



## PHARMACOLOGICAL EVALUATION OF ANALGESIC ACTIVITY OF N-BUTANOL AND ETHYL ACETATE EXTRACT OF *MATRICARIA CHAMOMILLA* IN RATS

Swati<sup>1</sup>, S D Singh<sup>2</sup>, Shashi Bhooshan Tiwari<sup>3\*</sup>

### Abstract

*Matricaria chamomile* is one of the oldest known herbs of traditional medicine and belongs to Asteraceae family. The present study was based on the selection, extraction, purification, and pharmacological evaluation of analgesic action of extracts of *Matricaria chamomile* in rats. Diclofenac sodium was purchased from Bharat medical store, Bareilly, UP. Alcohol (ethanol and methanol), distilled water, ethyl acetate, n-butanol, petroleum ether was obtained from the local chemical supplier. Clevenger apparatus, rotatory evaporator, and desiccator were used in this study. Flowers and stem from plants- *Matricaria chamomilla* selected to perform the study. The fresh whole plant of *Matricaria chamomilla* collected from the local nursery in Bareilly. The plant was identified and authenticated by a Dr. Alok Shrivastava, Professor (Department of plant science), Faculty of sciences, M.J.P. Rohilkhand University, Bareilly. UP. Eighteen adult any sex Wistar rats (150-120g) were obtained from Laboratory animal center at IVRI, Bareilly, U.P. the animal was housed at a controlled environmental temperature (23± 1°C, a 12-h light alternating with 12-h darkness cycle) and allowed food and water ad libitum. Hot plate and tail flick parameters were used for screening of analgesic and paw edema model for anti-inflammatory. Chemical constituents were characterized by TLC and UV spectroscopy techniques. The % inhibition of oedema (at 3 hours) was recorded as highest as 72.14\*\* in the n-butanol *Matricaria chamomilla* extract at the dose of 400mg/kg and compared with control that was found Nil. While ethyl acetate extract exhibited the % inhibition as 64.76\*\* in the ethyl acetate extract of *Matricaria chamomilla*. In conclusion, significant analgesic activity was observed with all four fractions and the extracts were more effective than diclofenac sodium. This study suggests that after successful clinical trials it might be incorporated in suitable dosage forms with better adaptability in terms of anti-inflammatory and analgesic action.

**Keywords:** *Matricaria chamomile*, analgesic, Clevenger, Hot plate, paw edema

<sup>1</sup>Research Scholar, Department of Pharmacy, MJP Rohilkhand University Bareilly, IN

<sup>2,3\*</sup>Professor, Department of Pharmacy, MJP Rohilkhand University Bareilly, IN

**\*Corresponding Author:** Shashi Bhooshan Tiwari

Professor, Department of Pharmacy, MJP Rohilkhand University Bareilly, IN

Email id: [s.tiwari@mjpru.ac.in](mailto:s.tiwari@mjpru.ac.in)

**DOI:** - 10.31838/ecb/2023.12.si5.026

## INTRODUCTION

The effect of plants on human being health has been documented for thousands of years. Herbs has been integral to both traditional and no-traditional forms of medicine dating back at least 5000 years (Newman et al. 2003). Medicinal and aromatic plants have been used for thousands of years in the traditional system of medicine for the cure of many ailments (Schilcher, 1973; Taberna, 1664). Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of diseases and clinical disorders, and relatively little knowledge available about their exact effective doses and mode of action (Saieed, 2006). Natural molecules are much safer than synthetic molecules. Medicinal plants derived from natural compounds such as flavonoids, steroids, lignans, polyphenols, coumarins, terpenes and alkaloids are scientifically proven to relieve inflammation, pain and fever (Trivedi, 2014; Kelly, 2009). Chamomile is also extensively consumed as tea or tonic. Chamomile. Herbal tea is used internally to treat anxiety, hysteria, nightmares, insomnia and other sleep problems, convulsions and even delirium tremens. And its essential oil is also a treatment for malaria and parasitic worm infections, cystic, colds and flu (Nemecz, 1998). Analgesic - Pain is an unpleasant, sensory and emotional experience usually associated with noxious stimuli and a response of a threatening condition in the tissues of the body, like hunger or thirst which is subjective, protective and modified by developmental, behavioral personality and culture factors (Rainville et al., 1997). Pain and inflammation are associated with pathology of various clinical conditions like arthritis, cancer, and vascular diseases (Malmberg et al., 1997). The special nerve endings that sense pain is very sensitive to prostaglandin. When prostaglandin is released, the nerve endings respond to it through prostaglandin E2 receptor by picking up and transmitting the pain and injury messages to the brain and causing visceral writhing stimuli in rats. Therefore, it has been suggested that the inhibition of prostaglandin synthesis is remarkably an efficient antinociceptive mechanism in visceral pain (Atta et al., 1998). **Anti-inflammatory** Inflammation reaction consists of three components (triple response), vasodilation (flash), emigration of leukocytes from venules & capillaries to the site of injury (flare) and edema due to increased capillary permeability. Inflammation is accompanied by pain, stiffness and swelling or edema of the affected area. Edema depends partially on volume of exudates. Thus, an anti-inflammatory drug can act by decreasing vascular permeability or cell accumulation or both.

It can also act by interfering with antigen antibody reaction. (Singh M., et al., 2014).

## Plant description

*Matricaria chamomilla* (synonym: *Matricaria recutita*), commonly known as chamomile, blue chamomile, German chamomile, or Scented mayweed. It is an annual plant of composite family Asteraceae often referred to as the “star among medicinal herbs” (Sing et al., 1995). The word chamomile comes from Greek, meaning- ‘earth-apply’ because of the apple-like scent of the plant (Mann et al., 2002). *Matricaria chamomile* is one of the oldest known herbs of traditional medicine and belongs to Asteraceae family. *Matricaria chamomilla* is a low growing aromatic herb with branched, erect and smooth stem, grows to a height of 15-60 cm., double feathery shared, long and narrow, bipinnate or tripinnate leaves and tiny, soft, hollow, head lettuce flowers. Chamomile essential oils derived from head lettuce flowers paniculate flower heads (capitula) of two types i.e., yellow disc florets and White ray florets, this flower has characteristic smell due the presence of blue essential oil. This color and smell are shown by chamazulene. Chamomile grows to 20 inches and has feathery foliage with daisy-like flowers like its cousin. The flowers are scented, but the foliage is not. Roman chamomile is an aromatic creeping perennial which grows only one foot in height. The flower heads are one inches in diameter, with a broad conical disk that is covered in yellow florets surrounded by white florets. It has many freely branching hairy stems and finely divided leaves (Das et al., 1998).



**Fig 1. Depiction of *Matricaria chamomilla* dried flowers**

## Phytochemical Constituents

There are approximately 120 secondary metabolites, including 36 terpenoids and 36 flavonoids have been identified in plant *Matricaria chamomilla*. It contains essential oil (0.2–1.9%), which has an intense blue color owing to its chamazulene content (1–15%). Other major constituents include  $\alpha$ -bisabolol, sesquiterpenes,

coumarins, Apigenin and related flavonoid glycosides are considered the most important constituents. There are eleven bioactive phenolic compounds such as herniarin, umbelliferon, cholinergic acid, caffeic acid, apigenin, apigenin-7-O-glycoside, luteolin and luteolin-7-O-glycoside (flavones), quercetin and rutin (flavanols), and naringenin are found in chamomile plant extracts. Essential oils extracted from the flowers are (E)- $\beta$ -farnesene (4.9-8.1%), terpene alcohol (farnesol), chamazulene (2.3-10.9%),  $\alpha$ -bisabolol (4.8-11.3), and  $\alpha$ -bisabolol oxides A and  $\alpha$ -bisabolol oxides B, these are known for their anti-inflammatory, antiseptic and spasmolytic properties.

## MATERIALS AND METHODS

### Experimental requirements

**Drugs-** Diclofenac sodium (Harson Laboratories) was purchased from Bharat medical store, Bareilly, UP. Drugs were given at the doses reported to exert significant action. **Solvents-** Methanol, Ethyl acetate, n-butanol, Distilled water, toluene, ethyl methyl acetate, were obtained from the local chemical supplier. **Apparatus-** Clevenger apparatus, rotatory evaporator, desiccator, hot plate, and plethysmometer were used in this study.

### Collection, Identification and Authentication of Plant

Flowers of *Matricaria chamomilla* selected to perform the study. The fresh flowers of *Matricaria chamomilla* collected from the local nursery in Bareilly. The plant was identified and authenticated by a Dr. Alok Shrivastava, Professor (Department of plant science), Faculty of sciences, M.J.P. Rohilkhand University, Bareilly. U.P. One hundred grams (100 gm) of flowers and stem obtained from the plants. Undesirable leaves were removed and samples were dried. The plant material placed in an airtight container, to protect them from moisture. Dried samples (at room temperature;  $25 \pm 2^\circ\text{C}$ ) were powered by a mechanical grinder and sieve the material then kept these in a dark place.

### Clevenger apparatus

The Clevenger apparatus based on distillation is the best method to determine the essential oil content of the plant. The name of this apparatus was derived from its inventor, Joseph Franklin Clevenger in 1928. It has three main parts- a round bottom flask in which the organic material is placed, a separator for automatically separating the distilled solution and a condenser. The apparatus is available in varied sizes to facilitate organic material (Clevenger J.F., 1928).



**Fig 2. Extraction of essential oil from *Matricaria chamomilla* flower by using Clevenger apparatus**

### Preparation of plant extract

Hydro-distillation by using Clevenger apparatus using n-butanol and ethyl acetate as solvents, Fine powder of chamomilla flower were subjected to hydro-distillation with the Clevenger under optimal operation conditions with a temperature of  $40^\circ\text{C}$  as described. 25 gm of dried powder (coarse powder) of plant material were placed in to a 1000 ml round water flask and added a few pieces of porcelain disk, add 250 ml of n-butanol and ethyl acetate in different flask. (Cica et al. 2017). The distillation process was performed for 6 hours, and the obtained essential oil was collected and at the end of the procedure the vapors of essential oil were condensed and recovered. Recovered volatile oil was stored on the top of the hydrosol and removed by using the closure tap. and dehydrated using anhydrous sodium sulphate. (Pino, 2002; Pirzad et al., 2006).

### Moisture content

Moisture content of collected essential oil/volatile oil was examined and used for future references.

### Extraction Yield

For each extraction technique, the total yield is defined as gram of extracted per kg of the dried material flower into the extraction apparatus. The extraction n yield of the essential oil obtained from both methods were calculated as follows:

$$\text{Extraction Yield (\%)} = \frac{\text{Mass of extracted oil}}{\text{Mass of Dried flowers}} * 100$$

### Phytochemical screening

Phytochemical test of *Matricaria chamomilla* was performed and it was evaluated that the n-butanol and ethyl acetate extract of plant shows the several phytoconstituents e.g., glycosides, saponin, flavonoids, phenols, alkaloids, these compounds found in this plant.

### Chromatographic Techniques

TLC study aimed to investigate phytochemicals present in n-butanol and ethyl acetate extract of *M. chamomilla*. Essential oils (Bisabolol) confirm by Developing solvent system for both extract in ratio of methanol: Ethyl methyl acetate: Toluene (3:6:11) v/v/v, in order to determine the presence of volatile oil in compounds.

### UV spectroscopy

The present study was carried out to characterize the bioactive constituents (essential oil) present in plant extract of *M. chamomilla* using UV – spectroscopic techniques. The plant extract was scanned in the wavelength ranging from 300-800nm by using UV-spectrometer (Shimadzu - 1400) and the characterized peaks were detected. Essential oil of *Matricaria chamomilla* was extracted by hydrodistillation Clevenger apparatus, which is common conventional method for extraction of essential oil from flowers of plant. Since the extraction procedure significantly varies in different solvent, and time, thereby these parameters were studied in order to determine the effect of each factor and consequently find the optimum condition, in which highest yield of essential oil is extracted.

### Group design

Table 1. Group design-

Group (n=6)	Dosing
STANDARD GROUP	Diclofenac sodium (50 mg/kg, i.p)
VEHICLE	Normal saline, orally
TEST GROUP-1 (ethyl acetate extract of <i>M. chamomilla</i> )	200 mg/kg, orally
TEST GROUP-2 (ethyl acetate extract of <i>M. chamomilla</i> )	400 mg/kg, orally
TEST GROUP-3 (n-butanol extract of <i>M. chamomilla</i> )	200 mg/kg, orally
TEST GROUP-4 (n-butanol extract of <i>M. chamomilla</i> )	400 mg/kg, orally

### Evaluation of Pharmacological activity

Painful reaction in experimental animals can be produced by applying noxious (unpleasant) stimuli such as (i) thermal (radiant heat as a source of pain), (ii) chemical (irritants such as acetic acid and bradykinins) and (iii) Physical pressure (tail compression). In the laboratory, commonly used procedures are tail-flick (tail-withdrawal from the radiant heat) method using the hot water, analgesiometer, hot plate (jumping from the hot plate at 55 C) method and acetic acid-induced writhing.

#### A. Analgesic activity

**Hot plate mode** - The animals were weighed and randomized into six groups of six animal each. Basal reaction time was taken by observing hind paw licking or jump response (whichever appears first) in animals when placed on the hot plate  
*Eur. Chem. Bull.* **2023**, *12*(Special Issue 5), 214 – 223

### Calculation of dose regimen:

A vehicle is any substance that act as a medium which a drug is administered. Vehicle should be biologically inert, no toxic effect on animal, no any influence on results. Determine by OECD guidelines (Pandey V.; (2020).

$$\text{Dosage in ml} = \frac{\text{Body wight of animal (g)}}{1000 \text{ g}} * \text{dose (ml)}$$

The dosage of the drug (mg/ ml) should be constituted in an appropriate volume not usually exceeding 10ml/kg body weight of experimental animals for non-aqueous solvent in oral route of administration. Dosage calculation for plant extract with selected concentration as 200 mg/kg, 400mg/kg, 600mg/kg, for a rat weighing 100 gm.

### Biological parameters

Biological such as age, sex, weight, temperature, and hematological parameters such as blood glucose level were assessed before conducting experiment. All animals are Age of one month, Female rats with body temperature  $37.1 \pm 0.2^\circ\text{C}$ , blood glucose level of all rats found to be normal that determines all rats were non-diabetic.

maintained at constant temperature ( $55^\circ\text{C}$ ), animal showed this response in 6-8 sec. A cutoff period of 10 sec was observed to avoid damage to the paws, taken as the end point. Diclofenac sodium were injected by intraperitoneal injection to the positive control and vehicle to negative control group, remaining third fourth fifth and sixth group received test compound in dose dependent manner. (Siegmond, E., C., at al., 1957).

### Tail flick response

The animals were weighed and randomized into six groups of six animal each. First group received diclofenac sodium as a standard drug, the second group received vehicle, remaining group received test drug in dose dependent manner through oral route of administration. Basal reaction time was taken to radiant heat by placing the tip (last 1-2 cm) of the tail on the radiant heat source, into hot water



maintained at 58°C. The tail-withdrawal from the heat (flicking response) is taken as the end point, normally rat. withdraws its tail within 3-5 sec. A cutoff period of 10-12 sec is observed to prevent damage to the tail. At least 3-5 basal reaction time was taken for each rat at a gap of 5 min to confirm the normal behavior of the animal.

Diclofenac sodium were injected by intraperitoneal injection to the positive control and vehicle to negative control group, remaining third fourth fifth and sixth group received, test compound in dose dependent manner. The reaction time at 15, 30, 45, 60 and 90 min after the drug were noted. As the reaction time reached 10n sec it was considered maximum analgesia and the tail was removed from the source of heat to avoid tissue damage (Siegmund, et al., 1957).

**Anti-inflammatory response** Rats treated with normal saline (negative control) did not show any significant difference in the reaction time on tail flick throughout the 60 min observation. In comparison with the baseline values within the same treatment group, the increase in reaction time

at different time points significantly differed ( $P < 0.05$ ) for Diclofenac sodium (positive control) and n-butanol & ethyl acetate on dose dependent manner. Duration of the reaction time in diclofenac sodium and plant extract treated animal significantly higher compared to saline treated

## RESULTS AND DISCUSSION

**Extraction yield-** Extraction procedure was taken 6 hours by hydro distillation. The ultimate yield of essential oil obtained from *M. chamomilla* was  $0.5 \pm 0.02\%$  for both (n-butanol and ethyl acetate) extracts. Essential oils obtained blue colored with characteristic pleasant aroma. Yields are expressed as in grams of essential oil per 100g of *M. chamomilla*

**Phytochemical test-** Preliminary investigation revealed the presence of essential oil/volatile oil, flavonoids and phenols in both solvent fraction of plant (i.e., ethyl acetate and n- butanol).

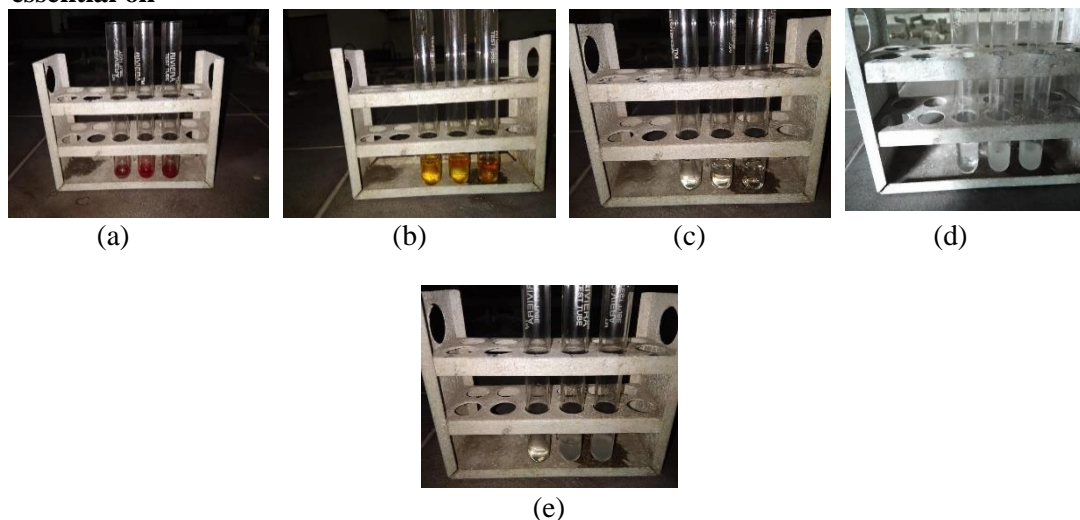
Following data confirms the presence of essential oils, flavonoids, phenols whereas all the test for tannins and alkaloids shown negative that indicated

**Table 2. Constituents of plant *M. chamomilla***

S. No.	Constituents	Test	n- butanol extract	Ethyl acetate extract
1.	Glycosides		+	+
2.	Volatile oil	Sudan red III	+	+
3.	Saponin	Foam test	+	+
4.	Flavonoids	Lead acetate	++	++
		Alkaline reagent test	+	+
5.	Terpenes		++	++
6.	Tannins		--	--
7.	Phenols		++	++
8.	Alkaloids	1. Dragendroff's test 2. Wagner's test 3. Hager test	-- -- --	-- -- --

Here: (+) present, (++) moderately present, (--) absent.

### Test for essential oil -



**Fig 3. (a) Sudan red test for Volatile oil/essential oil and (b) Triterpenes test for Glycosides (c) Frothing test for saponins and (d) Phenol test for Flavonoids.**

### TLC evaluations

The RF values were found as 0.311 and 0.283 for ethyl acetate and n-butanol (sample 1 and sample

2) respectively when observed. The following figure depicts the RF value screening.

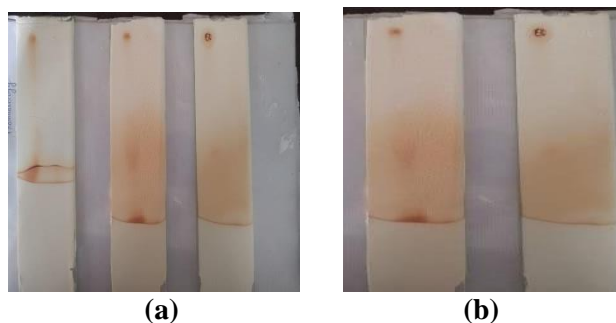


Fig 4 (a) TLC evaluation of ethyl acetate (Sample A), (b) N- butanol (Sample B) with distilled water (sample C)

### UV Spectrophotometry

The n- butanol extract and ethyl acetate extract of *Matricaria chamomilla* were determined in terms of UV spectrophotometry. In the same order, n-butanol extract showed the absorption spectra at

$\lambda_{max}$  of 543.5 and 575.5 and ethyl acetate extract exhibited the absorption spectra at  $\lambda_{max}$  of 495, 526.5 and 575.

Both the spectral peaks are showed in below figure as a) and b)-

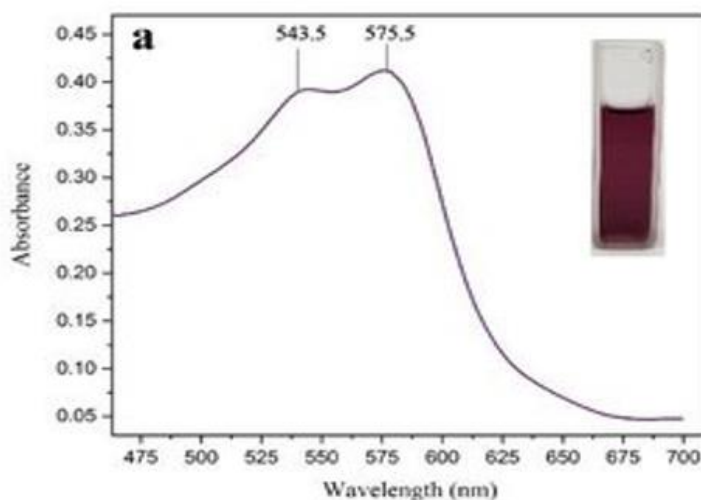


Fig 5. UV-spectra for n-butanol extract of *Matricaria chamomilla*

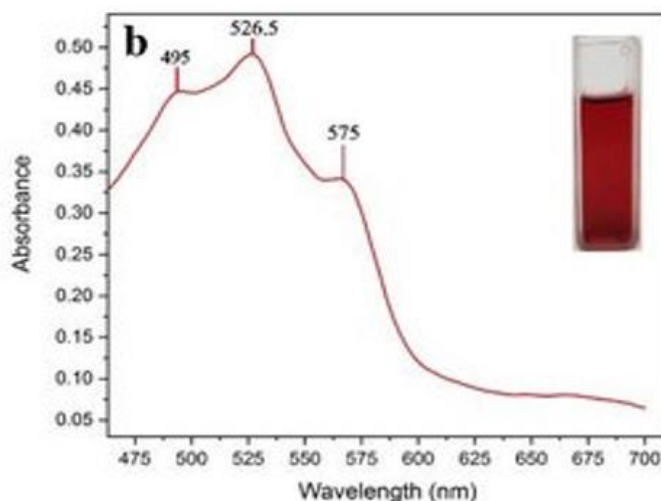


Fig 6. UV-spectra for ethyl acetate extract of *Matricaria chamomilla*

In the same order, n-butanol extract showed the absorption spectra at  $\lambda$ -max of 543.5 and 575.5 and

ethyl acetate extract exhibited the absorption spectra at  $\lambda$ -max of 495, 526.5 and 575.



Fig 7. Jumping response recorded at Hot plate

At 90 min, the maximum analgesic response was recorded paw licking as  $4.16 \pm 0.37^{**}$  seconds and  $6.20 \pm 0.75^{**}$  seconds in ethyl acetate (400mg/kg) and n-butanol extract (400mg/kg) of *Matricaria*

*chamomilla*, respectively when compared with control group that was observed as  $3.66 \pm 0.33^{*}$  seconds.

Table 3. Analgesic activity recorded at tail flick method.

Group	Basal reaction time (sec)									
	0 min		30 min		45 min		60 min		90 min	
	Jump response	Paw licking	Jump response	Paw licking	Jump response	Paw licking	Jump response	Paw licking	Jump response	Paw licking
Vehicle	$2.33 \pm 0.33^{*}$	$2.46 \pm 0.33^{*}$	$2.33 \pm 0.33^{**}$	$2.66 \pm 0.33^{*}$	$2.65 \pm 0.32^{*}$	$3.33 \pm 0.14^{*}$	$3.23 \pm 0.29^{*}$	$3.66 \pm 0.27^{*}$	$3.16 \pm 0.31^{*}$	$3.66 \pm 0.33^{*}$
Diclofenac sodium (10mg/kg)	$2.33 \pm 0.32^{*}$	$2.21 \pm 0.75^{**}$	$3.6 \pm 0.3^{*}$	$3.67 \pm 0.98^{*}$	$4.33 \pm 0.13^{**}$	$4.15 \pm 0.89^{**}$	$4.66 \pm 0.33^{***}$	$4.55 \pm 0.89^{*}$	$5.66 \pm 0.23^{**}$	$6.20 \pm 0.75^{**}$
Ethyl acetate (200mg/kg)	$2.33 \pm 0.32^{*}$	$2.14 \pm 0.33^{**}$	$2.36 \pm 0.27^{*}$	$3.66 \pm 0.30^{*}$	$3.46 \pm 0.52^{**}$	$3.66 \pm 0.33^{**}$	$3.03 \pm 0.30^{**}$	$3.6 \pm 0.6^{**}$	$3.33 \pm 0.33^{*}$	$3.6 \pm 0.51^{**}$
Ethyl acetate (400mg/kg)	$3.16 \pm 0.33^{*}$	$3.66 \pm 0.33^{*}$	$3.33 \pm 0.33^{***}$	$3.66 \pm 0.30^{**}$	$3.14 \pm 0.31^{***}$	$3.85 \pm 0.19^{**}$	$3.66 \pm 0.30^{**}$	$4.01 \pm 0.33^{**}$	$4.33 \pm 0.48^{**}$	$4.16 \pm 0.37^{**}$
n- butanol (200mg/kg)	$2.66 \pm 0.31^{**}$	$3.6 \pm 0.33^{*}$	$3.6 \pm 0.3^{*}$	$3.6 \pm 0.33^{**}$	$4.33 \pm 0.13^{**}$	$3.6 \pm 0.33^{**}$	$4.66 \pm 0.33^{***}$	$3.6 \pm 0.3^{**}$	$3.33 \pm 0.23^{*}$	$3.6 \pm 0.33^{**}$
n- butanol (400mg/kg)	$3.33 \pm 0.33^{**}$	$2.21 \pm 0.75^{**}$	$3.50 \pm 0.32^{***}$	$4.17 \pm 0.98^{**}$	$4.26 \pm 0.13^{**}$	$4.17 \pm 0.98^{**}$	$4.66 \pm 0.33^{**}$	$4.15 \pm 0.89^{***}$	$5.66 \pm 0.14^{*}$	$6.20 \pm 0.75^{**}$

Results are presented as mean  $\pm$  SEM, (n=3), \*p<0.01, P<\*0.05 test as compared to control. The control group at 0 min, 30 min, 45 min, 60 min and

90 min shows jump response and paw licking response on hot plate.



Fig 8. Tail flick response

**Table 4. Analgesic activity recorded at hot plate response**

Treatment and dose (mg/kg) (N=3)	Basal reaction time	Reaction time				
		15 min	30 min	45 min	60 min	90 min
Vehicle(2ml/kg)	3.9±0.23**	3.9±0.24**	4.0±0.25**	4.0±0.21**	4.1±0.54**	4.2±0.27**
Diclofenac sodium 10mg/kg)	3.24±0.54*	6.97±0.21**	9.8±0.28*	10.6±0.54**	11.9±0.20**	12.4±0.26**
Ethyl acetate (200 mg/kg)	3.7±0.26**	4.4±0.25**	5.8±0.28***	7.2±0.23**	8.1±0.26*	9.5±0.23***
Ethyl acetate (400 mg/kg)	3.9±0.47*	4.7±0.23**	7.1±0.25**	8.2±0.54**	9.3±0.23**	10.4±0.25**
n-butanol (200mg/kg)	3.7±0.26**	4.9±0.54**	6.3±0.23**	7.9±0.28**	8.7±0.23**	9.8±0.54***
n-butanol (400 mg/kg)	4.1±0.28**	6.2±0.25**	8.3±0.62***	9.8±0.54**	11.6±0.25**	12.6±0.43**

Results are presented as mean± SEM, (n=3), \*p<0.01, P<\*0.05 test as compared to control. The control group at 0 min, 30 min, 45 min, 60 min and 90 min shows tail flicking response by using radiant heat as a source.

At 90 min, the maximum analgesic response was recorded tail flicking 10.4±0.25\*\*seconds and 12.6±0.43\*\* in ethyl acetate (400mg/kg) and n-butanol extract (400mg/kg) of *Matricaria chamomilla*, respectively when compared with control group that was observed as 4.2±0.27\*\* seconds.

Comparison with the baseline values within the same treatment group, the increase in reaction time at different time points significantly differed (P<0.05) for Diclofenac sodium (positive control) and n-butanol & ethyl acetate on dose dependent manner. Duration of the reaction time in diclofenac sodium and plant extract treated animal significantly higher compared to saline treated animals, except for the extract treated group at 90 min.

**Table 5. Anti-inflammatory activity in carrageenan induced paw edema**

Treatment and dose (mg/kg)	Mean increase in paw oedema (ml)			% Inhibition of oedema (ml)		
	1h	2h	3h	1h	2h	3h
Vehicle	0.46±0.10**	0.73±0.18*	0.94±0.86*	-	-	-
Diclofenac sodium (10mg/kg)	0.16±0.82**	0.22±0.45*	0.28±0.39**	65.21*	69.86**	70.21*
Ethyl acetate Extract (200mg/kg)	0.18±0.38*	0.24±0.43**	0.29±0.49*	32.60**	34.24**	38.29***
Ethyl acetate extract (400mg/kg)	0.21±0.66**	0.29±0.38**	0.35±0.28**	60.34**	62.27**	64.76**
n-butanol extract (200mg/kg)	0.18±0.65***	0.24±0.47**	0.29±0.58**	34.60***	36.24**	40.29***
n-butanol extract (400mg/kg)	0.31±0.86***	0.48±0.36**	0.58±0.19***	68.86***	70.12***	72.14**



**Fig 9. Anti-inflammatory response**

The data were analyzed statistically. The results were expressed as mean ±SD; then the differences in weight of both paws (oedema) among groups were calculated and statistically evaluated using multiple way ANOVA; values with p<0.05 are considered significantly different. Result- The % inhibition of oedema (at 3 hours) was recorded as

highest as 72.14\*\* in the n-butanol *Matricaria chamomilla* extract at the dose of 400mg/kg and compared with control that was found Nil. While ethyl acetate extract exhibited the % inhibition as 64.76\*\* in the ethyl acetate extract of *M. chamomilla*.

### CONCLUSION

The extraction yield of the flower was determined by using electrical weighing machine, and it gives an approximate 0.5±0.02% yield of essential oils. Phytochemical evaluation revealed the presence of essential oil, flavonoids, and phenols. For further evaluation TLC chromatographic methods used to confirm the presence of essential oil in both fraction (i.e. ethyl acetate and n-butanol). UV analysis determines λ-max value of both fractions at 200-800nm spectroscopic range.

In conclusion, significant analgesic and anti-



inflammatory activity was observed with all four fractions and the extracts were more effective than diclofenac sodium. The use of higher dose (400 mg/kg) of the extracts abolish the pain completely, the LD50 of the total extract orally given in rat is 400 mg/kg. Dose- response study required to determine the optimal doses with low side effects and toxicity. The n-butanol extract of chamomile produced significant less pain than ethyl acetate extract, it indicates that the compounds present in then-butanol extract of chamomile have better analgesic activity than that observed with ethyl acetate fraction of the chamomile extract. The highly significant anti-inflammatory activity of chamomile extract, may be due to the interference with the mediators of inflammation such as histamine, serotonin and prostaglandins and may therefore be responsible for its analgesic activity.

The pharmacological responses, both analgesic and anti-inflammatory were found as dose dependent.

This study suggests that after successful clinical trials it might be incorporated in suitable dosage forms with better adaptability in terms of anti-inflammatory and analgesic action. It would be a great change towards medication system of inflammation induced pain with the better availability of *Matricaria chamomilla* being cost effective too.

This study suggests that after successful clinical trials it might be incorporated in suitable dosage forms with better adaptability in terms of anti-inflammatory and analgesic action.

#### FUNDING

Nil.

#### CONFLICT OF INTEREST

Authors have declared none of the conflict of interest.

#### REFERENCES

1. Atta, A.H., and Alkofahi, A., (1998). Antinociceptive and anti-inflammatory effects of some Jordanian medicinal plant extracts, *J Ethnopharmacol*; page no. 117-124.
2. Cica V., Karl-Heinz G., Jurgen A., and Karen N. (2017). Springer-Verlag Wien.
3. Das M., Mallavarapu G.R., and Kumar S., (1998). Chamomile (*Chamomilla recutita*): Economic botany, biology, chemistry, domestication and cultivation. *J Med Aromatic Plant Sci*; page no. 20: 1074-109.
4. Karunakaran D., Rashmi R., Kumar T.R., (2005). Induction of apoptosis by curcumin and

its implications for cancer therapy. *Current Cancer Drug Targets* 5: 117-129. Kelly K. (2009) *History of medicine, Facts on File*; New York; page no. 29 –50.

5. Mann C., Staba E.J., Cracker L.E., and Simon J.E. (2002) *The chemistry, pharmacology and commercial formulations of chamomile. And Herbs, spices and medicinal plants- recent advances in botany, horticulture and pharmacology. USA*; Haworth Press Inc; page no. 235-80.
6. Malmberg A.B., Chen C., Tonegawa S., and Basbaum A.I., (1997). Preserved Acute Pain and Reduced neuropathic pain in mice lacking PKC $\gamma$  Science; page no. 278: 279–283.
7. Nemezc G., (1998). chamomile, *US pharm.*, 23:115-116.
8. Newman D J, Cragg G M, Snader K M (2003). Natural products as sources of new drugs over the period 1981-2002. *J Nat Prod*; 66:1022-1037.
9. Pino J.A., Bayat F., Marbot R. and Aguerro J., (2002). Essential oil of *Chamomilla recutita* (L.) Rausch. From Iran. *J Essent Oil Res*; 14,407-8.
10. Pirzad A., Alyari M.R., Shaliba S., Zehtab-Salmasi and Mohammadi A., (2006). Essential oil content and composition of German chamomile (*Matricaria chamomilla*) at different irrigation regimes. *J Agron*; 5:451-5.
11. Rainville P., Duncan G.H., and Price D.D., (1997). Carrier B. and Bushnell M.C. Pain effect encoded in human anterior cingulate cortex.; *Science*; page no. 277, 968–971.
12. Saieed P., Reza R.M., Abbas D., Seyyed ail R., and Ali Asghar H. (2006). Inhibitory effect of *Ruta graveolens* L. extract on guinea pig liver aldehyde oxidase.; *Chem Pharm Bull*; 2.
13. Schilcher H., (1973). Neuere Erkenntnisse bei der Qualitätsbeurteilung von Kamillenblüten bzw. Kamillenöl — Einteilung der Hundskamille in vier chemische Typen. *Planta Medica*; page no. 23, 132.
14. Siegmund, E., Cadmus, R. and Lu, G. (1957). *proc. Soc. Exp. Biol. Med.*; page no. 95,729.
15. Singh S., and Majumdar D.K., (1995). Analgesic activity of *Ocimum chamomile* and its possible mechanism of action. *Int J Pharmacology*; page no. 33,188.
16. Tabernae montanus, J.T., (1664). *New vollkom männlich Kräuter-Buch*, Jacob Werenfels, Basel; page no. 1529.
17. Trivedi P., Pal A., Shanker K., Singh M., and Sharma P., (2014). Protective mechanism of lignans from *Phyllanthus amours* against galactosamine / lipo- polysaccharide - induced

hepatitis: An in-vivo and in-silico studies.; Curr  
Top Med Chem 1.