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QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL SCREENING, OPTIMIZING THE EXTRACTION AND FRACTIONATION OF *TANACETUM UMBELLIFERUM* BY USING RESPONSE SURFACE METHODOLOGY

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Abstract

Phytochemicals are characterized as bioactive nutritional plant compounds that advantages extend beyond basic nutrition. The presence of various phytochemicals such as saponins, tannins, steroids, coumarins, terpenoids, anthocyanins, fatty acids emodins and leucoanthocyanins, are confirmed by qualitative phytochemical analysis of some significant medicinal plants. These findings suggest that these plants have potential pharmacological properties as well as biochemical properties for treating various diseases. In current study qualitative phytochemical analysis was performed for the detection of existing phytochemicals in *T. umbelliferum* hydroethanolic extract followed by quantitative analysis for quantitizing these phytochemicals. The plant was further subjected to fractionation in different polarity based solvents i.e. 1-butanol, chloroform and n-hexane and percentage yield was measured for each fraction. The experimental design was determined at optimum level using response surface methodology. The predicted values of percentage yield by means of RSM were close to experimented results therefore optimization was achieved at its maximum output. The phytochemical trials affirm the occurrence of carbohydrates, terpenoids, glycosides, phenols, saponins, alkaloids, flavonoids and tannins whereas proteins and amino acids were not reported in extract of *T. umbelliferum*. In quantitative analysis the phytochemicals in high concentration were determined as phenolic compounds followed by flavonoids, saponins, tannins and alkaloids. The fractions formation suggested that highest variety of phytochemicals can be achieved by using mixture of hydroethanolic solvent that declines while moving towards either extreme pole and least percentage yield was obtained by nonpolar solvent i.e. n-hexane. This analysis also paves the way for the isolation of new and novel compounds.

Keywords

Phytochemical screening, Medicinal plant, Quantitative analysis, Alternative therapy, *Tanacetum umbelliferum*

1. Introduction

Phytochemicals are chemical elements that occur naturally in plants and can have either beneficial or harmful effects on human health. Medicinal plants being used for varying types of ailments are the richest bio reservoirs of various phytochemicals. The medicinal properties of the plants are determined by the phytochemical constituents. Some of the significant phytochemicals include alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenes, etc. are dispersed in diverse regions of the plants {Shaheen, 2019}. Nature is a unique source of structures of high phytochemical diversity representing phenolics (45%), terpenoids and steroids (27%), and alkaloids (18%) as major groups of phytoconstituents Whereas these derivatives appear to be optional for the plant that produces them, they are crucial for survival by mediating ecological functions with rivals, protecting the plant from diseases, stress, pollution, and UV rays, and contributing to the color, aroma, and flavor of the plant. The metabolites produced by plants to protect themselves against biotic and abiotic stresses have turned into medicines that people can use to cure various ailments {Basit, 2021}. The prestigious statement of Hippocrates "Let prescription be your food and food your medicine shed the light on the meaning of local medications. The chronicled setting of healing plants is essentially just about as old as human advancement. That is the clarification World Prosperity Affiliation (WHO) proclaimed that the focal clinical benefits structure for the 80% individuals all through the planet contains the customary medications {Holst, 2020}.

Different extraction methods can be used to isolate phytochemicals from the plant's constituents. *Tanacetum umbelliferum L.* for the most part known as Bozidan. This is an enduring zest of the “Asteraceae” family, nearby to gentle Europe and Asia, where it creates close by the street, hedgerows and waste spots. *T. umbelliferum* is a sweet-smelling, herbaceous plant that can reach up to 5 ft. (1.5 m) in height. *T. umbeliferum* from Lithuania is segregated into four principal phytoconstituents: 1,8-cineole, trans-thujone, camphor and myrtenol. In Norway, seven chemotypes of *T. umbelliferum* could be recognized: α-thujone, β thujone, camphor, cysteinyl acidic corrosive determination/ chrysanthenol, chrysanthemum, artemisia ketone/artemisia alcohol, and 1,8-cineole. Also, more than 15 undeniable chemotypes have been depicted from Scandinavia and the Baltic up until this point, and the connection between the genetic distance networks of comparable genotypes was significantly colossal {Bernal, 2022}.

2. Material and Methods

2.1. Assortment of plant material

The research plant *T. umbelliferum* (Figure-1) was taken from the Rawalakot Azad Kashmir, in November 2020. Voucher specimens of the collected plant were deposited at the Botany Department, The Islamia University of Bahawalpur, to identify and authenticate the plant species. Dr. Ghulam Sarwar (Lecturer, Department of Botany, The Islamia University of Bahawalpur) identified the *T. umbelliferum* plant, and the identification number of the voucher is “Ref. No. 39/

Botany".
Identification sources:
"Diagn. PI. Orient. ser. 2, 3: 30", 30,1856
gbif/org/species/118674924/verbatim
http://rs.tdwg.org/dwc/terms/specificEpithet

2.2. Study design of the hydroethanolic extraction

All the useful part i.e. roots of the selected plant, was washed carefully and dried under shade shadow for a time of 72 h and processed, utilizing an electric mill operator and from there on sieved (size No. 40). The selected plant was extracted using the maceration method. The solute to solvent ratio of 1:10 with a particle size of 5 mm was soaked for 3 days in hydroethanolic solvent mixture (30:70) and suspension was passed through cotton muslin cloth and filtrate was centrifuged at 4000 rpm for 10 min, then supernatant was passed through paper filtration using Whatman filter paper. To evaporate solvents, rotary evaporation was used with a temperature of 50-60 ˚C the condensed extract was stored at 4 ˚C for further study {Chuensun, 2021}.
As per guidelines of WHO the extract was prepared serially as per requirement to avail fresh extract with all the metabolites and phytochemicals in active form. Each time the plant powder of 500mg was subjected to extraction and percentage yield was calculated using the following formula {Mumed, 2022}.

W1/ W2× 100
W1= weight of extract
W2= weight of plant powder

2.3. Experimentation optimization

Temperature (X1), extracting duration (X2), and ethanol concentration (X3) are three different variables that have an impact upon that a range of input values. Throughout this research, the correlation between the independent and dependent variables was established using a central composite design (CCD). The CCD has 27 tests including Six runs just at centerline for the three factors temperature (X1), extraction time (X2), and ethanol concentration (X3). To minimize the consequences of unanticipated variation in dependent variable, each sequence in which these 20 trials were conducted was randomised. Table 1 provides an illustration of how the tests were set up.

2.3.1. Calculating the extraction efficiency

A quantity of reductive extract multiplied by the quantity of powdered by using the formula:

% yeild= Weight of dry extract/ Weight of dry plant powder × 100

2.4. Phytochemical Examination

The extract was subjected to thorough phytochemical analysis and standard techniques were used to determine the presence of carbohydrates, amino acids, proteins, lipids, saponins, flavonoids, alkaloids, tannins, and phenols in the plant (Table-2).

2.5. Fractions Preparation

Solvent selection was polarity based but other characteristics of solvent are also of keen interest while fractionation. Therefore, following solvents given in Table-1 were selected on the basis of given properties.

The preformed crude hydro-ethanolic extract (30:70) was dissolved in distilled water (pH 7.1) with a ratio of 5g/ml, the literature shows 0.05g/ml and 5g/ml concentration of water and crude extract to make a suspension. Then this suspension is added to separating funnel and add n-hexane (Merk) solvent usually i.e. with lowest polarity at a concentration of 50/50 (50ml suspension and 50 ml solvent) {Mir, 2013}. Make sure that the inlet and outlet is closed tightly then gently shakes and mix the solution with in separating funnel approx. 50-80 times and loose pressure in between. Fix the separating funnel in stand and allow the mixture to separate in two layers for about 60 min. Note down the relative density of solvents and one that is heavier than water will make lower layer and lighter will form upper layer. Chloroform is heavier and others are lighter so lower will be the aqueous layer except for chloroform in current study. After 20 min, carefully collect the two layers in separate beakers and repeat the same procedure with this aqueous suspension while solvent will be fresh each turn until it become clear transparent to get maximum yield of fraction. Similarly, change the subsequent solvents and collect the fractions the final left over will be aqueous fraction. Evaporate using rotary evaporator for collected fractions or at room temperature to get final semi solid fraction. It could be completely dry and grind to fine powder. Store the fractions at 4℃ temp in air tight container. Characteristics of solvents are discussed in Table No. 1.{Truong, 2019}.

Calculate the percentage yield of each fraction using formula:

% yield= weight of fraction obtained/weight of crud extract used x 100.

2.6. Quantitative estimation of phytochemicals

The hydro-ethanolic extract of *T. umbelliferum* were processed to estimate the total phenolic contents (TPC), total flavonoids contents (TFC), total alkaloids contents (TAC), total saponins contents (TSC), total tannins contents (TTC) using the Folin- Ciocalteu method as described by Jain, 2020 {Jain, 2020}

3. RESULTS AND DISCUSSION

3.1. Extraction

Hydroethanolic extraction of *T. umbelliferum* was optimized using RSM against the factors of temperature, time and ethanol concentration where out of 17 runs with values between minimum and maximum peaks, the most frequent predicted values were selected for calculation of mean that is given in table # 3,4. For maximum yield the mean values of dependent variables or factors were subjected to experiment. In table # 5 and Figure # 2 for selected output parameters the percentage yield was predicted by RSM as 76% where the observed value is very closer to it i.e. 79.5%. it represents accuracy and optimized scenario of experiment given by RSM is highly applicable and time saving. Figure # 3 shows the percentage yield of extraction.

Coding for factors is used.

sum of the squares Partial Type III

The model is suggested to be significant by the model's F-value of 5.12. The likelihood that noise would result in an F-value this big is merely 2.14%.

Model terms are considered significant when the P-value is less than 0.0500. A and A2 are important model terms in this instance. Model terms are not significant if the value is higher than 0.1000. Model reduction may enhance your model if it has a lot of unnecessary words (except those needed to maintain hierarchy).

The Lack of Fit F-value of 12.44 indicates that there is a 7.61% probability that noise might be the cause of a big Lack of Fit F-value. This is undesirable since we want the model to fit. This relatively low probability (<10%) is troubling.

3.1.1. Final Equation in Terms of Coded Factors

Y= +78.41+13.57+2.00-0.1897+3.38-2.38-4.62-8.86-6.38-4.08

It is possible to anticipate the reaction for certain amounts of each element using the equation expressed in terms of coded factors. By default, the factors' high values are written as +1 and their low levels as -1. By contrasting the factor coefficients, the coded equation may be used to determine the relative importance of the elements.

3.2. Percentage Yield of Test Samples (Extract and Fractions)

The plant powder subjected to extraction in hydroethanolic solvent generated a semisolid crude extract. The percentage yield of this crude extract was calculated using formula given above. Similarly, the obtained yield of crude HE extract was further subjected to dissolution for collecting fractions of three different solvents i.e. n-hexane, 1-butanol and chloroform. The percentage yield for each extraction was calculated and given in table 6. While among fractions the solvent having high polarity generated high yield of phytochemicals compared to nonpolar or least polar given in table 1. For crude hydroethanolic extract highest yield of phytochemicals justifies that being a combination of medium to highest polar solvent it dissolves almost all bioactive metabolites that are responsible for boosting the therapeutic activities of plant and its parts. The results are supported by another study conducted on three medicinal extracted in different solvents where polar solvent extracts produced superior pharmacological activity compared to others {Windmi, 2021}.

3.3. Phytochemical Investigation

The phytochemical investigation on the aqueous ethanolic extract of *T. umbelliferum* roots was performed to determine the presence of phytomolecules in table 7, which shows the presence (+) and absence (-) of phytomolecules in the extract. Multiple tests for authentication were implemented for each group of phytomolecules and compared with standards. The plant contains

alkaloids, carbohydrates, favonoides, tannins, glycosides, phenolic, saponins, and terpenoids. Protein content remained absent while comparing to the standards. Therefore, it is declared that *T. umbelliferum* is rich in flavonoids and phenols whereas protein content is diminished that is indicative of significant pharmacological activities such as antioxidant, antiarthritic, anti-inflammatory etc. {Kagambega, 2022 }

3.4. Quantitative estimation of phytochemicals:

In quantitative analysis the phytochemicals in high concentration were determined as phenolic compounds followed by flavonoids, saponins, tannins and alkaloids. Phenols were present in high concentration in *T. umbelliferum* at 350.9 mg/g (Table # 8, Graph # 1) and flavonoid as 98.7 (Table # 9, Graph # 2) that indicates high potential of plant for muscloskeletal disorders with evidence of low protein content in qualitative analysis. The intriguing alternative sources for pharmacological and medical uses include flavonoids and other phenolic chemicals. In various studies examples of these phytochemicals from various medicinal plants are also provided, along with a discussion of their potential uses in pharmaceutical and medical fields, particularly for the promotion of health. Examples include their antioxidant, antibacterial, anti-cancer, cardioprotective, immune-system-boosting, anti-inflammatory, and skin-protecting properties against UV radiation {Walters, 2021}. Tannins (Table # 10, Graph # 3), Alkaloids (Table # 11, Graph # 4) and saponins (Table # 12, Graph # 5) also shows some concentration of chemicals in *T. umbelliferum*.

4. Conclusion

The information on the substance constituents of plants assists with screening for biological applications. The phenolic and flavonoids are broadly distributed metabolites in plants having pharmacological potential and have wide scope of implementation in clinical practices as natural medicine. It was proved to be supportive in regard of hydroethanolic solvent as rich in metabolites or phytochemicals as compared to other solvents. The RSM provided high optimization for extraction method and predicted values were close to observed values to depict accuracy. Therefore, it is declared that to achieve optimized conditions for research studies RSM plays pivotal role and hydro-ethanol is the solvent of choice for obtaining maximum yield of phytochemicals.

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Figure 1: *Tanacetum umbelliferum* extraction

Table-1: Experimental factors with obtaining percentage yield by RSM

Serial No	X1 Ethanoic concentration	X2 Time	X3 Temperature	Percentage yield
1	75.00	4.77	37.00	49
2	100.23	30.00	37.00	85
3	75.00	55.23	37.00	73
4	60.00	15.00	34.00	45
5	90.00	15.00	34.00	69
6	60.00	15.00	40.00	62
7	75.00	30.00	42.05	70
8	75.00	30.00	37.00	81
9	90.00	45.00	34.00	76
10	75.00	30.00	31.95	65
11	90.00	15.00	40.00	65
12	60.00	45.00	40.00	37
13	75.00	30.00	37.00	79
14	60.00	45.00	34.00	50
15	49.77	30.00	37.00	23
16	90.00	45.00	40.00	65
17	75.00	30.00	37.00	75

Table-2: Methods for phytochemical test of *T. umbelliferum*

	TEST	REAGENT	EXTRACT	METHOD	RESULT
Proteins	Millon's test		Prepared HAE+D/W {Holst, 2020}	Crude extract mixed with 2ml of Millon's reagent {Shaikh, 2020} {Holst, 2020}	Appearance of white ppt , turn red on gentle heating , {Shaikh, 2020}

	Ninhydrin test	10 mg of ninhydrin in 200 ml of acetone {Holst, 2020}	Prepared HAE+D/W {Shaikh, 2020}, Aqueous extract {Holst, 2020}	Crude extract boiled with 2ml of 0.2% solution of Ninhydrin {Shaikh, 2020} Two drops of ninhydrin solution are added to 2 ml of aqueous filtrate {Holst, 2020}	Appearance of violet colour indicates presence of amino acid and protein {Shaikh, 2020} {Holst, 2020}
	Biuret test		Prepared HAE+D/W {Holst, 2020}	2 ml of filtrate is treated with 1 drop of 2% copper sulphate solution. To this 1 ml of ethanol (95%) is added, followed by excess of potassium hydroxide pellets {Holst, 2020}	Pink colour ethanolic layer indicates the presence of protein. {Holst, 2020}
Carbohydrates	Fehling's test		Prepared HAE+D/W {Shaikh, 2020}	Equal volume of Fehling A & B reagents were mixed together, 2ml of it was added to crude extract and gently boiled. {Shaikh, 2020}	brick red precipitate appeared to indicate the presence of reducing sugars. {Shaikh, 2020}
	Benedict's test		Prepared HAE+D/W {Shaikh, 2020},	Crude extract mixed with 2ml of Benedict's reagent and Boiled at water bath for 2 min {Shaikh, 2020} {Holst, 2020}	reddish brown precipitate {Shaikh, 2020} {Holst, 2020}
	Molisch's test	alcoholic solution of α-naphthol	Prepared HAE+D/W {Shaikh, 2020} {Holst, 2020}	Crude extract was mixed with 2ml of Molisch's reagent and shaken. Then 2ml of concentrated H2SO4 was poured carefully along the side of the test tube. {Shaikh, 2020} {Holst, 2020}	violet ring at the interphase {Shaikh, 2020}, formation of purple or reddish color {Bernal, 2022}.
	Iodine test		Prepared HAE+D/W {Shaikh, 2020},	Crude extract was mixed with 2ml of iodine solution {Shaikh, 2020}	Dark blue or purple coloration {Shaikh, 2020}

Tannins	$FeCl_3$ test		Prepared HAE+D/W or Aqueous extract {Dadhich, 2022} {Holst, 2020}	Crude extract was mixed with 2ml of 2% solution of FeCl3 {Shaikh, 2020} 0r 5% $FeCl_3$ {Holst, 2020}	blue-green or black coloration for tannins {Shaikh, 2020} {Dadhich, 2022} {Holst, 2020} {Bernal, 2022}.
Phenols	Gelatin test		Aqueous extract {Holst, 2020}	2 ml of 1% solution of Gelatin containing 10% NaCl is added to aqueous extract	White precipitate indicates the presence of phenols
	Lead acetate test		Aqueous extract {Holst, 2020}	3 ml of 10% lead acetate solution is added to aqueous extract	A bulky white precipitate indicates the presence of phenolic compounds
	$FeCl_3$ test		Prepared HAE+D/W {Shaikh, 2020} {Bernal, 2022}or Aqueous extract {Dadhich, 2022} {Holst, 2020}	Crude extract was mixed with 2ml of 2% solution of FeCl3a {Shaikh, 2020} 0r 5% $FeCl_3$ {Bernal, 2022}.	blue-green or black coloration for phenols {Shaikh, 2020} {Dadhich, 2022} {Holst, 2020} {Bernal, 2022}.
Flavonoids	Shinoda test		Prepared HAE+D/W or alcoholic extract {Holst, 2020}	Crude extract was mixed with few fragments of magnesium ribbon and concentrated HCl was added drop wise {Shaikh, 2020} {Holst, 2020}	Pink scarlet to crimson colour appeared after few minutes {Shaikh, 2020} {Holst, 2020}
	Alkaline reagent test		Prepared HAE+D/W {Shaikh, 2020} {Bernal, 2022}or aqueous extract {Holst, 2020}	Crude extract was mixed with 2ml of 2% solution of NaOH {Bernal, 2022} or 10% ammonium hydroxide {Holst, 2020}	intense yellow color was formed which turned colorless on addition of few drops of diluted acid, yellow fluorescence {Holst, 2020} {Bernal, 2022}

			Prepared HAE+D/W or Aqueous extract {Dadhich, 2022}	A few chop of 1% NH**3** solution is added to the aqueous extract in a test tube. {Dadhich, 2022}	A yellow coloration is observed if flavonoids compound are present. {Dadhich, 2022}
Saponins	Foaming test		Prepared HAE+D/W {Bernal, 2022} or Aqueous extract {Dadhich, 2022} {Holst, 2020}	A test tube containing crude extract and 5ml of distilled water was forcefully shaken. {Shaikh, 2020} {Holst, 2020} {Bernal, 2022}.	The formation of stable foam {Shaikh, 2020} {Holst, 2020} {Bernal, 2022}.
				The frothing is mixed with 3 drops of olive oil {Dadhich, 2022}	the formation of emulsion indicates the presence of saponin. {Dadhich, 2022}
Glycosides	Liebermann's test		Prepared HAE {Shaikh, 2020},	2ml of acetic acid and 2ml of chloroform were combined with the crude extract. Ice was used to chill the concoction. A carefully diluted H2SO4 solution was added.{Shaikh, 2020}	colour change from violet to blue to green {Shaikh, 2020}
	Salkowski's test		Prepared HAE {Shaikh, 2020},	2ml of chloroform was combined with crude extract. Next, 2ml of concentrated H2SO4 was cautiously added and gently mixed.{Shaikh, 2020}	A reddish brown colour {Shaikh, 2020}

	Keller-kilani test		Prepared HAE+D/W {Shaikh, 2020},	Crude extract was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of FeCl3. The mixture was then poured into another test tube containing 2ml of concentrated H2SO4. {Shaikh, 2020} {Dadhich, 2022}	A brown ring at the interphase indicated the presence of cardiac glycosides. {Shaikh, 2020} {Dadhich, 2022}
	Borntrager' s test		Acid extract. {Holst, 2020}	50 mg of extract is hydrolysed with concentrated HCl for 2 hrs on a water bath and filtered. To 2 ml of filtered hydrolysate, 3 ml of choloroform is added and shaken, choloroform layer is separated and 10% ammomia solution is added to it {Holst, 2020}	Pink colour indicates presence of Glycosides {Holst, 2020}
	Legal' s test		Prepared HAE {Holst, 2020}	50 mg of extract is dissolved in pyridine, sodium nitroprusside solution is added and made alkaline using 10% NaOH. {Holst, 2020}	Pink colour indicates presence of Glycosides {Holst, 2020}
			Prepared HAE {Bernal, 2022}	To 2 ml of extract, 3ml of chloroform and 10% ammonia solution was added. {Bernal, 2022}.	Formation of pink color indicates presence of glycosides {Bernal, 2022}.
Terpenoids	Salkowski' s test		Prepared HAE+D/W {Shaikh, 2020}	Crude extract was dissolved in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated H2SO4 was added and heated for about 2 minutes. {Shaikh, 2020}	A grayish colour indicated the presence of terpenoids. {Shaikh, 2020}

			Aqueous extract {Dadhich, 2022}	In a test tube, 5ml of the aqueous extract and 2ml of CHCl3 are combined. To create a layer, 3ml of concentrated H2SO4 is carefully added to the mixture.{Dadhich, 2022}	An interface with a reddish brown coloration is formed. {Dadhich, 2022}
Alkaloids	Mayer's test		Acid extract {Shaikh, 2020} {Holst, 2020} {Bernal, 2022}.	Crude extract was mixed with 2ml of 1% HCl and heated gently. Mayer's reagents were then added to the mixture {Shaikh, 2020} {Holst, 2020} {Bernal, 2022}. .	Turbidity of the resulting precipitate or green color or white precipitate {Bernal, 2022}.
	Wagner's test		Acid extract {Shaikh, 2020},	Crude extract was mixed with 2ml of 1% HCl and heated gently. Wagner's reagents were then added to the mixture {Shaikh, 2020} {Holst, 2020}	Turbidity of the resulting precipitate {Shaikh, 2020}. Reddish- Brown precipitate confirms the test as positive {Holst, 2020}

HAE= hydroalcoholic extract, D/W= distilled water

Table-1. Characteristics of Selected Solvents

Solvents	Relative Polarity	Density g/ml	Formula	Molecular weight	Solubility in H_2O(g/100g)
Water	01	0.998	H_2O	18.02	-
Ethanol	0.654	0.889	C_2H_6O	46.07	Miscible
1-butanol	0.586	0.81	$C_4H_{10}O$	74.12	7.7
Chloroform	0.259	1.498	$CHCl_3$	119.38	0.8
n-hexane	0.009	0.659	C_6H_{14}	86.18	0.6

Table No 3: Factors of extraction parameters with output

Ser no	Name	Unit	Minimum	Maximum	Mean	Std. Dev
1	Ethanolic concentration	ml	49.77	100.23	**70**	**13.86**
2	Time	Sec	4.77	55.23	**30**	**13.86**

3	Temperature	°C	31.95	42.05	**37**	**2.77**

Table No 4: Response of extraction parameters with output

Response	Name	Units	Observations	Minimum	Maximum	Mean	Std. Dev.	Predicted %	Observation %
R1	percentage yield	%	17.00	23	85	78.88	16.84	76	79.54

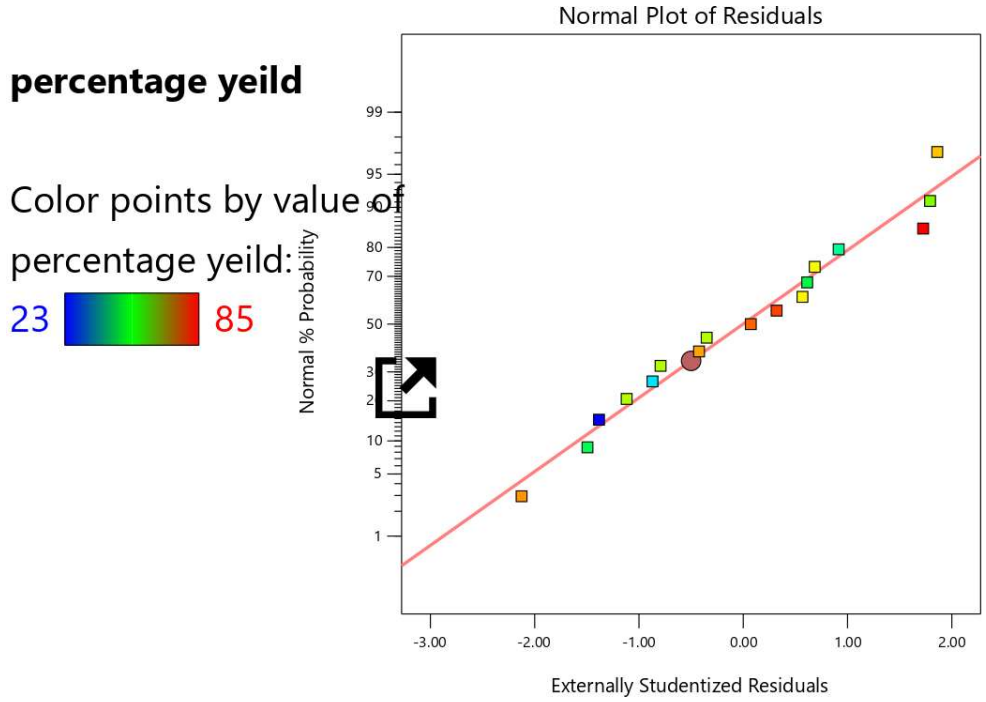


Fig No 2: Percentage probability of extraction's experimental models

Table No 5: ANOVA for Quadratic model

Response 1: percentage yeild

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	3940.64	9	437.85	5.12	0.0214	significant
A-Ethanol concentration	2513.42	1	2513.42	29.37	0.0010	
B-time	54.82	1	54.82	0.6406	0.4498	
C-Temperature	0.4916	1	0.4916	0.0057	0.9417	
AB	91.13	1	91.13	1.06	0.3365	
AC	45.13	1	45.13	0.5272	0.4913	

BC	171.13	1	171.13	2.00	0.2003	
A^2	884.50	1	884.50	10.33	0.0148	
B^2	459.29	1	459.29	5.37	0.0537	
C^2	188.10	1	188.10	2.20	0.1818	
Residual	599.12	7	85.59			
Lack of Fit	580.46	5	116.09	12.44	0.0761	not significant
Pure Error	18.67	2	9.33			
Cor Total	4539.76	16				

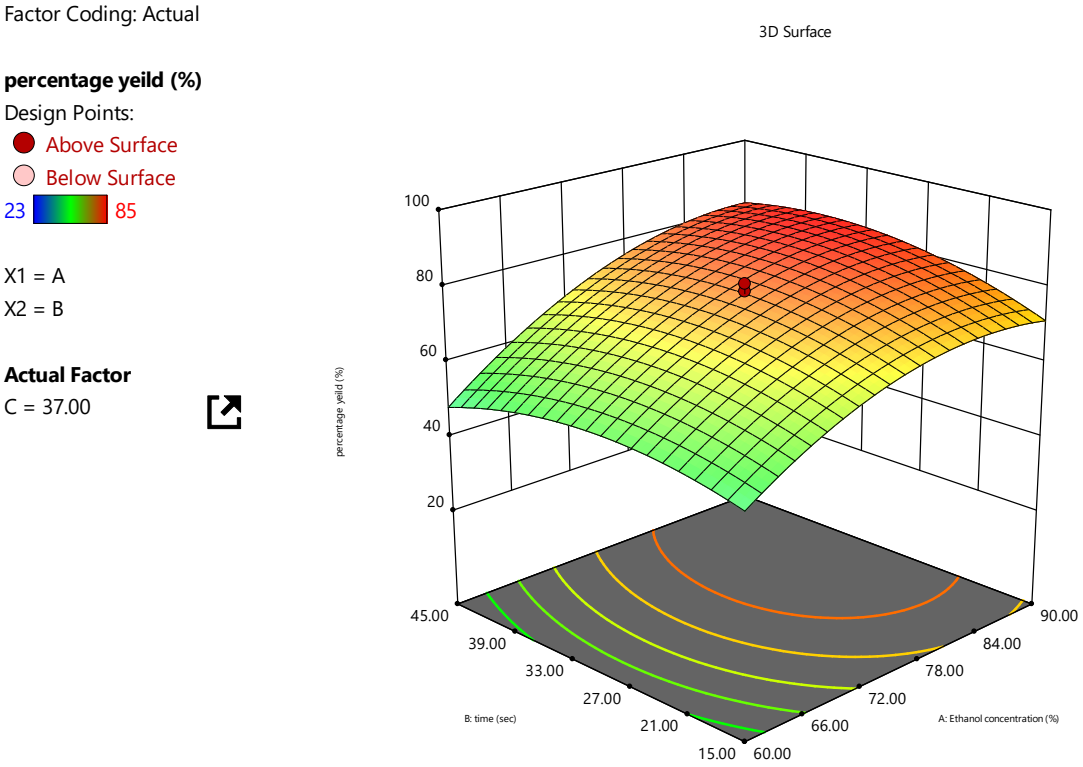


Fig No 3: 3D Percentage yield of extraction parameters

Table-6- Percentage Yield of Crude extract of *T. umbelliferum* and its Fractions

Test Sample	Solute in gram	Obtained yield (g)	Percentage yield (%)
HE extract	500	30g	06
nH	20	0.259	1.295
1B	20	0.345	1.725
Ch	20	0.484	2.4

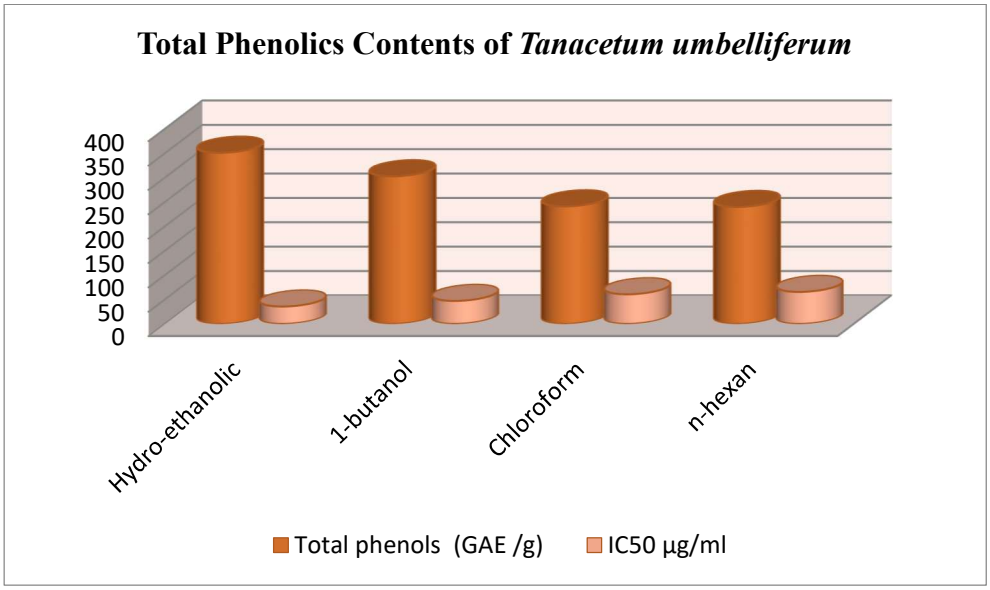
HE (hydro-ethanol); nH (n-hexane); Ch (chloroform); 1B (1-butanol)

Table 7. Phytochemical Analysis of *T. umbelliferum*

S. No.	Phytochemical Tests	HE crud Extract
1.	**Test for alkaloids** (a) Mayer's test (b) Wagner's test (c) Hager's test	 +ve +ve +ve
2.	**Test for protein** (a) Million test (b) Ninhydrin test (c) Biuret test	 -ve -ve -ve
3.	**Tests for carbohydrate** (a) Molish test (b) Iodine test (c) Fehling's test (d) Benedict's test	 +ve +ve +ve +ve
4.	**Test for reducing sugar** (a) Fehling's test (b) Benedict's test	 +ve +ve
5.	**Tests for flavonoids** (a) Alkaline reagent (b) Lead acetate	 ++ve ++ve
6.	**Test for glycosides** (a) Borntrager's test (b) Legal's test (c)Keller-kilani test (d) Salkowski's test (e) Liebermann's test	 +ve +ve +ve +ve +ve
7.	**Test for tannin and phenolic** (a) Ferric chloride test (b) Lead acetate test	 ++ve ++ve
8.	**Tests for saponin** Foaming test	 +ve
9.	**Test for terpenoids** Salkowski's test	 +ve
HE= Hydroethanlic; +ve= Positive (Presence); -ve= Negative (Absence)		

Table 8- Phenolic contents of *T. umbelliferum*

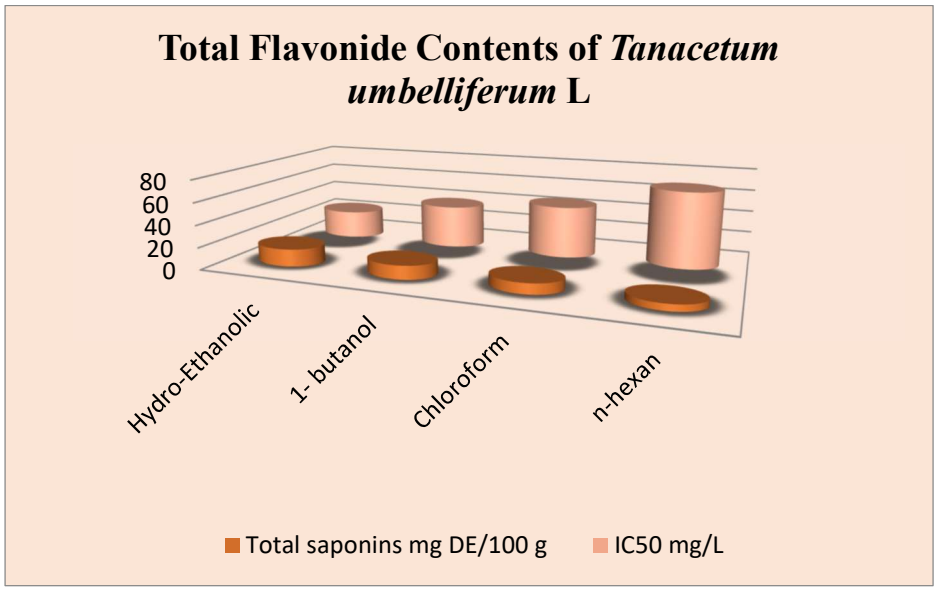
Plant Extracts		Total phenolic contents (mg/g) GAE	IC_{50} µg/ml
Tanacetum umbelliferum	Hydro-ethanolic	350.9	35.4
Fractions	1-butanol	302.6	46.9
	Chloroform	240.9	60.5
	n-hexane	239.7	65.4



Graph 1- Total Phenolic contents of *T. umbelliferum*

Table 9- Flavonides contents of *T. umbelliferum*

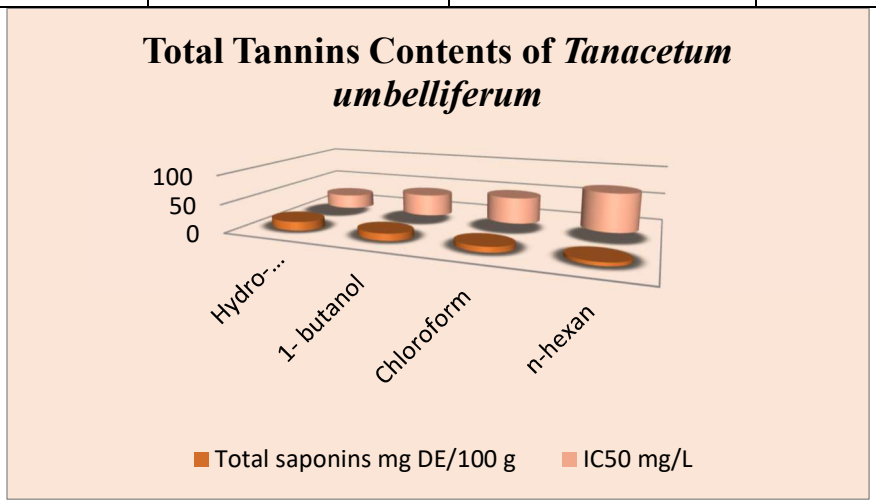
Plant Extracts		Total flavonoids contents (QE /g of extract)	IC_{50} µg/ml
***Tanacetum umbelliferum* L**	Hydro-ethanolic	98.7	39.5
Fractions	1-butanol	85.5	49.3
	Chloroform	78.7	56.7
	n-hexane	69.2	65.2



Graph 2- Total Flavonide contents of *T. umbelliferum*

Table 10- Tannin contents of *T. umbelliferum*

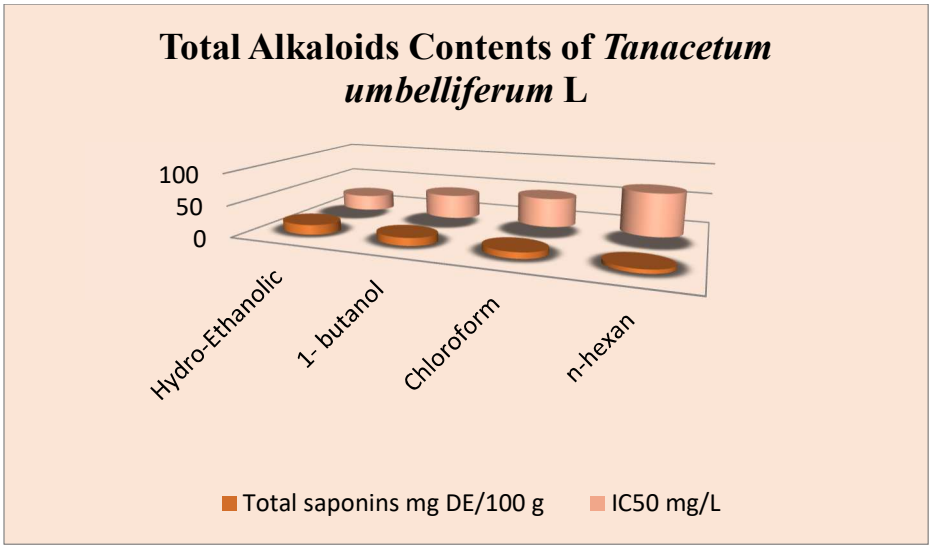
Plant Extracts		**Tannin contents (mg CE/g DW**	**IC_{50} µg/ml**
***Tanacetum umbelliferum* L**	Hydro-ethanolic	8.52	37.7
Fractions	1-butanol	6.73	56.0
	Chloroform	3.21	69.5
	n-hexane	2.10	77.6



Graph 3- Total Tannins contents of *T. umbelliferum*

Table 11- Alkaloids contents of *T. umbelliferum*

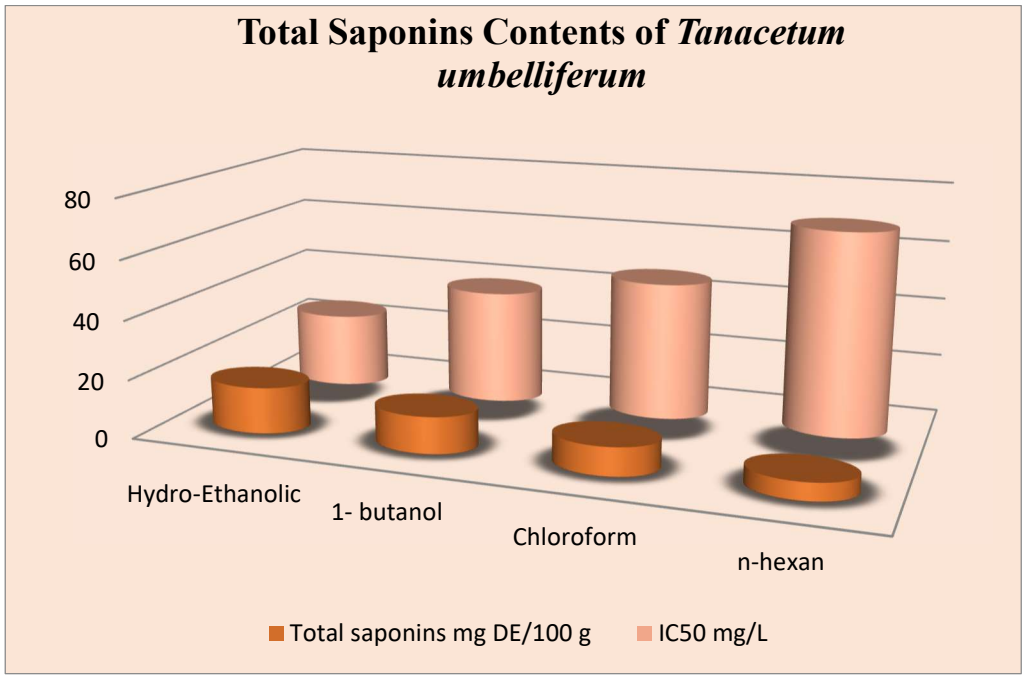
Plant Extracts		Alkaloids (mg PNE / g of dry mass)	IC_{50} mg/L
***Tanacetum umbelliferum* L**	Hydro-ethanolic	5.77	12.32
Fractions	1-butanol	4.21	28.67
	Chloroform	2.10	45.8
	n-hexane	1.56	68.42



Graph 4- Total Alkaloids contents of *T. umbelliferum*

Table 12- Saponins contents of *T. umbelliferum*

Plant Extracts		Total saponins mg DE/100 g	IC_{50} mg/L
***Tanacetum umbelliferum* L**	Hydro-ethanolic	15.69	25.6
Fractions	1-butanol	12.41	39.0
	Chloroform	9.56	47.0
	n-hexane	5.89	69.0



Graph 5- Total Saponins contents of *T. umbelliferum*