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OPTIMIZATION AND BIOLOGICAL EVALUATION OF *CROTALARIA MEDICAGINEA* CRUDE EXTRACT VIA MICROWAVE-ASSISTED EXTRACTION

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Abstract

This study aims to establish an herbal source based on microwave-assisted extraction (MAE) for the treatment of acute and chronic inflammatory diseases by using *in-vitro* and *in-vivo* studies. *In-vivo* studies were conducted on albino rats. MAE was optimized for the rapid recovery of phytochemicals compounds from *Crotalaria medicaginea*. Central Composite Design (CCD) was used with three factors (power 200–900 W, time 1–5 min, and temperature 40–70 ^{0}C) using response surface methodology. Total phenolic content (TPC), and total flavonoid content (TFC) were response factors. The maximum level of responses was 66.56 mg-eq gallic acid and 105.94 mg-eq quercetin in 3 minutes extraction at 300 W of microwaves. *In-vitro* and *in-vivo* antioxidant and anti-inflammatory activity was then checked by different methods for optimized extracts. The maximum antioxidant activity in DPPH, FRAP, HRSA and ABTS assay was observed as 36.74%, 94.49%, 70.96%, and 59.76% respectively comparable to standard antioxidants. Under *in-viro* anti-inflammatory studies, the maximum activity was observed at the highest concentration (1 mg/mL), which is 46.32%, and 96.55%. In carrageenan induced rat paw edema, xylene induced ear edema and cotton pellet induced granuloma in rats, the highest inhibition in plant extract was observed as 47.31%, 73.66% and 51.08% which was very close to the standard drug used (77.33%, 58.23%. 56.03%). It is the first time report of MAE of *C. medicaginea* as antioxidant and anti-inflammatory activity and the plant possess strong antioxidant and anti-inflammatory activity as compared to standard drugs used.

Key words: Antioxidants, Anti-inflammatory, Aspirin, *Crotalaria medicaginea,* microwave-assisted extraction, Phenolics

INTRODUCTION

Inflammation is a common body response to several problems in a visible form of redness or swelling, accompanied by pain. Simply, it is a symptom that is related to various ailments. To decrease the painful effects of such diseases, patients are treated with various anti-inflammatory drugs (AID). Various synthetic AIDs available in the market are of various types with mild to severe side effects. Individuals also have different responses to the AID according to their age, health, gender, and susceptibility to the medicine (Theken, 2008; Furst and Zundorf, 2014).

Plant-based AIDs should be explored to fight the increasing cases of new types of disease reactions and inflammations in the bodies. Therefore, ethnobotanical knowledge of plants can cause a great turnover in this field. One such plant is *Crotalaria medicaginea* (locally known as sap booti or snake plant) as it is used to relieve the pain and inflammation of snake bites (Rouamba *et al.,* 2018). This plant is an important member of family Fabaceae and is commonly dispersed in tropic and

sub-tropic areas of the world (Daimon, 2006). The medicinal value of this plant is unexplored, that is a major point of interest for future research (Ali *et al.,* 2023).

Overall, this genus is reported to be antioxidant, anti-cancerous, antifungal, antibacterial, and antispasmodic activities, but this plant needs to be explored for its anti-inflammatory potential. When all these medicinal points of importance are discussed, the first step of the process is usually ignored, i.e., the extraction of plant metabolites of interest from plants (Akhtar *et al.,* 2019). This step determines what the quality will be (in terms of biological activity) and the quantity of the final product. All these factors are also involved in determining the cost of that medicine directly or indirectly (Azmin *et al.,* 2016).

The history of extraction of plant based biochemical also goes back to the prehistoric times when plant-based decoctions and teas were used for various health issues (Paulsen, 2010). But the present era of the energy crisis, and environmental issues urges us to introduce protocols of extraction that are time and cost saving as well as friendly to the environment. Microwave assisted extraction (MAE) is one such energy efficient and environmentally friendly method that is based on the energy of microwaves in a certain range. Microwaves are used to heat a plant-solvent system that leads to higher extraction of the plant metabolite with undisturbed or even enhanced biological activity (Akhtar *et al.,* 2019; Akhtar *et al.,* 2020). Therefore, the present study was designed to establish an MAE protocol for rapid and efficient extraction of anti-inflammatory agents from the unexplored plant *C. medicaginea* followed by their antioxidant and anti-inflammatory analysis.

MATERIALS AND METHODS

Collection of plant material

Leaves and branches of *C. medicaginea* were carefully collected from different parts of the district Haripur, Khyber Pakhtunkhwa, Pakistan, situated between 72-73˚ E longitude and 33-34˚ N Latitude, during July and August of the year 2021. Haripur district is located at 520-610 m above sea level with 37 ℃ temperature on average in summer and 24 ℃ temperature in winter (Siddique *et al.,* 2021). After collection, plant material was washed carefully, shade dried, and ground into a fine powder followed by separation into a size of 80 µm by the respective sieve (Akhtar *et al.,* 2020) to be used for microwave assisted extraction.

Microwave Assisted Extraction (MAE)

An optimized protocol for the MAE of *C. medicaginea* is not available in the literature. Therefore, Central Composite Design (CCD) of Response Surface Methodology (RSM) was used to get a protocol for this purpose. Methanol was used as a solvent in this experiment as a polar solvent. Using Design Expert software, the design for the study was established using three factors i.e., the power level of microwaves (200-900 W), temperature (40-70 ℃), and time of extraction (1-6 minutes) as mentioned in Table 1. Model fitness was tested at a 5% level of significance. 3D response graphs were also built to understand the interaction of the factors of microwaves under study.

Table 1: Parameters analyzed for optimal yield of phenolics, flavonoids and antioxidants

	Parameters	Varied conditions
A	Power level	200, 400, 600, 800 and 900 W
B	Time of Extraction	1, 3, 5 min
C	Temperature	40, 50, 60 and 70℃

Whereas for the performance of this design as experimental work, a microwave extractor (MDS-6G) was used that carried Teflon vials with temperature and pressure sensors. Each time, for each run, 0.5 g of dried powder plant material was taken with 20 mL of the solvent, and conditions of the microwave were run according to the parameters of run. To study the effect of efficiency of MAE parameters on the extraction of *C. medicaginea,* two main parameters were selected i.e., total phenolic and total flavonoid content. Thereafter, the extracts or run number with maximum phenolics and flavonoids were selected for bioassays including antioxidant and anti-inflammatory activity.

Total phenol content

A slightly modified method of spectrophotometric assay of (Billah *et al.,* 2020) was used to estimate the phenolic content of the extract. The reaction mixture was made by mixing plant extract (80 µL), distilled water (630 µL), and Folin-Ciocalteu reagent (400 µL), mixed vigorously and placed for 10 minutes. After this incubation at room temperature, a 25% solution of sodium carbonate (1200 µL) was added to this reaction mixture and incubated in the dark at room temperature, and then the final-colored mixture was analyzed on the spectrophotometer to check its absorbance at 760 nm using gallic acid curve as standard phenolic.

Total flavonoid content

A colorimetric assay was used for flavonoid content as described by (Banothu *et al.,* 2017). The reaction mixture was made by mixing plant extract (250 µL), distilled water (400 µL), and sodium nitrite (90 µL), mixed vigorously, and placed for 10 minutes. After this incubation at room temperature, a 10% solution of aluminum chloride (200 µL) was added to this reaction mixture, followed by the addition of 1M sodium hydroxide (600 µL), and incubated in the dark at room temperature, and then the final-colored mixture was analyzed on the spectrophotometer to check its absorbance at 510 nm using quercetin curve as standard phenolic.

Gas Chromatography-Mass Spectrometry analysis

GC-MS analysis of crude methanol extract (1 mg/mL) of plant extract were carried out using GC 7890B, 5977A (Agilent USA) equipped with DB 5MS column (0.25 mm diameter x 0.25 μm thickness, 30 m length) with single quadrupole detection system. Helium (99.9%) was used as carrier gas, and flow rate was fixed at 1.0 mL/min. Injection temperature and oven temperature were maintained at 280 °C.

Oven temperature was maintained at 50 °C for 1 min then increased at 15 °C/min to 320 °C for 5 min. Injection volume was set at 1 μL and total run time was 24 min. Chemical constituents present in the extract were expressed as percentage based on peak area. Compounds were identified by comparing the data with existing spectra in National Institute of Standard and Technology (NIST) library database.

***In-vitro* antioxidant activity**

The antioxidant activity of selected extracts with higher TPC and TFC was carried out to check their stable biological activity. For this purpose, four main methods were used, namely the DPPH assay, hydroxyl radical scavenging assay (HRSA), ferric reducing antioxidant power assay (FRAP), and 2, 2 azinobis 3-ethylbenzothiazoline-6-sulfonic acid radical scavenging assay (ABTS). The main formula used in all four antioxidant assays was for the percent inhibition of the oxidation of the respective radicle (of each method) by the phytochemical of microwave assisted extract of *C. medicaginea*

$$\% \text{ inhibition} = \frac{Ac - As}{Ac} \times 100$$

Where A_c is represents the absorbance of a respective molecule of each method and A_s represents the absorbance of the sample + respective reagent.

DPPH assay

A slightly modified method by (Nurhaslina *et al.,* 2019) was used for this purpose. The reaction mixture was made by mixing plant extract (0.5 mL), and 0.05 mM DPPH solution (2.5 mL) and placing the mixture at 37 ℃ for almost half an hour. This caused a change in color of the DPPH solution from purple to yellow that was quantitatively checked on a spectrophotometer by observing the absorbance of each reaction mixture at 517 nm and ascorbic acid was used as a reference antioxidant.

HRSA assay

Method described by (Ashraf *et al.,* 2015) was used for this assay with slight modifications. For this purpose, reaction mixture was made by mixing plant extract (1 mL), 0.02% EDTA (0.5 mL), iron EDTA (1 mL), DMSO (1 mL). It was mixed thoroughly and tubes were made air tight before heating on water bath at 80-90 ℃ for 10-15 minutes. This was repeated with various concentration of plant extract. After heating, two reagents were added to the reaction mixture, at first ice-cold trichloroacetic acid (1 mL) was added, followed by the addition of Nash reagent. After almost 10-15 minutes, an intense yellow color was obtained and checked for absorbance at 412 nm. Ascorbic acid was checked as reference antioxidants.

FRAP assay

In this method, reaction mixture was made by mixing plant extract (1 mL), 0.2 M phosphate buffer with pH 6.6 (2 mL), and 1% potassium ferricyanide (2 mL), mixed and placed untouched at 50 ℃ for 20-30 minutes. Then 10% trichloroacetic acid was added and shaken. 2.5 mL was taken from this mixture, and mixed with 0.1% of ferric chloride (500 µL) and its absorbance was checked at 700 nm. Ascorbic acid was used as reference antioxidant (Tijani *et al.,* 2018).

ABTS assay

The ABTS radical scavenging assay was calculated based on the method of (Chan *et al.,* 2007) with some modifications. In this assay, fresh ABTS reagent was used every time by mixing 7 mM ABTS and 2.45 mM potassium persulfate (1:1) and placed in dark for 12-16 hrs. Quality of this reagent was strictly controlled by checking its absorbance at 745 nm and adjusting the absorption value at 0.700 by diluting the reagent. For analysis, plant extract (100 µL) and ABTS (2 mL) were mixed and incubated for 10 minutes, followed by observing the absorbance of the reaction mixture at 745 nm and Trolox was used as a standard.

***In-vitro* anti-inflammatory activity**

Three main assays were used to check the anti-inflammatory activity of microwave assisted extract of the plant namely inhibition of heat induced protein denaturation (IHIPD) and HRBC membrane stabilization assay. Blood sample for this purpose was taken from a healthy volunteer.

Inhibition of heat induced protein denaturation

This reaction is based on the activity of test sample where it inhibits the denaturation of present proteins and protects them, and method used here in this study is a slightly modified method of (Gorinstein, 2009). Aspirin was used as a standard in this experiment. For this purpose, plant extract (20 µL), egg albumin (20 µL), and phosphate buffer with pH 6.4 (3.28 µL) were mixed. For comparison, tests were repeated by replacing plant material with aspirin and incubated the mixtures at 37 ℃ followed by heating at 70 ℃. Then absorbance was checked at 660 nm and following formula was applied where A_c and A_s are the absorbance values of control and sample respectively.

$$\% \text{ inhibition} = \frac{Ac - As}{Ac} \times 100$$

HRBC Membrane Stabilization Assay

For this assay, plant extract was made in to dilutions of strength 31.25, 62.5, 125, 250, 500, and 1000 µg/mL. 10 mM phosphate buffer was mixed with NaCl (154 mM) to make an isotonic phosphate buffer (pH 7.4). Blood sample was centrifuged at 3000 rpm for 10 minutes followed by the washing of the pellet with 0.9% saline solution. Supernatant was discarded as it contained plasma and WBCs. Then, this pellet was mixed with the isotonic buffer. Then reaction mixture was made by mixing 0.5 mL of this pellet suspension in buffer, one mL of phosphate buffer, 2 mL of hyposaline solution, and half mL of microwave assisted plant extract. Each time a separate extract dilution was used. Distilled water was a control whereas in reference or standard solution acetyl salicylic acid replaced the plant extract. All the treated samples were incubated for 30 minutes at 37 ℃ followed by its analysis for absorption at 560 nm (Carter and Thornburg, 2000). Following formula was applied finally where A_c and A_s are the absorbance values of control and sample respectively

$$\% \text{ inhibition} = \frac{Ac - As}{Ac} \times 100$$

***In-vivo* Studies**

Experimental animal and ethical considerations

Male albino rats (180-200 g) were caged in the animal house of University of Health Sciences, Lahore, Pakistan under optimum conditions. The animals were adjusted to standard laboratory conditions (temperature 25 ± 2 °C and dark light cycle 14-10 h). They were allowed free access to standard dry pellet diet and water ad libitum. The animal care and experimental protocols were abided by the criteria outlined in the guide for the care and use of laboratory animals. Ethical authorization for this study was issued by the Institutional Ethical Committee of the Lahore College for Women University, Lahore (Protocol approval number DEC/LCWU/2023-03).

Grouping for *in-vivo* studies

Animals were divided into five groups and each group contained 6 rats (n =6). Group I served as positive control group and was treated with 25 mg/kg body weight diclofenac sodium. Group II was vehicle treated control and received only DMSO (1%). Group III was disease control group treated with

inflammatory substances (carrageenan, xylene, and cotton pellets). Group- IV was the test group and received 400 mg/kg body weight plant extract.

Group-V was the healthy group which did not receive any treatment throughout the study. At the end of the experiment, for getting blood, deep anesthesia was given to animals (60 mg ketamine/ 4 mg xylazine per kg). The blood was obtained by cardiac puncture and collected in heparin tubes. For obtaining serum, blood samples were centrifuged for 15 min, and rpm was set at 3000 rpm. Serum storage was done at −20°C and was used for studying antioxidant potential. Absorbance of all assays was measured by using Synergy HTX multimode reader (BioTek) and the % enzymatic activity was calculated by using following formula:

$$\% \text{ enzymatic activity} = \frac{Ac - As}{Ac} \times 100$$

Where A_c is represents the absorbance of a respective molecule of each method and A_s represents the absorbance of the sample.

***In-vivo* antioxidant activity**

CAT assay

The CAT activity in serum samples was determined using the method of (Hadwan & Abed, 2016). The reaction mixture contained serum (10 μL) and 50 mM potassium phosphate buffer (2.80 mL). Addition of 0.1 mL of freshly prepared hydrogen peroxide (30 mM) initiated the reaction. Hydrogen peroxide decomposition rate was measured at 240 nm for 5 min on Synergy HTX multimode reader (BioTek).

SOD assay

SOD activity in serum samples was estimated following the method of (Zhang *et al.,* 2016). A 3 mL reaction mixture contained 50 mM Tris-HCl buffer (pH 8.2), 1 mM EDTA, 100 µL serum, 0.4 mM pyrogallol and appropriate amount of distilled water. Blank contained all the reagents except pyrogallol. The activity was performed in triplicates in 96-well microtiter plate. The plate was kept in Synergy HTX multimode reader (BioTek) and absorbance was recorded at 420 nm.

***In-vivo* anti-inflammatory activity**

Carrageenan induced rat paw edema

For carrageenan induced rat paw edema assay, method of (Adeyemi *et al.,* 2002) was adopted. Induction of inflammation was done by subplantar injection of 0.1 mL carrageenan (1% carrageenan suspended in 0.9% NaCl) in the right paw of rat. After injection the rats were administered with the test substances such as DMSO, diclofenac sodium and crude extract. Paw size was measured immediately before and at regular intervals after carrageenan injection (30 min, 1 h, 2 h, 3 h and 4 h) using a digital vernier calliper. Evaluation of anti-inflammatory activity was done in treated and control group by using the equation:

$$\% \text{ inhibition} = \frac{(Dt - DO)\ control - (Dt - DO)\ treatd}{(Dt - Do)\ control} \times 100$$

Where D_o and D_t is the average diameter for each group before and after treatment.

Xylene induced ear edema

In this study, all the doses were administered by oral intubation. Test and standard drugs with same doses were injected to the right ear of each animal 1 hour prior the bilateral application of xylene (inflammatory substance). After 15 min of xylene application animals were sacrificed by dislocating their spinal cord from the neck and a 16-diameter circular area was cut from each ear of the animal. The

weight of the inflamed (right) ear was then compared to that of control (left) ear (Li *et al.,* 2011).

$$\text{Edema degree} = M_{(right)} - M_{(left)}$$

$$\% \text{ inhibition} = \frac{Edema\ degree_{(control)} - Edema\ degree_{(treated)}}{Edema\ degree_{(control)}} \times 100$$

Cotton pellet induced granuloma in rats

This method provides a measure to evaluate the anti-inflammatory effect of test compounds (Gupta *et al.,* 2013). The animals were placed in an animal house for 7 days and served with a normal water ad libitum diet. On the 8th day, rats were lightly anesthetized with 60 mg/kg ketamine plus 4 mg/kg xylazine, and two sterilized cotton pellets (20 mg) were implanted (subcutaneously) in the dorsal region.

After implantation of cotton pellets, animals were given all test doses by oral intubation for eight days. On the 9th day, rats were sacrificed as described earlier and cotton pellets were dissected out without affecting adjacent granuloma tissues. Weight of wet and dried cotton pellets was recorded where pellets were dried at 60°C for 48 h. The weight of granuloma formation was calculated and percent inhibition of granuloma by test samples and standard drug was calculated as per following formula

$$\text{Granuloma inhibition (\%)} = \frac{1 - Granuloma\ in\ treated\ group}{Granuloma\ in\ Control} \times 100$$

Statistical Analysis

RSM was used to optimize the factors for MAE where RSM was applied by using software "Design Expert" version 11.0. SPSS version 20.0 was utilized as a statistical instrument. Means were compared by applying one way ANOVA for studying biological activities of extracts and a post hoc test (DMR test) at 5% level of significance was applied to compare means.

RESULTS

Microwave assisted extraction

This study had mainly two focus points where the first one is to establish a protocol for extracting maximum flavonoids and phenolics from the plant under study by using a strong statistical tool. A statistical tool or design can help us to effectively understand the various extraction parameters and their interaction influencing the selected dependent parameters. Whereas the second focus point was to evaluate the antioxidant and anti-inflammatory potential of plant extract by using optimized protocol.

Results from Table 2 showed that power level and time of extraction and their interaction were the significant factors over all to extract maximum amount of TFC and TPC from the plant material. It was further noticed that there is a mean value of microwave power (60 W) and time (3 min) up to which amount of response factors increases, then it starts to decrease. The maximum amount of phenolics and flavonoids (66.56 mg/g and 59.39 mg/g) was observed at 300 W after 3 min of microwave assisted extraction (Table 2).

To calculate the effects of the involved parameters on biological processes is a challenge because these parameters are directly and indirectly correlated with each other, thus affecting each other's efficiency. Response surface methodology (RSM) was, therefore, used to construct an empirical model for extraction of phenolics, and flavonoids to overcome this problem. By using RSM the

collaborating results of three substantial variables (A: power level, B: extraction time, C: temperature) on the response production (R_1: phenolics, and R_2: flavonoids) was determined statistically.

Table 2: Three-factor Complete Composite Design (CCD) main data with actual and predicted values for response surface methodology (RSM)

Run	Power level (W)	Time of extraction (min)	Temperature	TPC		TFC	
				Actual	Predicted	Actual	Predicted
1	600	3	60	13.48	10.35	2.40	-5.71
2	400	5	50	21.50	22.20	33.67	26.00
3	600	3	60	21.02	22.20	32.14	26.00
4	400	1	50	11.09	10.92	14.01	20.82
5	600	3	60	21.11	22.20	28.32	26.00
6	800	1	70	12.04	11.14	101.60	98.71
7	600	3	60	23.43	28.42	68.76	80.08
8	400	1	70	36.78	39.43	26.27	25.74
9	800	5	50	19.29	16.34	113.21	105.94
10	800	1	50	20.73	18.09	80.13	69.74
11	400	5	70	22.91	22.59	17.00	18.44
12	800	5	70	25.81	25.07	13.49	11.31
13	263.641=300	3	60	66.56	59.39	75.64	65.55
14	936.359=1000	3	60	33.00	36.08	50.09	53.20
15	600	3	43.1821	21.61	21.24	70.89	76.86
16	600	3	60	29.87	22.20	34.45	26.00
17	600	3	76.8179	16.39	22.20	16.79	26.00
18	600	6.36359	60	24.07	27.30	70.66	74.47
19	600	-0.363586	60	32.68	36.54	41.92	50.71
20	600	3	60	22.77	22.20	10.40	26.00

The Analysis of Variance (ANOVA) was applied to acquired responses to study them using a response surface quadratic model. As clear from Equation 1, it is believed that the production of phenolics (Y_{TPC}) and flavonoids (Y_{TFC}) depends on power level (A), extraction time (B) and temperature (C).

Equation 1:

$$TPC = +22.20 + 0.1686A + 6.79B + (-1.46)C + (-4.09)A2 + 9.11B2 + (-1.21)C2 + 4.78)AB + (-1.12)AC + 0.8975)BC$$

Equation 2:

$$TFC = +26.00 + 7.89A + 4.41B + 8.65C + (-6.52)A2 + 11.36B2 + 23.12C2 + (-26.79)AB + 5.46)AC + (-0.3801)BC$$

The "probability > F" value is less than 0.05 (F-value = 11.32) which indicates the significance of model terms and the extraction of phenolics will be well explained by this model (Table 3). The lack of fit is a measure of fitness of experimental design to characterize the data. The value of "lack-of-fit" for F (p = 1.51) suggests that this value is insignificant as compared to the pure error. Suitability of model to study the optimized condition for maximum extraction of phenolics from *C. medicaginea* was

explained by Equation 2. High correlation was observed between actual and predicted values as explained by the R-squared value (0.9106).

Analysis of Variance (ANOVA) was also done for the acquired results of flavonoids (Table 3) to apply a response surface quadratic model. The F-value (17.65) for a model with a probability value less than 0.05 suggested the significance of model terms and flavonoid extraction will be well explained by this model. This also ensures the suitability of Equation 3 to find out TFC at any combination of independent factors. The R-squared value (0.9408) also confirmed the relationship between experimental and predicted values.

Table 3: Analysis of Variance (ANOVA) for a quadratic model of phenolic and flavonoid content

		Phenolics				Flavonoids			
Source	**D_f**	**Sum of Squares**	**Mean Square**	**F-value**	**p-value**	**Sum of Squares**	**Mean Square**	**F-value**	**p-value**
Model	9	2457.93	273.10	11.32*	0.0004	18380.06	2042.23	17.65*	< 0.0001
A-Power level	1	0.3881	0.3881	0.0161	0.9016	849.42	849.42	7.34*	0.0219
B-Time	1	630.17	630.17	26.11*	0.0005	265.89	265.89	2.30	0.1605
C-Temperature	1	29.06	29.06	1.20	0.2982	1020.74	1020.74	8.82*	0.0140
AB	1	182.69	182.69	7.57*	0.0204	5741.04	5741.04	49.62*	< 0.0001
AC	1	9.99	9.99	0.4140	0.5344	238.57	238.57	2.06	0.1815
BC	1	6.44	6.44	0.2670	0.6166	1.16	1.16	0.0100	0.9224
A^2	1	241.13	241.13	9.99	0.0101	612.96	612.96	5.30*	0.0441
B^2	1	1195.64	1195.64	49.55*	< 0.0001	1859.68	1859.68	16.07*	0.0025
C^2	1	20.97	20.97	0.8691	0.3732	7704.85	7704.85	66.60*	< 0.0001
Residual	10	241.32	24.13			1156.95	115.70		
Lack of fit	5	145.35	29.07	1.51	0.3299	655.57	131.11	1.31	0.3879
Pure error	5	95.97	19.19			501.39	100.28		
Corrected Total		2699.25				19537.01			

***indicates significance at p≤ 0.05 whereas for phenolics R-squared = 0.9106, Adjusted R-squared = 0.8301, Predicted R-squared = 0.5398, Adequate Precision ratio = 14.1179 and for flavonoids Model F-value = 17.65, R-squared = 0.9408, Adjusted R-squared = 0.8875, Predicted R-squared = 0.7076, Adequate Precision ratio = 14.6796**

The R-squared value in percentage shows that 94.08 % variation in flavonoid content is due to independent variables whereas the rest is not described by the model. For both responses, i.e., TPC and TFC, the values of actual and adjusted R-squared have a difference of less than 0.2. It supports our regression model for determining the trends of

response factors. The adequate precision value was higher than 4 that confirms the adequacy of model.

The response surface curves (three-dimensional) expose the interactions amongst power level, extraction time and temperature. The 3D response surface curves presented in Fig 1 and 2 are based on the functioning of concentration of two factors with third factor kept at its optimum level. Elliptical or saddle nature of contour plots was used for determine the significance of interactions between the corresponding factors.

Gas-Chromatography Mass Spectrometry analysis

The major chemical constituents of crude extract of MAE of *C. medicaginea* are given in (Fig 3). GC–MS analysis of crude extract identified fourty four (44) compounds. The major constituents were identified as pyrrolizidine alkaloid, ethyl benzene, p-xylene, benzene 1, 3 dimethyl, 9, 15-Octadecadienoic acid, methyl, 9, 11-Octadecadienoic acid, methyl, 9, 12-Octadecadienoic acid, methyl and Bis (2-ethylhexyl) phthalate. Results clearly showed that MAE is effective towards extraction of polar and nonpolar components because non-polar components are usually volatile components and are evaporated easily or degraded under the effect of heat. MAE is a solution to both problems with closed container action and controlled temperature option without boiling of the solvent (Akhtar *et al.,* 2020). Degradation of main components in MAE is avoided due to lesser time of exposure of components to the heat.

***In-vitro* antioxidant activity**

2,2 diphenyl-1-picryl hydrazyl radical scavenging activity (DPPH)

DPPH is a free radical and it forms a diamagnetic molecule by reducing or accepting an electron and hydrogen ion. Plant extracts and other antioxidant molecules can cause a decrease in absorbance values of DPPH by causing radical reduction. Results of the DPPH assay of *C. medicaginea* are shown in Figure 4. The maximum antioxidant activity is observed at highest concentration (36.74 ± 0.13%) having IC_{50} value of (346 ± 1.41 µg/mL). The results are significantly different from standard ascorbic acid.

Hydroxyl radical scavenging activity (HRSA)

C. medicaginea MeOH extract shows this activity in a dose dependent manner. Among all concentrations highest scavenging activity is observed at 1 mg/mL (94.49 ± 0.13%). The scavenging activity of ascorbic acid is also dose dependent. The maximum activity in ascorbic acid is observed at 1 mg/ mL (75.28 ± 0.53%). Results are demonstrating that scavenging activity of plant extract is higher than standard. The results are shown in Figure 4.

Ferric reducing antioxidant power assay (FRAP)

The reducing capacity of plant extract increases with increase in concentration. The maximum reducing capacity is observed at 1 mg/mL (70.96 ± 0.06%) and minimum activity at 0.03125 mg /mL (25.72 ± 0.06%). Previous studies also supported this relation (Ali et al., 2023). Results are explained in Figure 5.

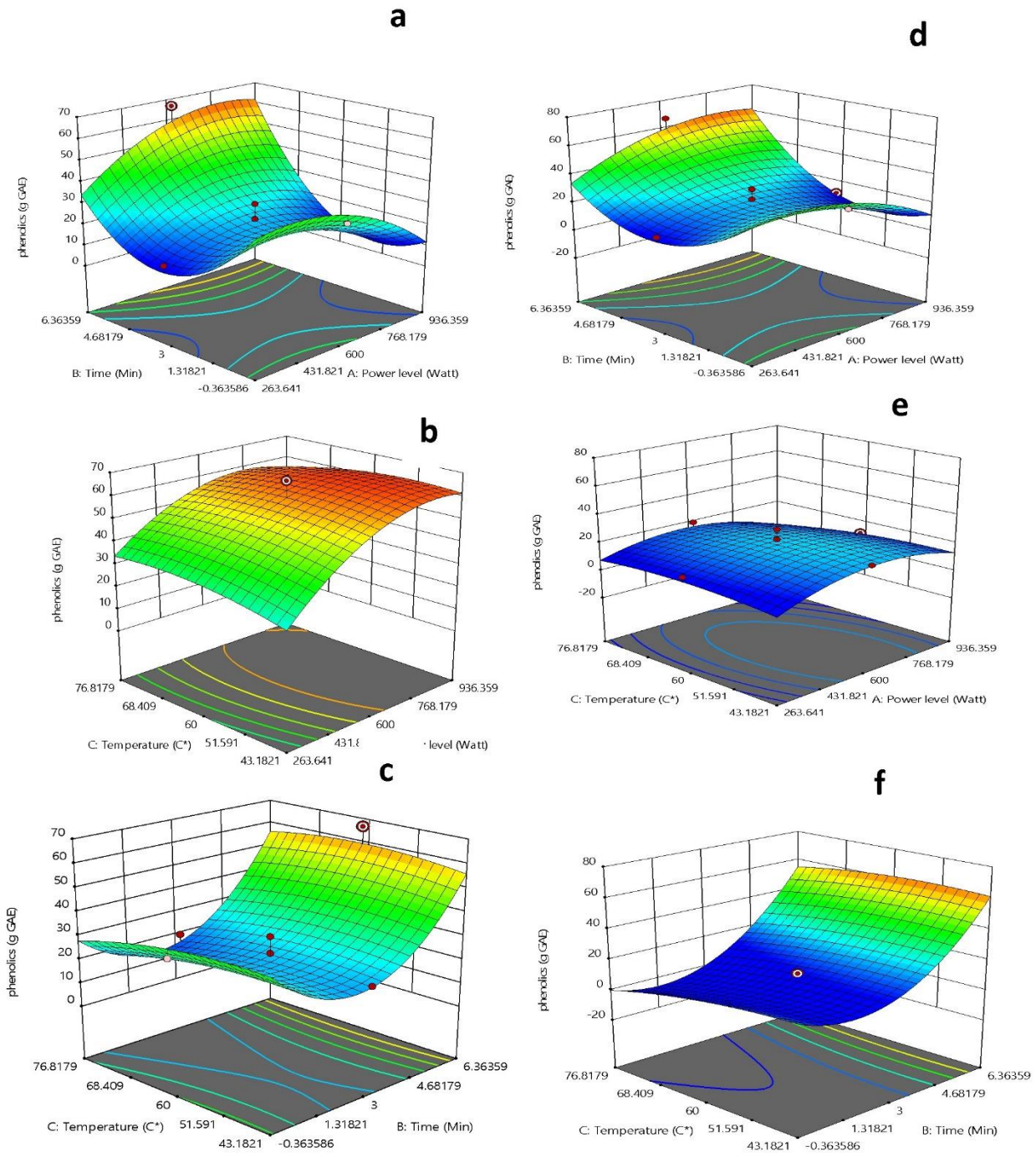

Fig. 1. Response surface plots showing the effects of variables on TPC response (R1)

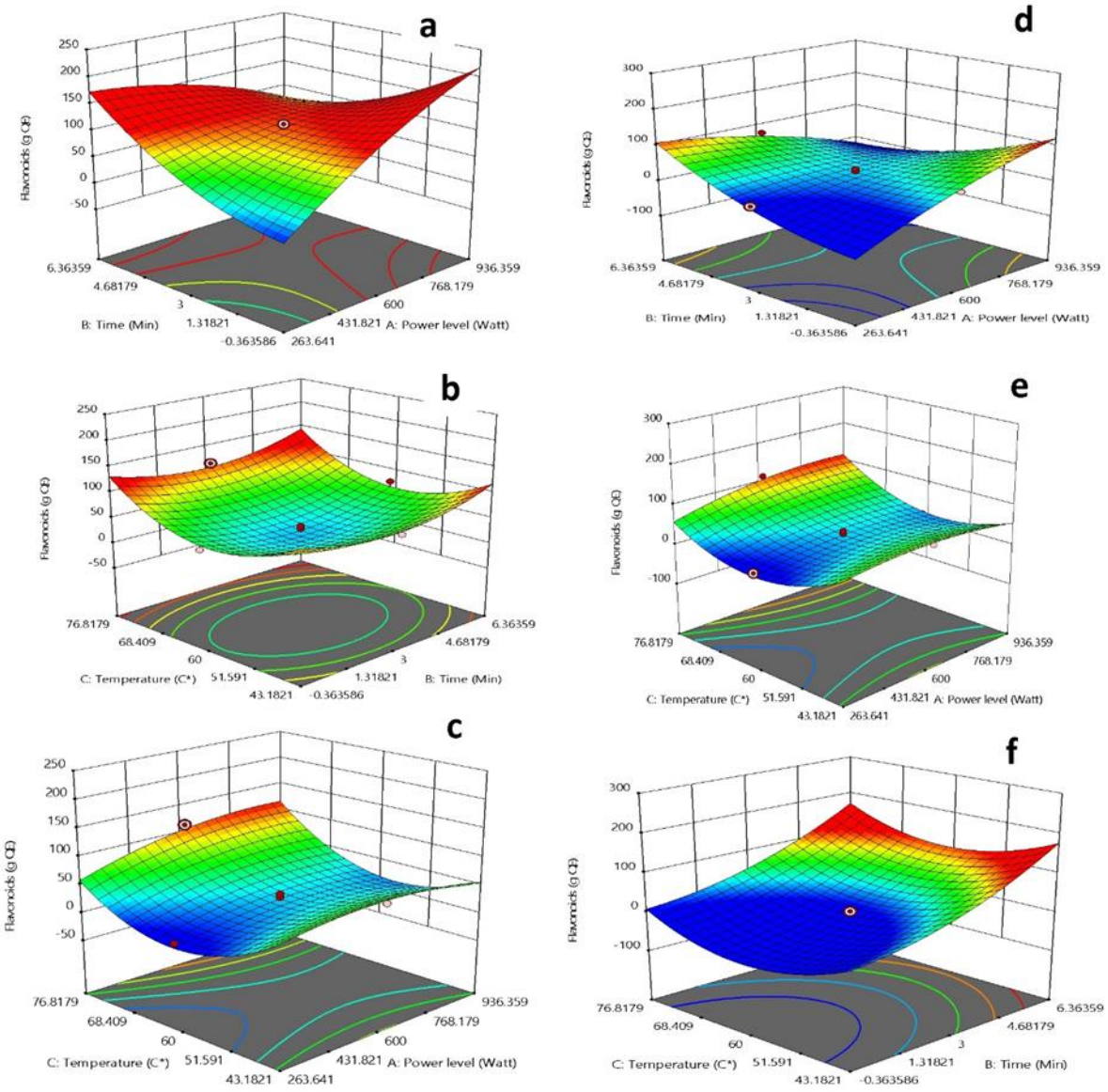

Fig.2. Response surface plots showing the effects of variables on TFC response (R2)

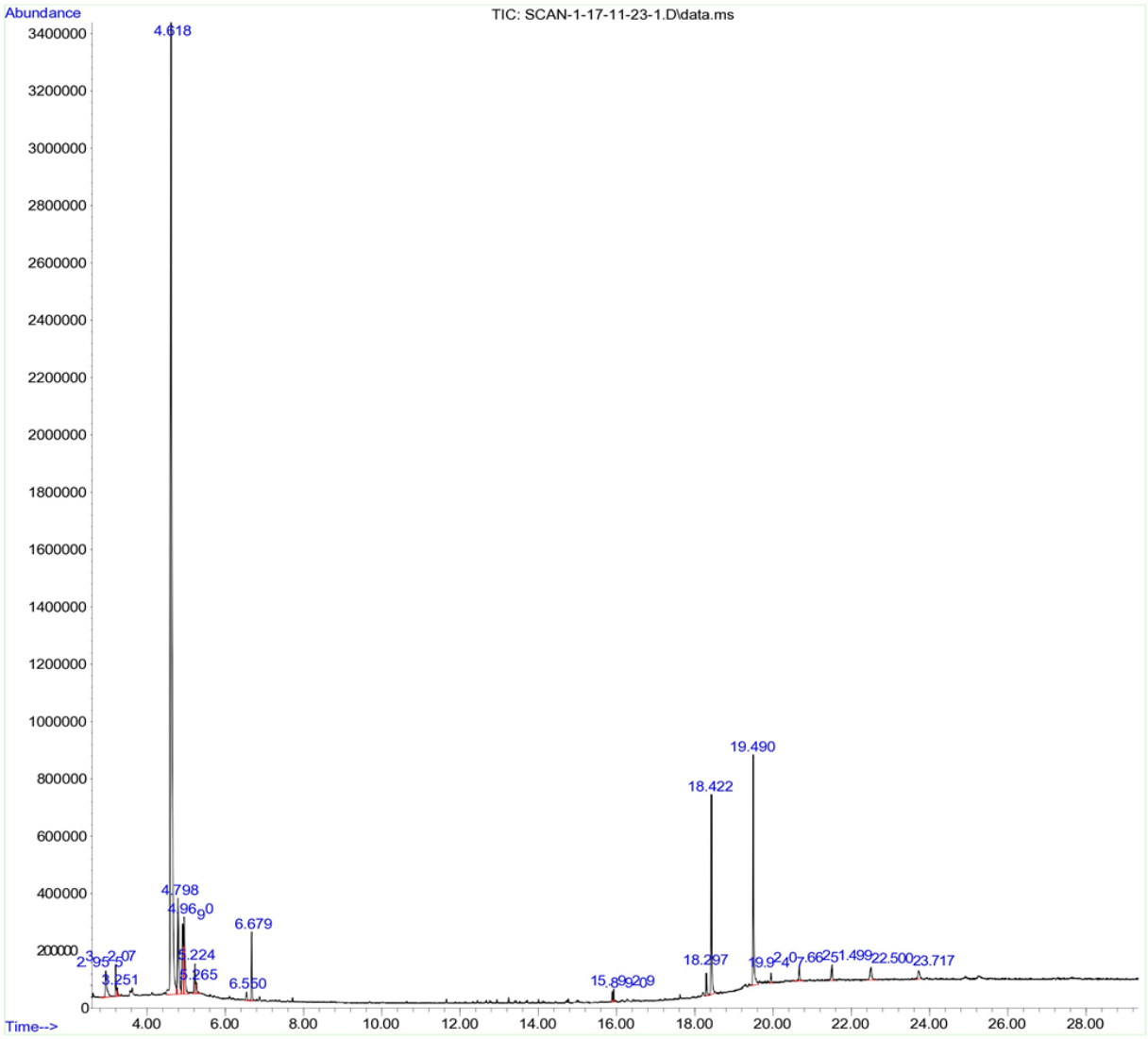

Fig. 3: GC-MS analysis of microwave assisted extract of *C. medicaginea*

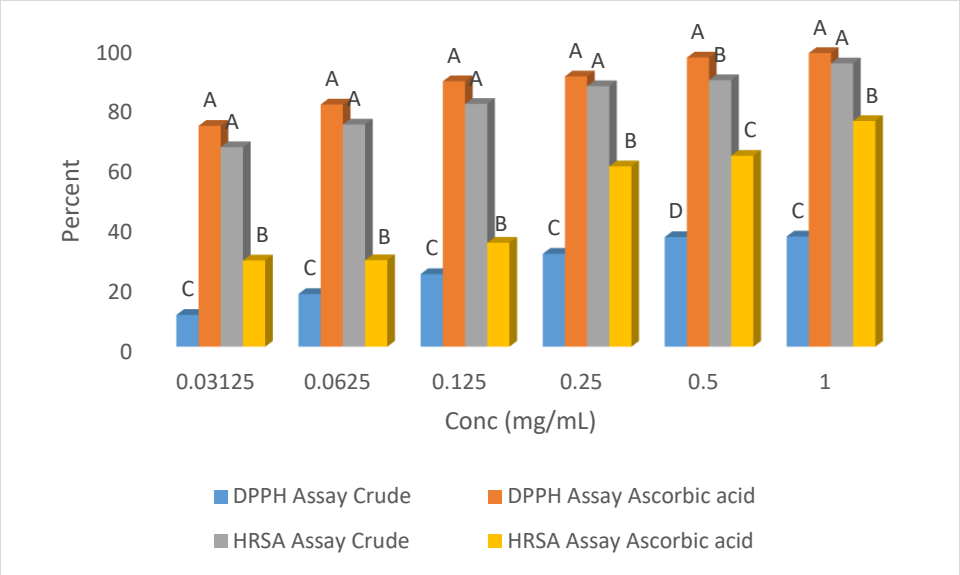

Figure 4: DPPH and hydroxyl radical scavenging activity of crude extract of C. *medicaginea*

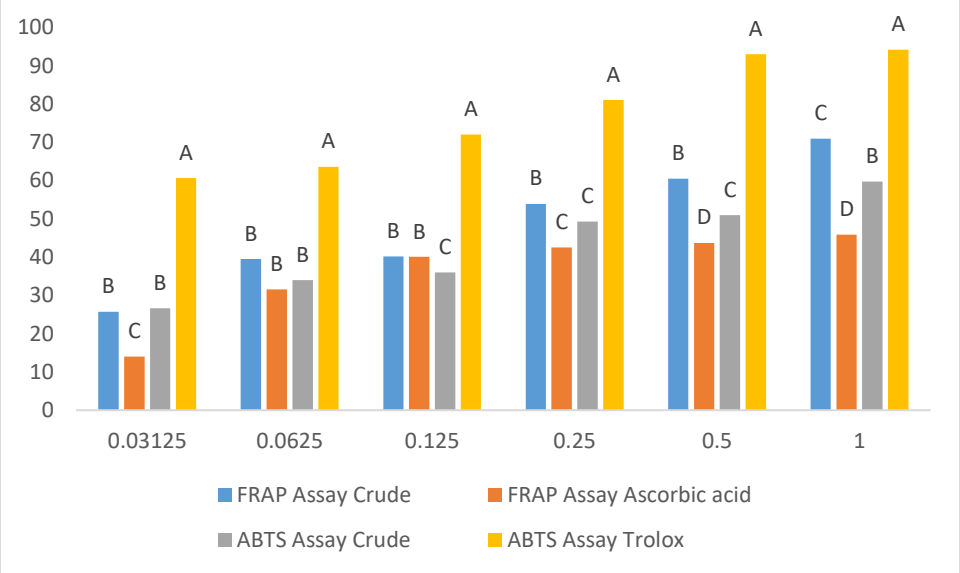

Figure 5: FRAP and ABTS activity of crude extract of C. *medicaginea*

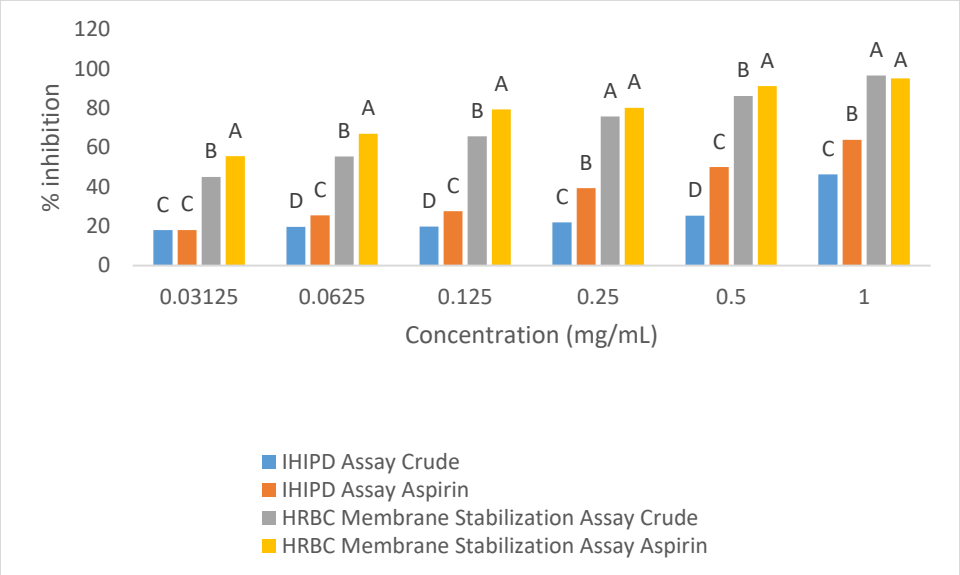

Figure 6: Inhibition of heat induced protein denaturation and HRBC membrane stabilization assay of crude extract of C. *medicaginea*

2, 2 azinobis 3-ethylbenzothiazoline-6-sulfonic acid radical scavenging assay (ABTS)

The ABTS scavenging activity of the methanolic extract of *C. medicaginea* is dose dependent. Among all the concentrations, the highest % age scavenging activity is observed at 1 mg/mL (59.76 ± 0.25%). The scavenging activity (%) of standard Trolox is also dose dependent. The maximum scavenging activity is observed at 1000 µg/ mL (94.16%) and minimum at 0.03125 mg/mL for standard (60.64%) and plant extract (26.67%). The results are presented in figure 5.

***In-vitro* anti-inflammatory activity**

Inhibition of heat induced protein denaturation

Protein-denaturation inhibition showed a statistically significant increase in plant extract and aspirin standard that was found to be dose dependent. In both cases the highest inhibition is observed at 1 mg/mL concentration (46.32% and 63.82%). The results exhibited that plant extract activity is relatively closer to the standard (Fig 6).

HRBC Membrane Stabilization Assay

The results of MAE-based methanolic plant extract is compared with the applied concentration of aspirin. A gradual increase in percent inhibition was observed and it is dose dependent. The highest dose of aspirin and microwave assisted extract of plant (1 mg/mL) showed maximum anti-inflammatory activity i.e., 95.06% and 96.55% respectively. The results are explained in Figure 6.

***In-vivo* antioxidant activity**

CAT and SOD assay

In the carrageenan induced rat paw edema, xylene induced ear edema and cotton pellet induced granuloma in rats the results of catalase (Table 4, 5 and 6) revealed an increase in serum of crude plant extract (400 mg/kg b.w.) with the value of 77.38 ± 3.06, 64.83 ± 1.95 and 54.46 ± 7.81% as compared to disease control group treated with carrageenan,

xylene and cotton pellet with the value of 50.35 ± 1.69, 48.59 ± 1.97 and 49.74 ± 1.93%.

In the carrageenan induced rat paw edema, xylene induced ear edema and cotton pellet induced granuloma in rats the percentage inhibition of superoxide dismutase were significantly increased in the crude plant extract (68.91 ± 1.41, 83.53 ± 0.02 and 90.58 ± 1.37%) as compared to disease control group (47.48 ± 1.97, 77.01 ± 2.64 and 49.74 ± 1.53%) and standard drug treated group (60.44 ± 1.09, 85.21 ± 4.87 and 84.63 ± 1.44%). The disease control group and diclofenac sodium treated group is not significantly different from each other ($p > 0.05$). The results were summarized in tables 4, 5 and 6.

***In-vivo* anti-inflammatory activity**

Carrageenan induced rat paw edema

Table 7 shows the gradual inhibition of degree of paw inflammation exerted by the crude extract of *C. medicaginea* against the standards where at a dose of 400 mg/kg b.w. the extract exhibited maximum activity (47.31%) that was lower than standard drug (77.33%). Diclofenac sodium that is a non-steroidal drug successfully resulted in highest suppression of rat paw edema among all the groups. The table 7 also shows the edema status of the inflamed right paw (n=6) of the rat in different time intervals which was induced by carrageenan. Hour 0 represents the base value and all other values were compared to this. All groups were compared to the control groups for the inhibition capacity. A comparison of means with $p< 0.05$ was considered significant.

Xylene induced ear edema

Plant extract at a dose of 400 mg/kg b.w. suppressed the inflammatory cascades. The table 8 shows %age inhibition of ear edema in different experiment groups where n=6 in each group and compared with control group. The data found not to be significant at 95% confidence interval and showed efficacy comparable to the standard. The maximum inhibition showed by plant extract is 73.66% while the standard drug inhibition percentage is 58.23%.

Cotton pellet induced granuloma in rats

C. medicaginea crude extract was able to decrease the granuloma formation process of inflammation in rats after the treatment as compared to control (Table 9). This was obvious because both wet and dry weights of the cotton pellets were reduced. Diclofenac sodium efficiently reduces the granuloma formation as compared to control groups. The results exhibited significant ($p < 0.05$) inhibition of cotton pellet granuloma at the tested concentration. The highest inhibition (56.03%) of granuloma tissue formation was observed for standard drug. The inhibition of test extract at a dose of 400 mg/kg b.w. was found to be 51.08%. Drug and plant extract significantly reduced inflammation as compared to the control that received no treatment.

Table 4: *In-vivo* antioxidant effects of carrageenan induced rat paw edema in rats

Groups	Treatments	% concentration	
		CAT	SOD
Disease Control group	Carrageenan	$50.35^{c} \pm 1.69$	$47.48^{b} \pm 1.97$
Negative Control group	Carr + 1% DMSO	$49.43^{c} \pm 0.03$	$44.85^{b} \pm 0.05$
Drug Control group	Carr + Diclofenac sodium (25 mg/kg b.w)	$65.04^{b} \pm 1.18$	$60.44^{a} \pm 1.09$
Plant Extract Treated group	Carr + Crude extract (400 mg/kg b.w)	$77.38^{a} \pm 3.06$	$68.91^{a} \pm 1.41$
	Healthy group	$37.34^{d} \pm 2.17$	$29.90^{c} \pm 2.80$

Values are mean ± STDEV of samples analyzed individually in triplicate.
Values in the same column with different superscripts are statistically significantly different from each other at $p < 0.05$.

Table 5: *In-vivo* antioxidant effects of xylene induced ear edema in rats

Groups	Treatments	% concentration	
		CAT	SOD
Disease Control group	Xylene	$48.59^{c} \pm 1.97$	$77.01^{b} \pm 2.64$
Negative Control group	Xyl + 1% DMSO	$44.90^{c} \pm 0.04$	$73.04^{b} \pm 0.07$
Drug Control group	Xyl + Diclofenac sodium (25 mg/kg b.w)	$54.05^{b} \pm 1.49$	$85.21^{a} \pm 4.87$
Plant Extract Treated group	Xyl + Crude extract (400 mg/kg b.w)	$64.83^{a} \pm 1.95$	$83.53^{a} \pm 0.02$
	Healthy group	$37.34^{d} \pm 2.17$	$29.90^{c} \pm 2.65$

Values are mean ± STDEV of samples analyzed individually in triplicate.
Values in the same column with different superscripts are statistically significantly different from each other at $p < 0.05$

Table 6: *In-vivo* antioxidant effects of cotton pellet induced granuloma in rats

Groups	Treatments	% concentration	
		CAT	SOD
Disease Control group	Cotton Pellet	$49.74^{c} \pm 1.93$	$75.13^{b} \pm 1.53$
Negative Control group	CP + 1% DMSO	$49.84^{c} \pm 0.03$	$74.01^{b} \pm 3.81$
Drug Control group	CP + Diclofenac sodium (25 mg/kg b.w)	$63.68^{a} \pm 2.01$	$84.63^{a} \pm 1.44$
Plant Extract Treated group	CP + Crude extract (400 mg/kg b.w)	$54.46^{b} \pm 7.81$	$90.58^{a} \pm 1.37$
	Healthy group	$37.34^{d} \pm 2.17$	$29.90^{c} \pm 2.80$

Values are expressed as mean ± SEM for 6 animals in each group.
Values in the same column with different superscripts are statistically significantly different from each other at $p < 0.05$.

Table 7: Measurement of paw thickness (cm) and % age inhibition in *C. medicaginea* crude extract and standard drug

Treatment	Paw thickness (cm) ± SD % inhibition					
	Before	30 min	1 h	2 h	3 h	4h
Untreated	0.661 ± 0.05	0.661 ± 0.05	0.661 ± 0.05	0.661 ± 0.05	0.661 ± 0.05	0.661 ± 0.05
Carrageenan	0.702 ± 0.08	0.788 ± 0.04	0.822 ± 0.04	0.864 ± 0.02	0.894 ± 0.02	0.938 ± 0.04
Carr + Diclofenac Sodium (25 mg/kg)	0.644 ± 0.07	0.664 ± 0.11 24.95 ± 0.37^{c}	0.726 ± 0.05 33.60 ± 2.75^{c}	0.766 ± 0.08 34.27 ± 1.33^{bc}	0.772 ± 0.02 45.20 ± 2.70^{b}	0.782 ± 0.01 77.33 ± 1.25^{a}
Carr + Crude extract (400 mg/kg)	0.658 ± 0.03	0.704 ± 0.08 12.98 ± 3.63^{d}	0.748 ± 0.09 27.06 ± 2.56^{c}	0.772 ± 0.07 30.92 ± 1.83^{c}	0.791 ± 0.05 45.30 ± 1.76^{bc}	0.831 ± 0.14 47.31 ± 1.13^{abc}

Table 8: Percentage inhibition of inflammation by *C. medicaginea* extract and standard drug in xylene induced ear edema in rats

Treatment	Wt. of Treated ear (g)	Wt. of Control ear (g)	Edema Degree	% inhibition
Xylene	0.370^{a} ± 0.12	0.228^{a} ± 0.04	0.142^{a} ± 0.08	-
Xylene + Diclofeanc Sodium (25 mg/kg)	0.240^{b} ± 0.09	0.180^{b} ± 0.06	0.060^{b} ± 0.03	58.23^{b} ± 1.40
Xylene + Crude extract (400 mg/kg)	0.205^{c} ± 0.03	0.165^{c} ± 0.03	0.039^{c} ± 0.05	73.66^{a} ± 2.19

Table 9: Percentage inhibition of inflammation by *C. medicaginea* extract and standard drug in cotton pellet induced granuloma in rats

Treatment	Wet Wt. (mg)	Dry Wt. (mg)	Granuloma inhibition (%)
Cotton Pellet	1334^{a} ± 1.00	618^{a} ± 1.52	-
CP + Diclofeanc Sodium (25 mg/kg)	1130^{c} ± 6.00	294^{c} ± 6.55	56.03^{a} ± 1.01
CP + Crude extract (400 mg/kg)	1217^{b} ± 1.15	343^{b} ± 4.35	51.08^{a} ± 0.69

Values are expressed as mean ± SEM for 6 animals in each group $p < 0.05$.
Values in the same column with different superscripts are statistically significantly different from each other at $p < 0.05$.

DISCUSSION

Plant exposure time to microwave radiations is an important factor due to which cell wall weakens and plant tissues also become soft which results in easy and enhanced release of phenolics, and flavonoids. Optimization of power level is, therefore, a necessary factor as it reduces the extraction time. But higher power levels can also cause burning of extracts or breaking down of extract constituents (Akhtar *et al.,* 2019).

Effect of extraction time on TPC, TFC and their antioxidant activity were analyzed at 263, 400, 600, 800, and 936 W and the obtained results revealed the presence of direct relation between power level and extraction time which result in an increase in number of extracted phenolics, flavonoids and their antioxidant activity. Maximum production of desired compounds can be obtained through MAE by optimized combination of power and time. In this type of extraction heating of sample occurs through microwave energy and after a definite time evaporation of water takes place and a fast increase in pressure and temperature occurs which expands cell wall and bioactive compounds are released in the extracted solvent without over heating (Akhtar *et al.,* 2019; Akhtar *et al.,* 2020; Ghaffar *et al.,* 2020).

This helps in sustaining the biological activity of the extracts and their biological molecules as proven in present study with the help of antioxidant activity.

The effect of varying power levels on extraction yield was evaluated. It is observed that increased power levels led to a gradual increase in the TPC and TFC values over time. This is attributed to the fact that at low temperatures kinetic energies are low and as a result mass transfer is also lower. Lower mass transfer leads to decreased solubilization of phenolics and flavonoids. These results confirmed other research works, e.g. the findings of (Cruz *et al.,* 2022).

As temperature increases cell sap and metabolites, internal pressure also increases due to microwaves. Moisture present in plant cells adds to the phenomenon. This leads to a rapid rupture of cells at a certain power and temperature which causes an earlier release of plant metabolites such as phenolics into the surrounding solvent than in conventional methods (Juodeikait *et al.,* 2022).

In GC-MS analysis of crude microwave assisted extract of *C. medicaginea* 44 different compounds have been identified. In terms of %peak area n-propyl acetate (1.84), 3-Octene Z (1.01), 3-Octene E (1.01), heptane 3 methylene (1.01), pyrrolizidine alkaloid (0.39), pyrrolizidine alkaloid (59.14), ethyl benzene (5.45), p-xylene (3.99), benzene 1,3 dimethyl (3.99), 1-Butanol, 3-methyl-, acetate (3.73), benzene 1,3 dimethyl (1.36), o-xylene (1.36), pyrrolizidine alkaloid (0.52), pyrrolizidine alkaloid (0.41), decane (0.41), Cyclohexane, 1,1-dimethoxy (2.17), 1,3-Dioxolane, 4-ethyl-2-pentade (2.17), Dimethylamine (N-B) bis trifluoro (2.17), 9,15-Octadecadienoic acid, methyl (0.28), 9,11-Octadecadienoic acid, methyl (0.28), 9,12-Octadecadienoic acid, methyl (0.28), pyrollodizine alkaloid (0.38), 5-Methyl-3-isoxazolyl methanamine (0.38), N-Benzoylglycine ethyl ester (0.80), Benzamide, N-5,6,7,8-tetrahydro (0.80), 2-Benzoyloxysuccinic acid, dimethyl (0.80), Bis(2-ethylhexyl) phthalate (6.38), Phthalic acid, di(2-propylpentyl (6.38), Bis(2-ethylhexyl) phthalate (6.38), 1,4-Benzenedicarboxylic acid, bi (8.47), 4-(4-Hydroxy-2,5-dimethylbenzyl (0.39), Tetrasiloxane, decamethyl (0.39), Cyclotrisiloxane, hexamethyl

(0.72), Methyltris(trimethylsiloxy)silane (0.72), Arsenous acid (0.72), Ethane (0.85), Tetrasiloxane, decamethyl (0.85), Cyclotrisiloxane, hexamethyl (0.85), Methyltris(trimethylsiloxy)silane (1.02), Ethane (1.02), Tetrasiloxane, decamethyl (1.02), 2'-Hydroxypropiophenone, TMS der (0.68), 2'-Hydroxy-5'-methylacetophenone (0.68) and Methyltris(trimethylsiloxy)silane (0.68). From the study pyrrolizidine alkaloid indicated the highest peak value in extract with retention time 4.618 min and %peak area 59.14. This alkaloid is a secondary metabolite and possess strong antioxidant, anti-inflammatory, anti-cancer, anti-HIV and acetylcholinesterase inhibitors activity (Moreira *et al.,* 2018).

While considering the antioxidant activity of plant-based compounds, one simple method is not enough because every method has different mechanisms of action. Therefore, present study included 4 antioxidant assays. Various antioxidants assays have diverse principle and experimental conditions. For example, DPPH uses organic radical producers and FRAP uses metal ions for oxidation.

Chemical reactions that are responsible for production of these different radicals are also quite different. Every technique has different procedure and experimental conditions that's why numerous antioxidants are used as standards or controls. They differ from each other according to their mode of action, rate of reaction, and time of reaction. Non-polar (Vitamin E) and polar antioxidant (phenolics etc.) with diverse scavenging potential exist as they have potential of being donate electrons or protons. Therefore, for different antioxidant assays different standards such as ascorbic acid, gallic acid and Trolox were used (Hwang *et al.,* 2022).

Hydroxyl radicals directly interact with DNA and show the mutagenic capacity of free radicals that results in breakdown of DNA and play a major role in inflammation (Scully, 1993; Rahman *et al.,* 2015). Iron-EDTA premixture is incubated with ascorbic acid and hydroxyl radicals are formed which damages the 2-Deoxy-d-ribose and formation of malondialdehyde takes place.

After addition of microwave assisted plant extract in reaction mixture hydroxyl radicals were removed that controlled the prevention of cell damage. The significantly higher hydroxyl radical scavenging activity is a major cause of its antioxidant agent as it can protect DNA from the damage of ROS. These extracts are also involved in the prevention of lipid peroxidation as they are OH ion scavengers. For the assessment of antioxidant activity reducing power is also an important parameter. By donating electrons antioxidant compounds can reduce reactive radicals in stable species. Phenolic compounds in extracts that are responsible for antioxidant activity, scavenging free radicals and ferric ion reduction are not directly involved in chelation of ferrous ion. Nitrogen containing compounds are better chelators than phenols (Wong and Kitts, 2006).

Antioxidants are compounds that react with free radicals and neutralize them. They prevent free radicals from causing cellular damage in the biological system (Diplock *et al.,* 1998). Endogenous antioxidants are those that neutralize free radicals in body. Exogenous antioxidants are those that are linked with diet and are commonly known as dietary antioxidants. Fruits, vegetables, and grains are rich sources of these antioxidants (Bouayed *et al.,* 2012). These results suggest that the application of crude plant extract was involved in

cellular protection directly as a source of antioxidant molecules and indirectly as an activity stimulator and the expression of antioxidant enzymes. During the inflammatory process monoterpenes are produced and act as free radical scavengers (Dapkevicius *et al.,* 2002). The antioxidant effect of crude plant extract is due to its secondary metabolites which present a high antioxidant capacity such as polyphenols, tocopherols, and sterols. Phenolic compounds have the capacity to trap radical species and reactive oxygen species (Heim *et al.,* 2002). Phenolic compounds interact with biological systems and act as bioactive molecules that are important inhibitors of lipid peroxidation (Jemai *et al.,* 2008).

In *in-vitro* anti-inflammatory activity studied it is observed that due to protein denaturation, a chronic inflammatory response occurs that results in reduced tissue strength and function. During chronic inflammation lysosomal membranes are lysed that releases pro-inflammatory markers that activate proteases, histamines, and neutrophils where injury or tissue damage occur. Such inflammatory responses also lead to hypersensitivity reactions and diseases like serum sickness (Anyasor *et al.,* 2009). Main mode of action of acetyl salicylic acid (aspirin) includes the endogenous synthesis of prostaglandin PGE2 by blocking the cyclooxygenase COX enzyme. Therefore, protein denaturation process is suppressed.

However, in the present study, the plant extract simulated the non-steroidal anti-inflammatory drug to a moderate extent showing a comparable pattern of results in responding to inflammation. Haemolytic activity causes the production of ROS and free radicals (lipid peroxides and superoxides) due to destabilization of membranes of red blood cells (Ranasinghe *et al.,* 2012). Plant extract contains various secondary metabolites including glycosides, tannins, steroids, flavonoids, saponins, and other phenolic content etc., that can reduce the ROS produced. It is assumed that plant extract can help the cell membrane of RBCs to stabilize their structures. This property that can be linked with these chemicals that prevents the stress induced destruction of plasma membrane. The present study demonstrates that MAE is rapid extraction method for biologically active constituents of the *C. medicaginea* without harming or damaging their biological activity. This method can save our time, energy and cost.

While studying *in-vivo* anti-inflammatory activity, it targets receptor bindings and mediators involved in development of inflammation. Carrageenan and xylene induced inflammatory methods are well-known and extensively used for the assessment of drugs or samples for their anti-inflammatory potentials (Hajhashemi *et al.,* 2011).

Mediators involved in inflammatory pathways prompted by these two agents are still under investigation. Two phases of biological response are involved in carrageenan treatment. Firstly, it releases inflammatory mediators (serotonin, histamine and kinin) and secondly, it produces cyclooxygenases (COX), prostaglandins and oxygen free radicals (Ashok *et al.,* 2010).

Xylene induced inflammation relates to P substance that is extensively dispersed in central and peripheral nervous system. The release of P substance (neuromodulator) causes plasma extravasations and vasodilatation that is directly linked with neurogenic inflammation and it causes ear swelling in rats (Agbaje *et al.,* 2012). In both processes diclofenac sodium is involved for suppression of mediators (Amann and Schuligoi, 2000).

Diclofenac sodium is a common NSAID with high efficacy. Now in comparison, *C. medicaginea* crude extract was also found to possess potential activity against the mediators involved. Conventional NSAID like diclofenac sodium and acetyl salicylic acid (aspirin) not only block COX enzyme and endogenous PGE2 synthesis but also suppress the protein denaturation (Insel, 1990). Crude methanol extract of *C. medicaginea* might have suppressed the inflammation response by the same method. The result of study demonstrated that *C. medicaginea* crude extract possess considerable anti-inflammatory activity as capable of inhibiting the auto antigen production to significant extent compared with the standard. The crude extract contains tannins and phenolic content that can be the attributor for these activities.

Subcutaneously implanted cotton pellet in rats is divided into three phases transduative, exudative, and proliferative phase (Swingle and Shideman, 1972). The cotton pellet induced granuloma is extensively used to evaluate the transduative and proliferative components of chronic inflammation (Winter and Porter, 1957).

Host inflammatory response is produced by implanted material, and it reduces the release of inflammatory mediators which causes the proliferation of tissues and granuloma formation. Wet cotton pellet weight relates to transduate material and dry cotton pellet weight is associated with granulomatous tissue (Namita *et al.,* 2012; Sireeratawong *et al.,* 2012). Transduative weight can be reduced through anti-inflammatory drugs because the permeability response of the blood vessels around the cotton pellet implantation can be reduced through the drugs. Granuloma formation can also be inhibited through this process because it also interferes with the proliferative components of the inflammatory process. NSAIDs such as diclofenac sodium stimulate a minor inhibition whereas SAIDs have a strong inhibition on both phases of inflammation (Swingle and Shideman, 1972). During the study, the reduction in weight of granuloma in rats treated with plant extract specifies that the proliferative phase was efficiently suppressed by the crude extract of *C. medicaginea.*

CONCLUSION

The current research revealed that *C. medicaginea* extract investigated possessed strong anti-inflammatory activity better than the standard drug (diclofenac sodium). The plant also possesses strong antioxidant activity. The results concluded that *C. medicaginea* must be tested against various types of inflammation and explored further to identify and isolate novel natural products from this plant as drug leads for anti-inflammatory therapies.

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COMPETING INTERESTS

Authors have no conflict of interest

AUTHOR'S CONTRIBUTION

SB Performed the research work, ZS conceptualized and supervised the work, KA supervised the work

and drafted the manuscript, KN analyzed the results, KH and TK worked on statistical analysis and manuscript final write up.

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