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Research Article

ADVANCEMENT OF TOTAL PHYTOCONSTITUENT EXTRACTION PROCESS BY NEW WAY FACTOR ANALYSIS FOR *TRIDAX PROCUMBENS* L.

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ABSTRACT

The majority of literature hails the potential of natural products, and their derivatives are still in the forefront of drug discovery. In spite of the exceptional success of natural products in the history of drug development, some voices which have pointed out significant challenges such as extraction time, low yields, limited supply, and complex structures precluding practical synthesis. In this work, we added fuel to extraction challenges through focusing on extraction end time identification using correlation studies of numerous factors for the *Tridax procumbens* aqueous leave extract. The factorial analysis resulted that six factors such as temperature, microwave irradiation power, absorbance, Oxidation/reduction potential, and total dissolved solids are most useful to the description of extraction end time except pH and volume water displacement. The correlation study between yield and microwave irradiation power quantified that more yield obtained at a 7-microwave power between 30-40 minutes with 80 °C temperature. A positive correlation (r^2) of favourable factors are directly proportional to the yield of extract and the total phytoconstituents extraction end at 30^{th} to 40^{th} minute interval in microwave 7 power. Further microwave irradiation leads to degradation of phytoconstituents. The results concluded that this new way analysis specifies the extraction end time of phytoconstituent with the lead of shorter extraction time, minimal solvent usage, and higher yield. These findings were shed light to the furthering of natural product discovery endeavors through uprooting bottlenecks.

Keywords: *Tridax Procumbens L,* Microwave assisted Extraction, Extraction optimization, End time analysis, Factor analysis, yield correlation.

INTRODUCTION

Throughout the ages, humans have trusted on nature for their primary desires and not least, their medicines. Plants have fashioned the premise of refined traditional medication systems that are breathing for thousands of years. There is no doubt that plants are faultless "natural laboratories" for the synthesis of precise molecules starting from simple skeleton to substantially complex chemical structures. In fact, approximately 40% of drug scaffolds found in natural products are absent in today's medicinal chemistry due to it's huge structural and chemical diversity.[1] The records, written on natural products as sources of recent medicine from 1981 to 2014 revealed that a significant number of all NCEs approved by FDA has still fallen into the categories of unaltered natural product (N), natural product derivatives (ND), and mimics of a natural product (NM). Among the 1562 FDA approved drugs 791 (50.64%) drugs are from nature over the full 34-year period.[2] These statistics strongly endorse the exploration of nature as a supply of novel active agents which will serve as the leads and scaffolds for urgently required medicine.[3]

To remain and become modest with other synthetic medicine, natural product research needs to advance the speed of the screening, extraction, isolation, and structure explication processes, as well as spouting the suitability of screens for phytoconstituent extracts and dealing problems involve in large-scale compound supply. The study of medicinal plants begins with pre-extraction and extraction which is a major step in the processing of phytoconstituents from plant materials. Traditional methods such as maceration and Soxhlet extraction are used commonly at the small research setting level medicine[4] and major problem with these conventional procedures are higher extraction times, organic solvent consumption, more energy and costs feed.[5] Hence, Significance advances made in the extraction methods; microwave-assisted extraction (MAE), ultrasound assisted extraction and supercritical fluid extract, in which these advances are aimed to eradicate those issues.[6]

As our contribution in this troubleshooting, the identification of time required to extract all phytoconstituent from the *Tridax procumbens L*. using microwave assisted extraction method was performed. Total extraction time identification helps the next generation to save time, cost, energy and solvent usage in extraction process. *Tridax procumbens* is a medicinal and ornamental plant that exhibits wide range of pharmaceutical activities and the leaves are commonly used for medicinal purposes, because of their myriad of pharmacological properties. These include analgesic[7], anti-anemic[8], anti-arthritic[9], anti-diabetic[10,11], antihypertensive[12,13], anti-inflammatory, antioxidant[14] antimicrobial[15], antipyretic, hepatoprotective[16], hypocholesterolemic, and weight reducing[17] properties. Additionally, optimization of the extraction conditions was carried out with time, temperature and microwave power variables for effective extraction. Furthermore, there is no literature reported about end time of the total phytoconstituent extraction from the natural products. Hence, in this study initiation effort was taken to determine the extraction end time of total phytoconstituents from *Tridax procumbens L*. leaves by analysing the various factors such as colorimetric absorbance,

pH, mV, total dissolved solid (TDS) and water volume displacement analysis. Correlation statistics was performed to relate all the factors and yield of extraction. This new way factor analysis will solve existing problems in the phytoconstituents extraction from the medicinal plants.

EXPERIMENTAL SECTION

General Experimental Procedures

Plant Material

The Arrow headed, opposite, pinnate, oblong to ovate and 1-2 inches long with cuneate bases, coarsely serrate marginal and acute apexes leaves of *Tridax procumbens* was collected from Anna University, BIT Campus, Tiruchirappalli (latitude 10.65774, longitude 78.74545) province in August 2016. Assistant Prof. S. Soosairaj, Department of Botany, St. Joseph's College, Tiruchirappalli, Tamilnadu, India, has authenticate that the plant belongs to the *Tridax L*. genus, *Tridax procumbens* species, cited pl. 900. 1753 and Asteraceae family. The collected plant leaves were washed with double distilled (DD) water to remove the dust and other external matters for subsequent use.

Reagents and apparatus

The reagents used for this experiment included ethanol, methanol, n-hexane, all of the first grade and HPLC grade. The Microwave Assisted Extraction (MAE) of plant material was performed in specially designed industrial open type CATA – 4RI microwave synthesizer. It can emit 140 – 700 watt in 15 watt microwave increments with beam reflector for perfect distribution of microwave. The dipping type, flexible temperature probe was used to calculate temperature up to 600 °C. The extraction samples were stored in appropriately labeled pyrogen-free tarsons click lock 2ml microcentrifuge tubes for further factor analysis. Analysis of the pH/mV/TDS was carried out in adwa's microprocessor based AD800 bench meter. Further, Water volume displacement was measured by using IITC's 520MR digital plethysmometer. Also, the percentage of absorbance of specific wavelength was measured by Elico CL-63 photometer. Filtration of the final extract was carried out by 20-25µm pored Whatman filter papers and IKA RV 10 rotary vacuum evaporator used to separate the phytoconstituent from the solvent.

Sample collection for optimization

Preliminary MAE work starts with 25 g of fresh leaves of *Tridax procumbens* and 150 mL of DD water was taken in a flat bottom flask (FBF). The temperature fixed at 70 °C, microwave power kept 5 (425 watts,50%) irradiation level and the pressure frozen at 150 mmHg. After shooting the microwave irradiation for 10 minutes through the flask content. The extract was cooled to room temperature and dried under rotary vacuum evaporator. The dried extract was collected and weighed in *grams* unit. Similarly, the temperature and microwave power were reformed for fresh flat bottom flask content, and the process was continued to optimize a process. The temperature was fixed based on the microwave absorbing capacity (dielectric constant), and boiling point of DD water and vacuum pressure fixed at 150 mmHg (Table 1)

Sample collection for factor analysis

Factorial analysis starts with, 2ml of a sample collection at each 5-minute interval up to the factors such as absorbance, pH, mV, total dissolved of solids in TDS, water volume displacement (WVD) of this study reach constant values. Once the extraction end time was judged from the analysis, the whole solvent drained out from the FBF and collected in a volumetric flask for further studies.

Factor analysis

Diverse factors such as absorbance (Abs), pH, mV, TDS and VWD were measured and analyzed to identify the extraction end point of the *Tridax procumbens* leaves extract. Each collected sample extracts were poured in a photometric sample tube, Abs and % T were measured. Subsequently, 1mL of collected sample was mixed and made up to 10 mL in a standard flask with DD water and the pH electrode, and conductivity probe tip was immersed into the solution. The pH, mV and TDS measurements displayed on the LCD monitor of the calibrated bench meter were noted and tabled. Consequently, volume displacement analysis was performed in IITC water digital plethysmometer. One mL of extract was taken in the small tube and kept in the orifice of the plethysmometer. The water displacement level was measured in mL and noted down. All the measurement of the various factors was compiled and the extraction end time was arbitrated for *the Tridax procumbens* aqueous leave extract.

Correlation studies

Graph Pad Prism 6.0 was used to examine the correlation statistics between the factors and extraction yield. The yield of extract was kept as a dependent variable and other factors such as temperature, microwave power, absorbance (Abs), pH, mV, TDS and WVD were considered as an independent variable. The linear correlation coefficient (r) calculate the strength and the direction of a linear relationship between two variables. The mathematical formula for computing r is

$$r = \frac{n\sum xy - (\sum x)(\sum y)}{\sqrt{n(\sum x^2) - (\sum x)^2} - \sqrt{n(\sum y^2) - (\sum y)^2}}$$

The final correlated r value was tabled and analyzed to identify the significant between the variables.

Table 1. Parameters used to analyze the *Tridax procumbens* leaves extract

FBF content	Microwave power	Temperature
25 gm of leaves + 150 mL of DD water	5 (425 watts,50%)	70 °C
25 gm of leaves + 150 mL of DD water	6 (510 watts, 60%)	70 °C
25 gm of leaves + 150 mL of DD water	7 (595 watts, 70%)	70 °C
25 gm of leaves + 150 mL of DD water	8 (680 watts, 80%)	70 °C
25 gm of leaves + 150 mL of DD water	5 (425 watts,50%)	80 °C
25 gm of leaves + 150 mL of DD water	6 (510 watts, 60%)	80 °C
25 gm of leaves + 150 mL of DD water	7 (595 watts, 70%)	80 °C
25 gm of leaves + 150 mL of DD water	8 (680 watts, 80%)	80 °C

Table 2. *Tridax Procumbens* extraction Yield of the extract depended on microwave power and temperature optimization

FBF content	Microwave power	Temp.	Pres.	Yield (g)				
				10 Min.	20 Min.	30 Min.	40 Min.	50 Min.
25 gm of leaves + 150 mL of DD water	5 (425 watts ,50%)	70 °C	150 mmHg	0.097	0.117	0.201	0.293	0.253
25 gm of leaves + 150 mL of DD water	6 (510 watts ,60%)			0.121	0.198	0.286	0.346	0.315
25 gm of leaves + 150 mL of DD water	7 (595 watts ,70%)			0.186	0.249	0.302	0.367	0.329
25 gm of leaves + 150 mL of DD water	8 (680 watts, 80%)			0.134	0.214	0.289	0.337	0.298
25 gm of leaves + 150 mL of DD water	5 (425 watts , 50%)	80 °C		0.137	0.204	0.287	0.325	0.291
25 gm of leaves + 150 mL of DD water	6 (510 watts, 60%)			0.178	0.244	0.309	0.364	0.316
25 gm of leaves + 150 mL of DD water	7(595 watts, 70%)			0.188	0.247	0.328	0.397	0.341
25 gm of leaves + 150 mL of DD water	8(680 watts, 80%)			0.124	0.189	0.276	0.331	0.288

Table 3. Various factor data of *Tridox procumbens* extract at 70 °C and different power level

Minutes	Power	Temp.	Abs	%T	pH	mV	TDS	WVD
0	0	38	0.06	86	6.35	34.4	21.8	0.58
5	5	70	0.34	34	6.82	55.5	48.1	0.62
10		68	0.5	31	6.88	61.9	48.2	0.62
15		68	0.53	29	6.78	63.3	49.4	0.63
20		68	0.55	27	6.81	65.0	51.4	0.64
25		68	0.61	25	6.83	66.2	53.3	0.65
30		68	0.64	24	6.94	66.4	55.4	0.66
35		68	0.66	22	6.91	68.3	54.9	0.66
40		68	0.66	22	6.86	69.7	55.8	0.67
45		69	0.65	23	6.86	58.6	53.6	0.67
5	6	69	0.54	39	6.74	56.4	48.5	0.61
10		69	0.56	38	6.56	59.2	49.0	0.62
15		68	0.56	28	6.26	63.4	51.8	0.62
20		69	0.58	26	6.35	65.5	52.2	0.65
25		69	0.6	25	6.3	67.1	53.6	0.66
30		69	0.65	23	6.64	68.5	54.7	0.68
35		68	0.68	21	6.63	70.7	55.2	0.68
40		68	0.68	20	6.63	70.8	55.8	0.67
45		69	0.60	27	6.76	61.5	51.1	0.68
5	7	69	0.56	26	6.44	59.3	51.6	0.63
10		69	0.59	28	6.42	60.5	53.4	0.66
15		69	0.64	26	6.35	64.7	56.9	0.66
20		68	0.64	26	6.49	66.0	55.6	0.67
25		68	0.66	24	6.51	67.3	56.8	0.67
30		68	0.71	19	6.96	70.5	57.1	0.68
35		68	0.7	19	6.84	71.7	57.3	0.69
40		68	0.69	20	6.92	69.6	53.7	0.69
45		68	0.66	24	6.87	64.3	43.5	0.70

Abs-Absorbance, T% - Percentage of transmittance, TDS – Total Dissolved Solids, VWD – Volume of Water Displacement

Table 4. Various factor data of *Tridox procumbens* extract at 80 °C and different power level

Minutes	Power level	Temp.	Abs	T%	pH	mV	TDS	VWD
0	0	30	0.07	84	6.39	34.4.7	21.2	0.57
5	5	78	0.38	42	6.61	55.4	40.8	0.63
10		78	0.46	38	6.55	57.8	41.7	0.65
15		78	0.54	30	6.6	58.5	44.0	0.66
20		78	0.56	28	6.53	61.5	47.3	0.66
25		78	0.58	26	6.62	64.5	48.5	0.67
30		78	0.61	25	6.65	67.1	50.2	0.68
35		78	0.61	25	6.62	68.4	52.9	0.68
40		78	0.60	26	6.85	70.7	55.3	0.69
45		78	0.58	29	6.96	67.8	54.9	0.67
5	6	78	0.41	40	6.89	56.6	43.7	0.66
10		78	0.49	34	6.87	59.5	44.8	0.67
15		78	0.56	28	6.72	62.1	45.9	0.66
20		78	0.57	27	6.93	65.2	48.2	0.68

25		78	0.58	26	6.92	66.7	50.9	0.69
30		78	0.66	21	6.95	68.1	54.5	0.70
35		78	0.65	22	6.95	70.4	56.1	0.68
40		78	0.63	25	6.88	70.9	55.3	0.67
45		78	0.61	27	6.96	69.8	53.4	0.68
5	7	76	0.48	30	7.05	62.6	44.9	0.66
10		78	0.57	26	7.06	65.7	46.3	0.68
15		78	0.61	24	6.95	66.7	49.8	0.69
20		78	0.65	22	6.98	68.6	53.1	0.68
25		78	0.69	22	6.9	69.9	56.9	0.69
30		78	0.69	20	6.97	71.4	57.4	0.70
35		78	0.70	19	7.05	70.7	57.6	0.69
40		78	0.61	26	7	70.3	56.7	0.69
45		78	0.58	27	6.49	69.6	55.2	0.68

Abs-Absorbance, T% - Percentage of transmittance, TDS – Total Dissolved Solids, VWD – Volume of Water Displacement

Table 5. Correlation and significant data of yield and microwave power at 70 °C for *Tridox procumbens* aqueous extract

Correlation	Time (min) vs. 5 MWP	Time (min) vs. 6 MWP	Time (min) vs. 7 MWP	Time (min) vs. 8 MWP
R-value	0.9117	0.9188	0.9226	0.8836
R-square	0.8311	0.8442	0.8547	0.7808
p-(two-tailed)	0.0311	0.0274	0.0359	0.0468
Significant	Yes	Yes	Yes	Yes

Table 6. Correlation and significant data of yield and microwave power at 80 °C for *Tridox procumbens* aqueous extract

Correlation	Time (min) vs. 5 MWP	Time (min) vs. 6 MWP	Time (min) vs. 7 MWP	Time (min) vs. 8 MWP
R-value	0.8840	0.8823	0.8817	.8894
R-square	0.7814	0.7784	0.7773	0.7910
p-(two-tailed)	0.0466	0.0476	0.0480	0.0434
Significant	Yes	Yes	Yes	Yes

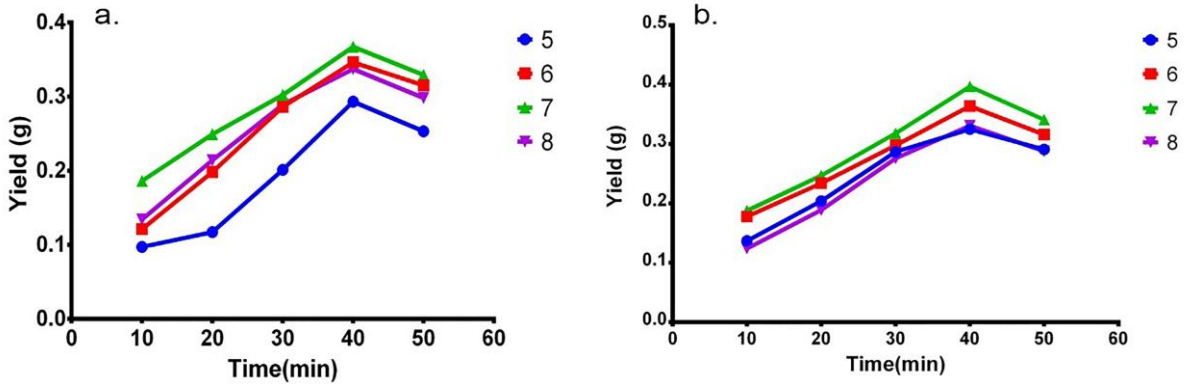

Figure 1. a) Correlation between the *Tridox procumbens* aqueous extract yield and microwave irradiation power at 70 °C at different time intervals (p<0.05) b) Correlation between the *Tridox procumbens* aqueous extract yield and microwave irradiation power at 80 °C (p<0.05). 5, 6, 7 and 8 indicates the different microwave irradiation power level.

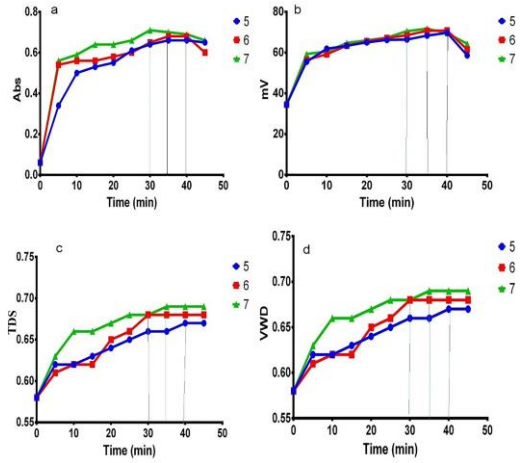

Figure 2. a) Correlation of absorbance of *Tridox procumbens* aqueous extract at different time and MWP. b) Correlation of mV of extract at different time and MWP. c) Correlation of TDS of *Tridox procumbens* aqueous extract at different time and MWP. d) Correlation of VWD of *Tridox procumbens* aqueous extract at different time and MWP. 5, 6, 7 and 8 indicates the different microwave irradiation power level. (P<0.05) (Temperature 70 °C).

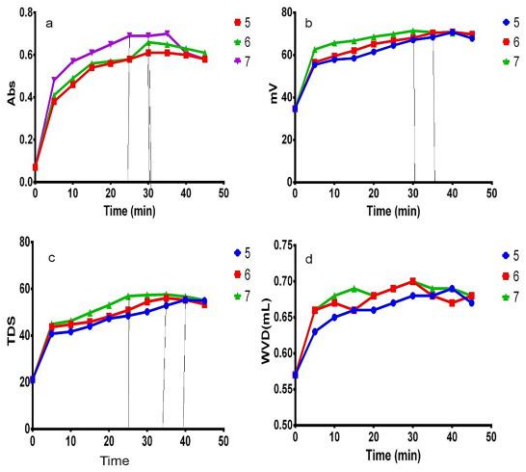

Figure 3. a) Correlation of absorbance of extract at different time and MWP. b) Correlation of mV of *Tridox procumbens* extract at different time and MWP. c) Correlation of TDS of *Tridox procumbens* extract at different time and MWP. d) Correlation of VWD of extract at different time and MWP. 5, 6, 7 and 8 indicates the different microwave irradiation power level. (P<0.05) (Temperature 80 °C).

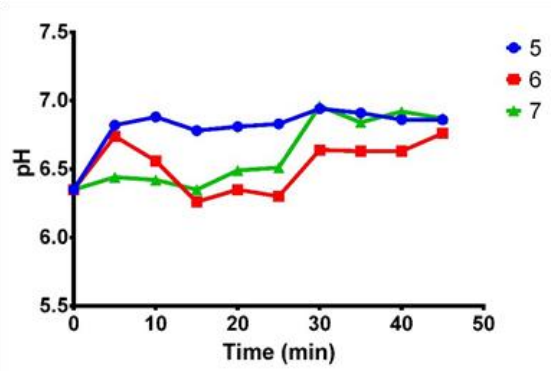

Figure 4. Correlation of *Tridax procumbens extract* pH with 5, 6 and 7 microwave power level (p>0.05)

RESULT AND DISCUSSION

Microwave power and temperature optimization

The microwave irradiation power and temperature of *T. Procumbense* leaves extract significantly influence the yield of extract. The correlation between yield and microwave power clearly shows that the more yield obtained at 30 - 40 minutes when the 7 (595 watts,70%) microwave power irradiation and 80 °C temperature compare to another parameters range. Increasing irradiation of the extract up to 50 minutes possibly degrade phytoconstituent of the leaves reflected in quantity of yield variation (Table 2).

Factor analysis

Factor analysis results showed that the extraction end time could be straightforwardly identified for the *Tridax Procumbense* phytoconstituent by analyzing the factors such as absorbance, oxidative reduction potential (mV), total dispersive solids (TDS), and water volume displacement (WVD) level. The pH factor of extract was not showing any favored influence in exaction due to the ionic nature of extractive phytoconstituent. The results of this study revealed that extraction of *Tridax procumbens* leaves completed between 30-40th minute interval. Additional time irradiation caused the denaturation of phytoconstituent which modified the value of the factor. The Tables 3 and 4 showed the complete value ranges of analyzed factors at temperature 70 °C and 80 °C, microwave irradiation at 5, 6, 7 and 8 power level of different time intervals.

Correlation statistics and significant level analysis

The correlation coefficient (r) is a degree of linear association between two variables. The value of r is such that -1 < r < +1. The positive and negative signs are used for positive linear correlations and negative linear correlations, respectively. If x and y have a robust positive linear correlation, r is close to +1. Positive values indicate a relationship between x and y variables such that as values for x variable is directly proportional to the y variable. If x and y have a strong negative linear correlation, r is close to -1. Negative r values indicate a relationship between x and y such that as values for x variable is indirectly proportional to the y variable. If there is no linear correlation or a weak linear correlation, r is close to 0. A correlation value >0.8 is described as strong, whereas a correlation <0.5 is described as weak.

Microwave irradiation power and yield correlation

In this present work, the correlation of MWP (5, 6, 7 and 8) and yield (g) at 10-50 minutes time interval with constant 70 °C temperature showed the r^2 value ranges between 0.7808 – 0.8547 (Table 5). It indicated that yield was more significantly related to the microwave irradiation power. Especially at 70°C with 7MWP found to be more suitable for extraction compare to other microwave range (Figure 1a). Similarly, Table 6 showed the correlation between yield and microwave irradiation at 80 °C (Figure 1b). This result concluded that 7 MWP and 80 °C parameters produced the more extract of *Tridax procumbens* at the 30 minutes period.

Microwave irradiation power and factors correlation

Figure 2 showed that the correlation relationship between the various factor at temperature70°C and 5, 6 and 7 MWP. Photometric green filter light absorbance of the extract possesses gradual increment in the values up to the concentration of reaches saturation level in the solvent. A 30th-minute sample of extract viewing maximum absorbance at 7 (595 watts,70%) power level. Further irradiation of leaves reduced the absorbance value, and correlation of absorbance showed positive value indicated the absorbance was one of the significant factors for the extraction end time analysis (Figure 2a). Percentage of transmittance was directly proportional to the absorbance value and negative correlation value specified that increase of the phytoconstituent concentration in solvent reduce light transmittance through the sample. mV is the measurement of oxidative reduction potential in volts of the extract (Figure 2b). Higher number and concentration of phytoconstituent in extract increase the tendency of acquiring or losing the electrons. Seven (595 watts,70%) power irradiated sample showed higher mV potential at 30th minute compare to 5 and 6 MWP. TDS is used to measure the content of all inorganic and organic substances in a liquid as molecular, ionized or micro-granular (colloidal sol) suspended form.

TDS meter using the conductivity principle to calculate the dissolved solids values. The concentration of ionized solids in aqueous solution create the ability for the water to conduct an electric current which can be measured by the TDS meter. The first 0th-minute extract showed TDS of 28.8 mg/L; it reached up to 71.7 mg/L at 30 minutes while passing the 7-microwave power (Figure 2c). Digital plethysmometer was used to measure the volume of water displacement. The water volume displacement produced by immersion of the 1 mL extract containing tube, in measuring tube is displayed on the second tube, inducing a change in the conductance between the two platinum electrodes in a digital meter. The total factor analysis concluded that the extract of *Tridax procumbens* end at the 30-40 minutes irradiation (Figure 2d).

Consequently, the correlation between the different factors at 80 °C and 7 MWP resulted that the extraction of *Tridax procumbens* end at 25-30 minutes interval compare to the other MWP (Figure 3a-3d). Finally, the correlation of factors indicated that the MWP, temperature, absorbance, mV, and TDS significantly influence the exaction time and yield. The factors pH and volume of water displacement was not adaptive to identify the extraction time due to the fluctuation of the values (Figure 4).

CONCLUSION

This complete work concluded that, advancement of the natural product research was current need to compete with other synthetic research. In this research work, an innovative step taken in the primary level of phytoconstituent extraction. The end time of the total phytoconstituent extraction of *Tridax procumbens* was identified based on the various factor analysis. The results of this studies found that all the *Tridax procumbens* phytoconstituent extraction end at 30-40 minutes when 70% microwave irradiation and 80 °C temperature was applied. The factor such as absorbance, mV, and TDS was favour the identification of extraction end time analysis. This analysis completely solves the time, solvent and cost depended problems in the field of phytoconstituent extraction studies. Implementation of this method of analysis with other medicinal plants will make the new footpath to future generation to do best extraction.

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