.3.5 Effect of temperature in extraction of saponin:

1 gm of T. terrestris powder is taken and 100 ml of 70% ethanol is added to it. The Above solution is extracted at different temperature as 50, 55,60,65,70 ^{O}C are investigated for the present study. The mixture is then extraction for 4 hrs. After extraction, the sample are collected, filtered and maximum % percentage yield is found to be at 100 ^{O}C.

s.no	Temperature (°C)	Saponin concentration (gm/ml)	% extraction of saponin
1	50	4.78	40.25
2	55	3.92	51.76
3	60	2.42	69.75
4	65	1.35	83.13
5	70	0.92	85.69

Table 4.6 Effect of % yield of saponin at different temperatures

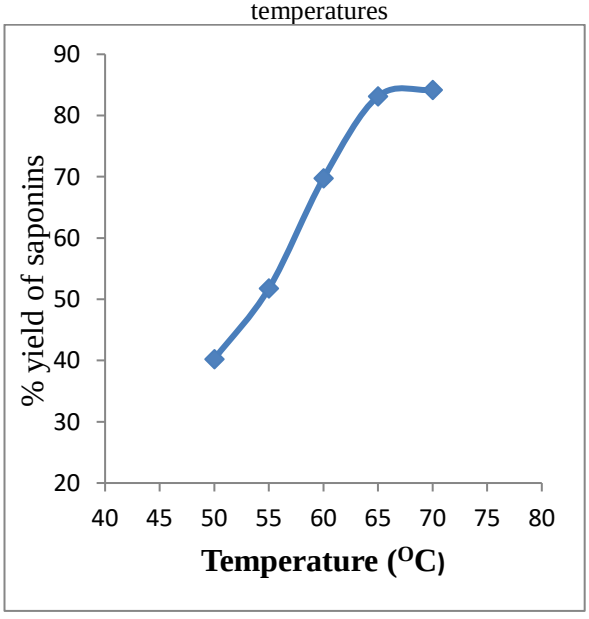

Fig no 4.9 Effect of % yield of saponin at different temperatures

CONCLUSION:

In the present work, Tribulus territris is a natural herb which contains high medicinal values is considered. The physical characterization like SEM, FT-IR is identified before and during the extraction process of saponin from tribulus powder. The saponin content in the tribulus is identified as high as of other herbs. As of which, saponin are extracted from the tribulus using different solvents like ethanol, methanol and aqueous and of different concentrations of the solvent resulted high % yield with70% ethanol. The optimum yield of saponin is observed for 1 gm of sample and 3M Hydrochloric acid at 70°C for 4 hrs. Tribulus Fruits are used in treatment of arthritis, diuretic, tonic and also in the treatment of calculus affections and painful maturation, aphrodisiac, gout. Saponin content of tribulus is preferable ingredient in various Ayurvedic preparations used in treatment of rheumatoid arthritis, it also have an anti inflammatory activity.

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