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OPTIMISED MICROWAVE ASSISTED EXTRACTION OF PHYTOCHEMICAL CONSTITUENTS FROM TINOSPORA CORDIFOLIA AND ASSESSMENT OF INVITRO ANTI-INFLAMMATORY ACTIVITY

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INTRODUCTION:

Microwave energy a new energy source application has become popular them since 1950(for food). From mid-1990s microwave synthesis is being exposed to the outside world of chemistry through various publications from professor. Richard Gedye and Professors Raymond j. Giguere and George Majetich and gained importance and implementation in organic synthesis.

Microwave assisted organic synthesis has revolutionized organic synthesis. Microwave assisted organic synthesis can achieve goals in a fraction of the time when compared to traditional conventional method.

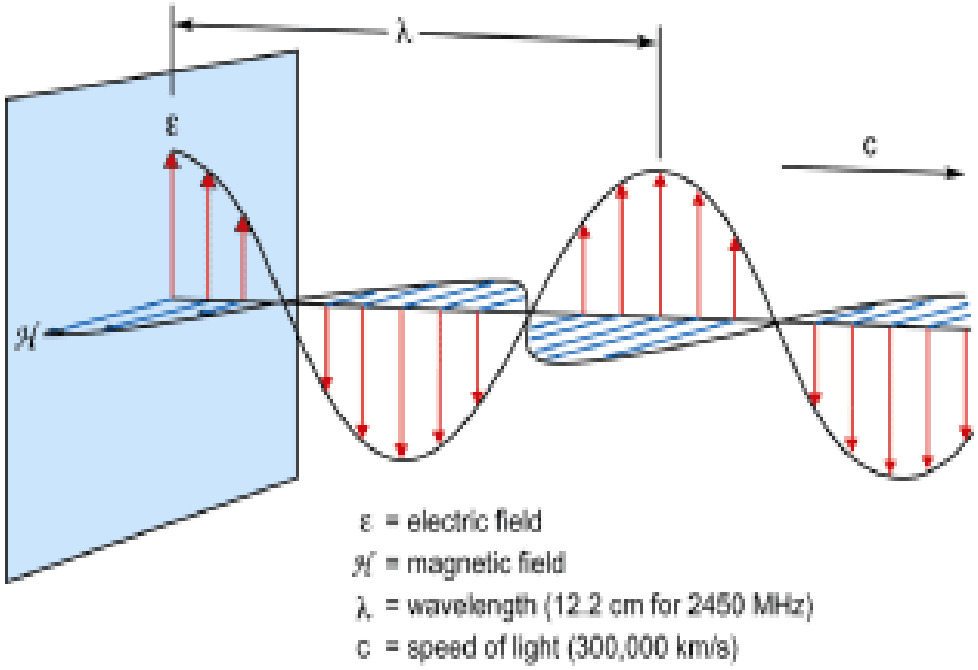

Figure1.1 : Electromagnetic spectrum

TERPENOIDS AND STEROIDS

This includes a vast group of substances – more than 35,000 are known derived biosynthetically from isopentenyl diphosphate.Terpenoids have an immense variety of apparently unrelated structures, while steroids are modified terpenoids,having a common tetracyclic carbon skeleton. These are biosynthesized from the triterpene,lanosterol.

ALKALIODS

Like terpenoids, there are a large and diverse class of compounds with more than 10,000 examples know at present.They contain a basic amine group in their structure and derived biosynthetically from amino acids.

TINOSORA CARDIFOLIA

PLANT PROFILE

- **Botanical name** :Tinospora cordifolia
- **Synonyms**: Giloy,tippateega,Guddchi,Giloe,amrita,
- **Vernacular names**:
- **Sanskrit**: Guddchi, madhuparni, Amrita, Chinnaruha,Vatsadaani,Tantrika, kundalini,Chakralakshanika.
- **Bengali**:Gulauncha
- **English**:Gulancha/Indian Tinospora
- **Gujarathi**:Galo.
- **Kannada**: Amruthaballi, Madhupa.

Marati:Shindillakodi.

- **Tamil**:Shindillakodi.
- **Telugu**:Thippateega.

TAXONOMY:

- **Kingdom**:plantae
- **Subkingdom**:Tracheophyta
- **Super division**:Spermatophyta
- **Division**:Mangoliaphyta
- **Class**: Mangoliopsia
- **Subclass**: Polypetalae
- **Series**: Thalamiflorae
- **Order**: Ranunculales
- **Family**: Menispermaceae
- **Tribe**: Tinosporeace
- **Genus**: Tinospora

Species: Cordifolia

RESPONSE SURFACE METHODOLOGY:

In many situations, the theoretical model that relates some controllable variables (factors) to a response either is not available or is very complex. In this case, the information about the relation between factors and response should be obtained in an empirical way. Response surface methodology (RSM), introduced by Box and Wilson,[1] is a collection of mathematical and statistical techniques whose purpose is to analyze, by an empirical model, problems as the one posed.

Inflammation word is first derived from Latin word inflammation. Inflammation means it is a complicated biological feedback of living tissues to harmful stimuli, such as pathogens, damaged cells, or irritants, and is a protective response involving immune cells, blood vessels, and molecular mediators.

MATERIALS AND METHODS:

Plant material

Fresh stem and leaves of *Tinospora cordifolia* were collected from during April and were identified by the Botanical Department.

Chemicals

Solvents chemicals and reagents

All the solvents and reagents used in the present work are of analytical grade required chemicals were procured from molychem and sd fine chemicals pvt .Ltd

All the glass ware used in the study was made by Borosil and equipment were used they are electric water bath, digital balance, heating mantle, desiccators, hot air oven, conical flask, funnel, Soxhlet apparatus.

Methanol, Ethanol, Petroleum ether, Chloroform, H2S04, Acetone, Wagner's reagent (Iodine in potassium iodide), NaOH, HCl, FeCl3, Chloroform, Glacial acetic acid and Ninhydrin.

The present work has been planned in to three stages

- **Optimisation of conditions**

- **Microwave assisted extraction**
- **Activity screening:**

In-vitro Anti-inflammatory activity by Egg albumin denaturation method

Preparation of plant extract and phytochemical screening

Collected plant material washed under running tap water to eradicate dust and microbes. The plant samples were then air dried under shade at room temperature for 15 days. The plant material were crushed well into fine powder in an electronic grinder and kept into air tight polythene bags for further use and stored at room temperature.

EXPERIMENTAL DESIGN FOR EXTRACTION:

Response surface analysis was performed to estimate the effects of independent variables on the response within the range of investigation.

RSM with the Box Behnken design was used to analyze the experimental data with 3 independent variables ($X1$, Irradiation power (∘C); $X2$, extraction time (min); $X3$ Solvent ratio at 3 levels in the extraction process. Investigated factors and tested levels are reported

Instrumentation: Microwave extraction experiments were performed in an open vessel microwave oven equipped with five power levels (140,210,240,280,350, 420,450, 480,560,700W) to give maximum flexibility and control for extraction.

Extraction of Tinospora Cordifolia:

Stems of *Tinospora cordifolia* were dried under shade for 7–10 days and pulverized using an electric grinder. Firstly, dried sample was extracted with solvent of methanol and acetone in different ratio, at different irradiation power, radiation time employing microwave synthesizer .The residue was dried under reduced pressure by using a rotary vacuum evaporator.

Finally the percentage yield were calculated of the dried extracts by following formula-

QUALITATIVE PHYTO CHEMICAL INVESTIGATION:

Qualitative phytochemical screening will help to understand a variety of chemical compounds produced by plants and quantification of those metabolites will help to extract, purify and identify the bioactive compounds for useful aspects to human beings. In phytochemical evaluation the powdered leaves were subjected to phytochemical screening for the detection of various plant constituents, characterized for their possible bioactive compounds, which have been separated and subjected to detailed structural analysis.

The various tests and reagents used are given below and observations are recorded.

Tests for Carbohydrates:

Preparation of test solution: The test solution was prepared by dissolving the test extract with water. Then it was hydrolyzed with 1 volume of 2N HCl and subjected to following chemical tests.

a) **Molisch's test:**

b) Fehling's test:

c) Benedict's test
d) Barfoed's test
f) Tests for Non-Reducing Sugars
g) Tannic acid test for starch
2) Tests for Proteins:
Preparation of Test Solution: The test solution was prepared by dissolving the extract in water.
a) Biuret test (General test) b) Millon's test (for proteinsc) Xanthoprotein test (For protein containing tyrosine or tryptophan
d) Precipitation test:
3) Tests for Steroids:
Preparation of test extract solution: The extracts were re fluxed separately with alcoholic solution of potassium hydroxide till complete saponification. Saponified extract was diluted with water and unsaponifiable matter was extracted with diethyl ether. The ethereal extract was evaporated and the residue (unsaponifiable matter) was subjected to the following test by dissolving the residue in the Chloroform.
a) Salkowski reaction
b) Libermann-Burchard test
c) Libermann's test
4) Tests for Amino Acids:
a) Ninhydrin test (General test)
b) Test for Tyrosine
c) Test for tryptophan5. Tests for Glycosides:-
Preparation of test solution: The test solution was prepared by dissolving extract in the alcohol or hydro-alcoholic solution.
Tests for Cardiac Glycosides:
a) Baljet's test
b) Bromine water test
c) Legal's test (For cardenoloids)
d) Test for deoxysugars (Kellar Killani test
e) Libermann's test (For bufadenolids)
Test for anthraquinone glycosides:
a) Modified Borntrager's test
b) Borntrager's test
c) Cyanogenetic glycosides:
a) Grignard's test
6. Tests for Alkaloids:-
a) Dragendorff's test
b) Mayer's test
c) Hager's test
d) Wagner's test
7. Tests for Flavonoids:- The flavonoids are all structurally derived from the parent substance called flavones. The flavonoids occur in the free form as well as bound to

sugars as glycosides. For this reason, when analyzing flavonoids, it is usually better to examine the flavonoids in hydrolyzed plant extracts.

Preparation of test solution:

i. To a small amount of extract added equal volume of 2M HCl and heated in a test tube for 30 to 40 min. at 1000C.

ii. The cooled extract was filtered, and extracted with ethyl acetate.

iii. The ethyl acetate extract was concentrated to dryness, and used to test for flavonoids.

a) Shinoda test

b) Ferric chloride test

8. Test for Vitamins:

a) Test for Vitamin A

b) Test for vitamin C (Ascorbic acid

c) Test for Vitamin D

9. Saponins.

Preparation of test solution

a) Foam test

b) Haemolysis test

c) Test for steroidal saponins

d) Test for triterpenoid saponins

10. Tannins and Phenolic compounds

a) 5% FeCl3 solution: Deep blue-black color.

b) Lead acetate solution: White precipitate.

c) Bromine water: Discoloration of bromine water.

d) Acetic acid solution: Red color solution.

e) Dilute iodine solution: Transient red color.

PREPARATION OF DICLOFENAC SODIUM SOLUTION:

10 mg of standard diclofenac sodium powder was dissolved in 5ml of water in a 10 ml volumetric flask then adjust the volume up to 10ml water then 1ml of this solution was diluted to 50ml of diluted water the concentration of solution of this solution was 20µg

STATISTICAL ANALYSIS

Invitro data were expressed as mean percentage inhibition ± SD(N=3). All analysis were carried out in graph pad prism (version 5.0) software.

QUALITATIVE DETERMINATION OF ALKALOIDS: The qualitative estimation of alkaloids was made using Wagner's and Mayer's reagents. In Wagner's test, 1ml of plant extract was treated with 2 ml of Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml of water) and examined for the formation of a reddish-brown precipitate (or coloration). In Mayer's test, to 2 ml of extract, 2 ml of concentrated hydrochloric acid was added, followed by the addition of 3 drops of Mayer's reagent. The appearance of greenish cream colored precipitate indicated the presence of alkaloids.

ACTIVITY STUDIES:

Protein Denaturation Method

Principle:

Inflammation is a complex process, which is frequently associated with pain and involves occurrences such as the increase of vascular permeability, increase of protein denaturation and membrane alteration. Protein denaturation is a process in which protein lose their tertiary structure and secondary structure by application of external stress or compound such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of protein is a well documented cause of inflammation. As part of the investigation on the mechanism of the anti inflammatory activity, ability of the plant oil to inhibit protein denaturation was studied.

In-vitro anti-inflammatory activity:

Albumin denaturation assay:

Reagent:

Egg albumin solution was prepared by taking 1 gm of Egg albumin in volumetric flask and the remaining area filled with distilled water to make 100ml.

Preparation of Phosphate Buffer pH 6.4

Place 50 ml of 0.2 M potassium dihydrogen phosphate in a 200ml of standard flask and a specified volume of 11.4 ml of 0.2 M sodium hydroxide and add water to the volume.

Instruments:

U.V., Incubator

Procedure:

In Egg albumin denaturation method, the reaction mixture (5ml) consists of 0.2 ml of Egg Albumin (from fresh hen's egg), 2.8 ml of Phosphate-buffer saline(PBS, pl 64) and 2 ml of varying concentrations (25,50,100,200,400,600,800,1000µg/ml) of sample. Similar volume of double distilled water served as the control. Next, the mixtures were incubated at 37° 2° in a BOD incubator for 15 minutes and then heated at 70°C for 5 minutes. After cooling, their absorbance was measured at 660 nm by using the vehicle as a blank. Diclofenac Sodium in the concentrations of (25, 50, 100, 200, 400, 600, 800, 1000 µg/ml) was used as a reference drug and treated similarly for the determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula:

Percentage inhibition = 100 X 1-V/Vc

Where,

Vt =Absorbance of test sample

Vc= Absorbance of control

Calculation:

$$\%\text{inhibition} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}} \times 100}{\text{Abs}_{\text{control}}}$$

%Protection = (optical density of test/optical density of control)x100

RESULTS AND DISCUSSION:

In this work a new optimal design of extracting of total alkaloids in tinospora cardifolia has been studied. The purpose is to improve the alkaloid extraction yield. This new extraction process provides a theoretical basis for the resources utilization of the new medicinal plant.

Optimization of process variables: Based on previous experiments, ethanol concentration, extraction time, and irradiation power were selected as the main variables for achieving the maximum extraction of total alkaloid compounds from tinospora cardifolia during the extraction process. In order to determine the best combination of extraction conditions, a box behnken design was formed with extraction times from 1 to 6 min, ethanol concentrations from 10 to 30 %, and irradiation power ranging from 28- to 560 watt All 17 experimental runs, as well as the experimental results and predicted results of total alkaloid content under these runs are presented

Experimental design: Box behnken design with three levels and three variables was employed to determine the optimum combination of independent variables for the maximal extraction of total alkaloid compounds from tinospora cardifolia. Three independent variables selected after the preliminary experiments were extraction time (ET), ethanol concentration (EC), and Irradiation power). Box Behkhen design consisting of 3 Factors, 5 central points,3 levels , and 17 runs .Regression analysis was carried out on the data of dependent responses to fit the experimental data and fitted to an linear model as shown below:

Statistical Analysis: Design-Expert (Version 8.0) software was used to analyze the experimental data. Values are recorded as mean ± SD. The data obtained from different groups was analyzed by ANOVA. The values were considered statistically significant for all conduct experiments. Every determination was carried out in triplicate

Table 1.1: Experimental factors and levels used in the design

Factor name	**Level (-)**	**Level (0)**	**Level (+)**
A. Irradiation power(Watt)	280	420	560
B. Extraction time(min)	1	3.5	6
C.Solvent ratio	10	20	30

Table 1.2: Factors and Responses for Box Behnken design

		Factor 1	**Factor 2**	**Factor 3**	**Response 1**
Std	**Run**	**A:Irradiation Power**	**B:Extraction Time**	**C:Solvent ratio**	**Extraction Efficiency**
		Watts	min	ml	%
9	1	420	1	10	69.65
12	2	420	6	30	76.76
10	3	420	6	10	75.87

1	4	280	1	20	66.9
16	5	420	3.5	20	73.87
4	6	560	6	20	78.98
13	7	420	3.5	20	72.4
15	8	420	3.5	20	72.5
14	9	420	3.5	20	74.7
8	10	560	3.5	30	80.2
17	11	420	3.5	20	73.6
5	12	280	3.5	10	65.5
7	13	280	3.5	30	66.9
2	14	560	1	20	77.65
6	15	560	3.5	10	78.89
3	16	280	6	20	67.87
11	17	420	1	30	73.34

ANOVA for Linear model

Response: Extraction Efficiency

Table 1.3: Response Surface Models and Statistical parameters obtained from ANOVA

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value Prob > F**	
Model	319.10	3	106.37	73.18	< 0.0001	significant
A-Irradiation Power	294.64	1	294.64	202.70	< 0.0001	
B-Extraction Time	17.82	1	17.82	12.26	0.0039	
C-Solvent	6.64	1	6.64	4.57	0.0521	
Residual	18.90	13	1.45			
Lack of Fit	15.14	9	1.68	1.79	0.3016	not significant
Pure Error	3.76	4	0.9400			
Cor Total	338.00	16				

Factor coding is **coded**.
Sum of squares is **Type III – Partial**

The **Model F-value** of 73.18 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, B are significant model terms. Values greater than 0.1000 indicate the model terms are not

significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The **Lack of Fit F-value** of 1.79 implies the Lack of Fit is not significant relative to the pure error. There is a 30.16% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

Table 1.4:Fit Statistics

Std. Dev.	1.21	**R^2**	0.9441
Mean	73.27	**Adjusted R^2**	0.9312
C.V. %	1.65	**Predicted R^2**	0.8940
		Adeq Precision	25.8584

The **Predicted R^2** of 0.8940 is in reasonable agreement with the **Adjusted R^2** of 0.9312; i.e. the difference is less than 0.2.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 25.858 indicates an adequate signal. This model can be used to navigate the design space

Final Equation in Terms of Coded Factors

Extraction efficiency=+73.27+6.07A+1.4B+0.9113C

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients

A factor was considered to influence the response if the effects are significant ($p<0.05$). A positive value indicates a synergistic effect that favours optimization, while a negative sign represents an antagonistic effect or inverse effect of the factor on the selected response.

The fitness of the linear model was assessed by coefficients of R2, adj- R2, pred-R2, and lack of fit, as well as analysis of variance (ANOVA) using the F-test. The significance of each variable was evaluated by a hypothesis T-test. All statistical analyses were carried out using SAS software (version 9.1, SAS Institute). The level of statistical significance was set at 95% ($p <0.05$).

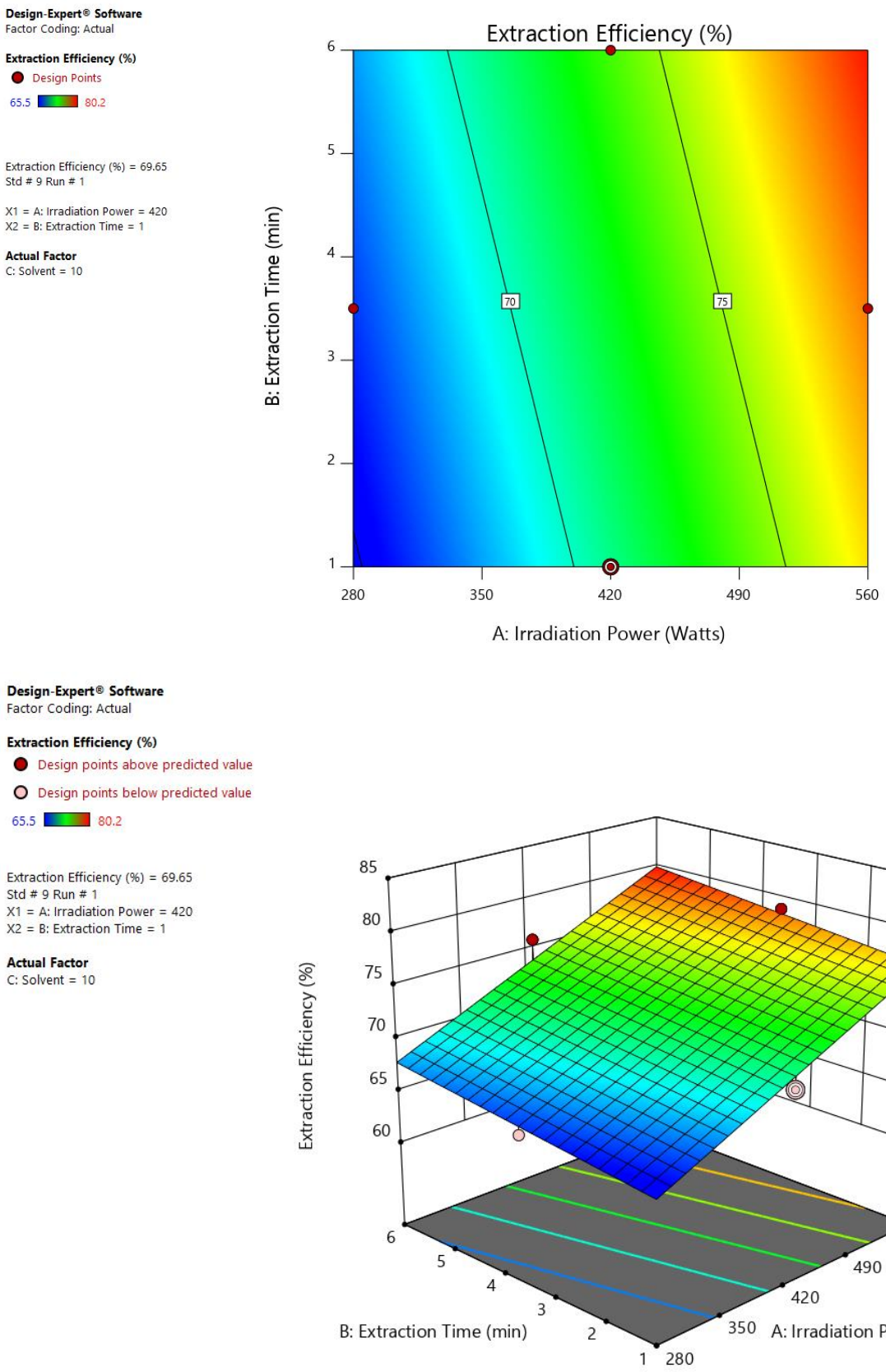

Figure 1.2: Contour plots and three dimensional response surface plots

Table No 1.5: Qualitative phytochemical analysis of ethanolic extract of *Tinospora cardifolia*

S.No	**CHEMICAL CONSTITUENTS**	**Ethanolic extract And Chloroform extract**
1	Alkaloids	+
2	Carbohydrates	+
3	Anthraquinone Glycosides	+
4	Cardiac Glycosides	+
5	Saponin Glycosides	+
6	Flavanoids	+
7	Steroids And Tri Terpinoids	+
8	Tannins	+
9	Proteins	+

Qualitative Determination of Alkaloids:

The qualitative estimation of alkaloids was made using Wagner's and Mayer's reagents. In Wagner's test, 1ml of plant extract was treated with 2 ml of Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml of water) and examined for the formation of a reddish-brown precipitate (or coloration). In Mayer's test, to 2 ml of extract, 2 ml of concentrated hydrochloric acid was added, followed by the addition of 3 drops of Mayer's reagent. The appearance of greenish cream colored precipitate indicated the presence of alkaloids.

Figure No. 1.3: Test for alkaloids

Effect of ethanol concentration on extraction of total alkaloid compounds.

Ethanol was selected as the extraction median due to its low toxicity, cheap price, and easy availability. Total alkaloid compounds were extracted from *T. cordifolia* using aqueous ethanol with different proportions of ethanol and chloroform, ranging from 0 to 95%. The *T. cordifolia* powder (1.0 g) was macerated with 30 mL of different concentrations of solution for 1 h, and then extracted employing microwave synthesizer under various conditions. The extract was filtered under vacuum, and the final fixed volume was used for the determination of total alkaloid content.. One gram of sample was mixed with different volumes of 50% ethanol ranging from 20 to 40 mL, and the effects of the different solvent to sample ratios on total alkaloid extraction are presented in Fig. 3. Total alkaloid content increased with increasing solvent to sample ratios from 20 (v/w) to 35 (v/w), and then remained constant from 35 (v/w) to 40 (v/w) with no significant changes

Effect of extraction time on extraction of total alkaloid compounds.

The subsequent extraction was performed at various irradiation levels for different extraction time periods ranging from 1 to 6 min. The extract was filtered under vacuum, and the final fixed volume was used for the determination of total alkaloid content.

Effect of extraction temperature on extraction of total alkaloid.

The alkaloid compounds contained in tinospora cardifolia are not heat-sensitive, so they do not easily decompose and can be extracted at high temperatures above 60oC. The irradiation power of 560 watt resulted in the highest total alkaloid content of 78.98%. Thus, the extraction temperature was assessed to be an significant process variable

Qualitative Determination of Alkaloids: The qualitative estimation of alkaloids was made using Wagner's and Mayer's reagents. In Wagner's test, 1ml of plant extract was treated with 2 ml of Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml of water) and examined for the formation of a reddish-brown precipitate (or coloration). In Mayer's test, to 2 ml of extract, 2 ml of concentrated hydrochloric acid was added, followed by the addition of 3 drops of Mayer's reagent. The appearance of greenish cream colored precipitate indicated the presence of alkaloids.

Results of protein Denaturation Assay

The Percentage protection from denaturation is calculated by using the
Formulae tabulated.

Figure No.1.4: Preparation of sample solutions

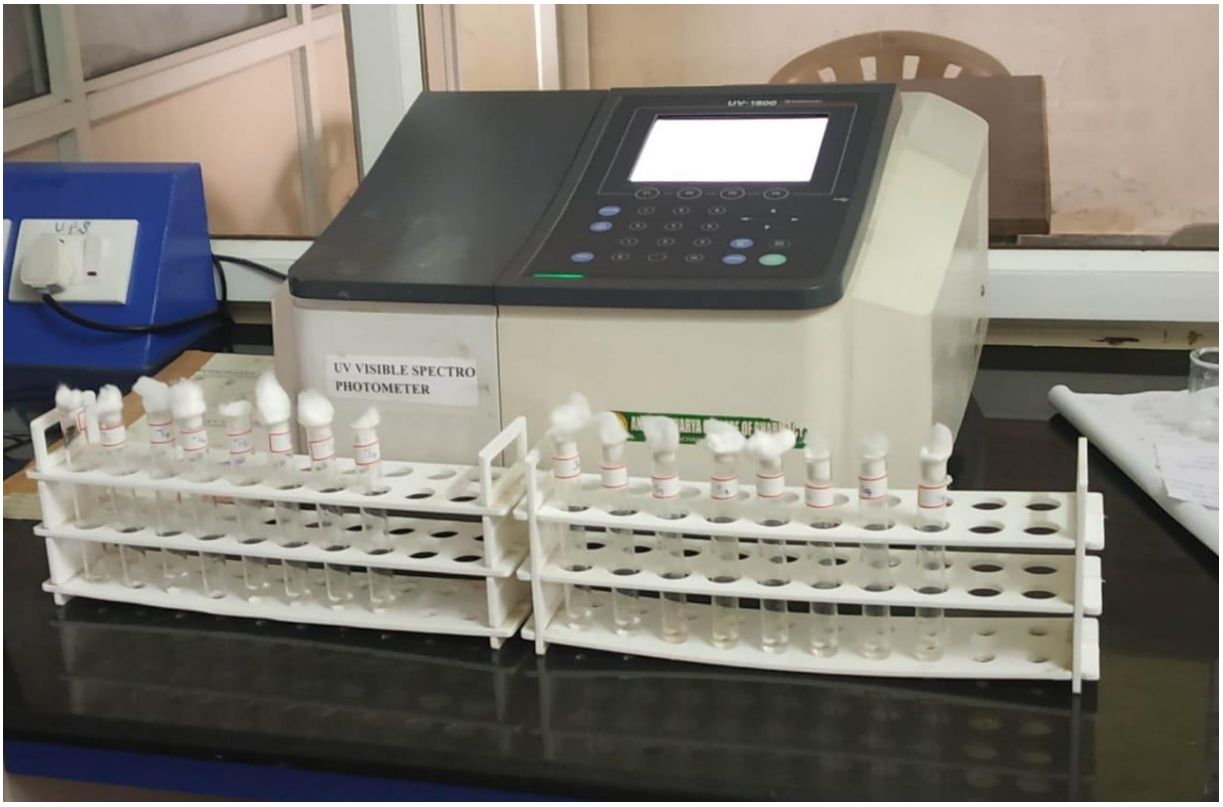

Figure.No.1.5. Determination of absorbance of samples by UV visible Spectroscopy

Table No 1.6. Egg albumin Protien denaturation Assay of Tinospora Cordifolia

S.No	Substance	Concentration	Absorbance	%Inhibition
1.	Control	---------	0.030	---------
2	Diclofenac Sodium	25	0.036 ±0.002	16.633 ±3.350
		50	0.037 ± 0.002	23.300 ±3.300
		100	0.038 ± 0.002	27.733 ±5.095
		200	0.041 ± 0002	35.533 ±5.085
		400	0.043 ± 0.001	44.400 ±1.905
		600	0.047 ± 0.001	55.533 ± 3.868
		800	0.049 ± 0.001	62.200 ±3.811
		1000	0.053 ± 0.005	71.067 ± 6.926
3.	Test sample	25	0.034 ± 0.0006	13.20±0.173
		50	0.035 ±0.0012	17.767± 3.868
		100	0.036 ±0.0000	22.20±3.811
		200	0.039 ±0.0020	29.967 ± 6.650
		400	0.041 ± 0.0010	36.633 ± 3.350
		600	0.044 ±0.0006	45.167 ±1.692
		800	0.046 ± 0.0025	53.733 ±7.360
		1000	0.051 ± 0.0053	62.967 ± 6.207

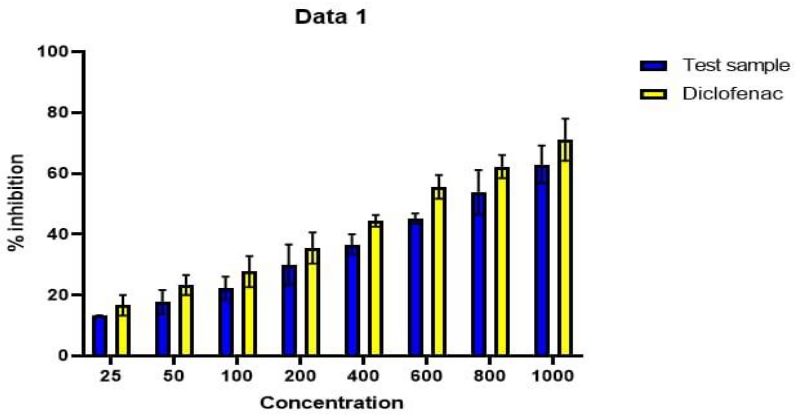

Figure No 1.6: Graph Showing % inhibitory Effect of tinospora cardifolia on inflammation

$$\left(\frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}}\right) \times 100$$

DISCUSSION:

In this work a new optimal design of extracting of total alkaloids in tinospora cardifolia has been studied. The purpose is to improve the alkaloid extraction yield. This new extraction process provides a theoretical basis for the resources utilization of the new medicinal plant. Extraction is an important step in the itinerary of phytochemical processing for the discovery of bioactive constituents from plant materials. Selection of a suitable extraction technique is also important for the standardization of herbal products, as it is utilized in the removal of desirable soluble constituents, leaving out those not required with the aid of the solvents.

Further, selection of suitable extraction process and optimization of various parameters are critical for up scaling purposes i.e., from bench scale to pilot plant level. Various extraction techniques most commonly used include conventional techniques, such as; maceration, percolation, infusion, decoction, hot continuous extraction and soxhlet extraction etc. Recently, alternative methods like ultrasound assisted solvent extraction (USE), microwave assisted solvent extraction (MAE), Accelerated Solvent Extraction (ASE) and Supercritical Fluid Extractions (SFE) have gained increasing interest during the last three decades but associated with certain limitations like during USE, ultrasound waves affect the extraction yield and during ASE high temperature leads to degradation of thermolabile constituents.

Microwave Assisted Extraction (MAE) is a relatively new extraction technique have shown promising results with certain advantages over other techniques like drastic reduction in organic solvent consumption and extraction time In MAE, the heating by microwaves is because of interaction of the radiation with the dielectric field associated with polar molecules and ions. The heated solvent accelerates, desorption of target compound from the matrix into the solvent The concerted forces applied by the electric and magnetic components of the microwave radiation are rapidly changing in direction (2.4-109 secG1) at a frequency of 2450 MHz, causing the heating of the polar molecules as these molecules try to orient themselves in the direction of the field. Because solids, semisolids and liquids cannot respond instantaneously to the changing directions of the microwave field, the friction among the molecules manifests itself as heat The microwave region of electromagnetic spectrum lies between infrared and radio frequency waves with frequencies from 0.3-300 GHz.

Based on previous experiments, ethanol concentration, extraction time, and irradiation power were selected as the main variables for achieving the maximum extraction of total alkaloid compounds from tinospora cardifolia during the extraction process. In order to determine the best combination of extraction conditions, a box behnken design was formed with extraction times from 1 to 6 min, ethanol concentrations from 10 to 30 %, and irradiation power ranging from 28- to 560 watt All 17 experimental runs, as well as the experimental results and predicted results of total alkaloid content under these runs are presented

Box behnken design with three levels and three variables was employed to determine the optimum combination of independent variables for the maximal extraction of total alkaloid compounds from tinospora cardifolia. Three independent variables selected after the preliminary

experiments were extraction time (ET), ethanol concentration (EC), and Irradiation power). Box Behkhen design consisting of 3
Factors, 5 central points,3 levels , and 17 runs .Regression analysis was carried out on the data of dependent responses to fit the experimental data and fitted to an linear model.
Design-Expert (Version 8.0) software was used to analyze the experimental data. Values are recorded as mean ± SD. The data obtained from different groups was analyzed by ANOVA. The values were considered statistically significant for all conduct experiments.
The qualitative estimation of alkaloids was made using Wagner's and Mayer's reagents.

CONCLUSION:

The WHO estimated that approximate 65% of the world population still depends mainly on herbal and traditional remedies. India is one of the richest countries in the world with huge diversity of medicinal plants, India has a large flora which require in traditional treatments of medical system. The medicinal properties of these plants could be based on the therapeutic and antioxidant effect of different phytochemicals present in them. The leaves and stem extracts of this plant have various phytochemicals such as Alkaloids, flavonoids saponins, which are responsible for these activities. These results revealed that alkaloids component were present in all solvent extracts of T. cordifolia. Total alkaloid content was high in chloroform and ethanolic extract of T. Cordifolia. Furthermore, these results of plant sources were found to be highly significant. Hence, there is more requirements to explore the applicability of these plant resources which are rich in phytochemicals and may have beneficial effect on health.

Phytochemical screening and analysis can be beneficial for drug discovery and development. Results obtained clearly reflects a linear increase in total alkaloid content with increase of extraction time , solvent to sample ratio at a fixed ethanol concentration, irradiation power and reached maximum at the highest extraction time. This indicates a linear relationship between the variables chosen and responses.
Hence, this plant can be use as a good source for beneficial drugs and its quantified values can be use as a tool for a drug to obtain a quality control profile. In this work a new optimal design of extracting of total alkaloids in tinospora cardifolia has been studied. The purpose is to improve the alkaloid extraction yield. This new extraction process provides a theoretical basis for the resources utilization of the new medicinal plant.

Our study revealed that important medicinal components present in the studied species and the developed microwave assisted extraction method during the present study is more efficient than the previous methods thus can be used as promising tool for extraction of *Tinospora cordifolia* because of high yield and fast extraction ability with less consumption of solvent as well as time.

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