# **ORIGINAL CONTRIBUTION**

# Phytochemical and pharmacological activities of methanol extract of Artemisia vulgaris L. leaves

Nurpen Meitei Thangjam, Jasmina Taijong and Awadhesh Kumar<sup>\*</sup>

# Abstract

Background: Artemisia vulgaris commonly known as "mugwort" is a very important medicinal plant which is used widely for the treatment of various ailments traditionally. The present studies aimed to conduct preliminary phytochemical screening and evaluate antioxidants and analgesic activities of leaves extract.

**Results:** Phytochemical screening showed the presence of saponins, glycosides, flavanoids, protein, triterpenoids in leaves extract. In DPPH, percentage inhibition of the scavenging activity (68.06%) by the extract in comparison with ascorbic acid (93.53%) at 60 µg/ml while in reducing power assay, the result shown the reducing power increases with the increase of concentration as  $(0.13 \pm 0.02)$ ,  $(0.27 \pm 0.03)$ ,  $(0.42 \pm 0.03)$ ,  $(0.62 \pm 0.05)$ ,  $(0.79 \pm 0.02)$ ,  $(0.95 \pm 0.05)$ at concentration of 5, 10, 15, 30, 60 and 100 µg/ml respectively. The evaluation of analgesic activities revealed that at the dose of 200 and 400 mg/kg of the extract showed significantly inhibited the writhing response induced by acetic acid by 8.60% and 32.03% comparatively with indomethacin at a dose of 10 mg/kg b. wt. exhibited 56.87% inhibition. Whereas, in the tail immersion methods, at the dose of 400 mg/kg b. wt. was showed higher deflexion value as  $3.40 \pm 0.300$ , while 200 mg/kg b. wt. was resulted at  $2.90 \pm 0.200$  value in dose dependent manner at the maximum time of the studies (60 min). Comparatively, the reference drug indomethacin at dose 10 mg/kg b. wt. has shown the highest deflexion value  $(5.134 \pm 0.351)$ .

**Conclusion:** This finding concludes that the methanolic leaves extract has a potent antioxidants and analgesic activities and it could be attributed to the presence of flavonoids, triterpenoids, saponin, glycosides and proteins. A further study is required to find out the novel bioactive compound of Artemisia vulgaris L. which can lead more effective in various biological activities.

Keywords: Antioxidants, Analgesic, Artemisia vulgaris, DPPH, Phytochemicals, Reducing power

# Background

Aromatics and Medicinal plants are one of the main significant sources of phytochemical constituent called secondary metabolites, which have been used widely in most of the pharmaceutical companies [1]. The World Health Organization (WHO) conducted a survey and reported that the total world's populations of around 80% were depends on traditional knowledge of medicines,

\* Correspondence: kumarawadhesh9@gmail.com

especially the medicinal plants for supporting their healthcare [2, 3]. In the plant kingdom, the genus Artemisia consists of small herbs and shrubs, found in northern temperate regions of South Asia, North America and European countries [4]. Artemisia vulgaris which is commonly known as "mugwort" is a perennial shrubby aromatic plant found wildly throughout the hills of India and it has a very unique medicinal values and it was recorded in the ayurvedic traditional system of medicine; the presence of phytoconstituent (terpenoids and sesquiterpene lactones) hold strong aromatic properties and bitter tastes makes discourage to the





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Department of Horticulture Aromatic & Medicinal Plants, School of Earth Sciences & Natural Resource Management, Mizoram University, Aizawl, Mizoram 796009, India

herbivorous and acts as a shield or defence mechanism and acts as a good larvicide like kerosene with insecticidal property too. Likewise, the plants has acknowledged with the high contain of the important compounds like flavonoids and terpenoids which used as a flavoring agents as well as highly antioxidant properties with several health benefits [5-9].

Since ancient period, the genus Artemisia from the family Asteraceae having more than 400 known species were commonly used as an antimalarial agent and as traditional medicine around the world because of its unique medicinal properties, and it also included in traditional Anatolian medicine system [10, 11]. Whereas, it was used in Korean traditional medicine for the treatments of various ailments like convulsions, fever, malaria and later polyphenolic chemistry was also investigated [12]. The plant extracts of Artemisia have been found to be rich in phenolic and flavanoids contents and were used for the cure of several diseases with sufficient scientific proof supports for its medicinal property as antidiabetic, anti-epileptic, antihelmintic, antimalarial, anti-microbial, antinociceptive, anti-oxidants anti-hysteric, diuretic, digestive and stimulant [13–16]. Additionally, there was a report that the Artemisia vulagris plant contains the important phytoconstituent such as oils, phenols, saponins, sesquiterpenoids as well as tannins, and has been evaluated for analgesic and anti-inflammatory activities [17].

Analgesic drugs mechanism is to interrupt nociceptive pathways that transmit impulses to be interpreted as pain in the central nervous system [18]. The pain relief therapies were progressing with the application of synthetic compound with the development of the science, the researcher is looking to target the compound isolated mostly from the plant resources to avoid the adverse side effects [19]. Along with analgesic effect, there is a believe that most of the medicinal plants possess antioxidant properties which can able to inhibit the free radicals from our human body and to protect from various harmful diseases caused by the free radicals [20]. The scientific investigations on the selected herbs were necessary in order to accomplish their antioxidant and analgesic properties. An increasing number of research work and evaluation have been done to find antioxidative drugs, which not only extend the shelf life of food products but also contribute as radical scavengers in living organisms. Therefore, the present study was carried out on Artemisia vulgaris L. to contribute for the development of modern analgesic and antioxidants drugs from the naturally occurring secondary metabolites derived from plants.

# Methods

# Collection and extraction of plant material

The fresh aerial parts of *Artemisia vulgaris* were collected from Mizoram University Campus, Tanhril. These

were then cleaned and removed the stalks, branches and stems. Only the leaves were taken and shade dried, and further the dried leaves were chopped and grinded with the help of an electronic grinder. The powder was extracted with 99.8% methanol by cold maceration technique. The liquid extract obtained was filtered by using Whatman filter paper 1 and allowed to evaporate in a rotary evaporator so as to get the plant extract in the crude form. This was collected and stored properly in refrigerator at 4 °C for further used.

# Preliminary phytochemical screening

The detection of phytoconstituent present in the methanolic extract of *A. vulgaris* (MEAV) leaves was carried out by standard methods.

# Test for proteins (Xanthoproteic test)

In 2 ml of extract add 2 ml of concentrated  $HNO_3$  observation of orange colour indicates the presence of proteins [21].

# Test for carbohydrates (Benedict's test)

In 2 ml of extract add 2 ml of Benedict's reagent and boiled. Formation of orange red precipitate indicates the presence of carbohydrates [21].

# Test for alkaloids (Mayer's test)

In 2 ml of the extract, drops of Mayer's reagent were slowly added by the side of the test tube, formation of a white or creamy precipitate indicates the presence of al-kaloids [22].

# Test for Flavanoids (alkaline reagent test)

2 ml of 2% sodium hydroxide solution was added to 2 ml of the extract. Appearance of yellow colour precipitation indicates the presence of flavonoids [22].

# Test for Triterpenoids (Salkowski's test)

In 2 ml of extract add 1 ml of chloroform followed by a few drops of concentrated  $H_2SO_4$  on the side of the test tube and shaken well, formation of yellow colour at the lower layered indicates the presence of triterpenoids [22].

# Test for Saponins (frothing test)

2 ml of extract was diluted with 10 ml of distilled water in a test tube and shaken for 5 mins, observation of stable foam indicates the presence of saponins [23].

# Test for tannins (ferric chloride test)

2 ml of the extract was mixed with few drops of 10% ferric chloride solution, the change of colour into dark blue or green indicates the presence of gallic tannins and catechol tannins [23].

# Test for glycosides (Keller-Kilani test)

A mixture of 4 ml of glacial acetic acid and 1 drop of 2.0% FeCl<sub>3</sub>was added to 10 ml of the extract followed by the addition of 1 ml of concentrated H<sub>2</sub>SO<sub>4</sub>. Formation of brown ring between the layers indicates the presence of cardiac glycosides [24].

# In-vitro antioxidant assay

# DDPH free radical scavenging activity

Radical scavenging activity of methanolic extract of *A. vulgaris* (MEAV) leaves was determined by Calorimetric assay using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a source of free radical according to the method of Blois with a slight modification [25, 26]. Stock solutions of MEAV extract were prepared at a concentration of 1 mg/ml and for test sample; it was serially diluted to make the concentrations of 10, 20, 30, 40, 50 and 60  $\mu$ g/ml respectively.

0.1 mM DPPH solution was prepared by dissolving 3.94 mg in 100 ml of methanol and then 1 ml of this solution was mixed with 150 µl methanol, which was used as control. The above mentioned concentration of MEAV was taken and mixed with 1 ml DPPH solutions to each test tube. After 15 min of incubation, the absorbance of control and test sample was measured at 516 nm in UV- visible spectrophotometer and the compound ascorbic acid was used as a reference. The percentage inhibition and IC<sub>50</sub> were calculated. The free radical scavenging activity (% inhibition activity) was calculated using the given equation-

$$\% inhibition \ activity = \frac{Control \ absorbance - Sample \ absorbance}{Control \ absorbance} \times 100$$

# Reducing power by FeCl<sub>3</sub>

Reducing power of MEAV was measured by method of Oyaizu with slight modifications [27, 28]. Stock solutions of MEAV were prepared at a concentration of 1 mg/ml and the test sample concentrations as 5, 10, 15, 30, 60and 100  $\mu$ g/ml were made by serial dilution.

Here, 2 ml of above concentration were mixed thoroughly with 2.5 ml of phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide solution. These mixtures were warm up in the water bath at a constant temperature of 50 °C for 20 min and then allowed for cooling, added 2.5 ml of 10% trichloroacetic acid to the mixture and centrifuged for 10 min at 3000 rpm. After centrifugation, 2.5 ml of supernatant part was taken and mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride and incubated for 10 min. Then, absorbance at 700 nm was measured in spectrophotometer. Control value was also recorded with same procedure without the MEAV. The standard compound Ascorbic acid was used as a reference compound.

# Test animals

The Swiss albinos male mice weighing between 18 and 25 g obtained from the animal house of department of zoology, Mizoram University were used for testing of the present study. These animals were kept in a well-ventilated room at a maintained temperature of  $25 \pm 2 \,^{\circ}$ C with 12:12 h light/dark cycle in polypropylene cages containing sterile locally procured paddy husk as bedding. The animals were fed with commercially available food pellets and water ad libitum till the completion of the experiment. Animals were adapted in the laboratory environment 15 days earlier to initiation of studies.

All the investigation protocols followed in the experiment were revised by the Institutional animal ethical committee and followed accordingly under the guide-lines of CPCSEA (2003) [29]. The present research was approved by the Institutional Animal Ethical Committee, Mizoram University, India. (Approval No. MZU-IAEC/ 2018/10).

# Acute toxicity test

The acute toxicity study of the MEAV was carried out for the selection of dose according to the OECD guidelines [30]. The extract was administered orally at increasing doses of 500, 1000 and 2000 mg/kg and the observation was made for 48 h for its behavioral changes, any toxicity and mortality. No mortality and other acute toxicity effect were found. Therefore, the doses for the present investigation were selected at 200 and 400 mg/ kg body weight for analgesic evaluation [15, 31].

# In-vivo analgesic activity Acetic acid induced writhing

The In-vivo analgesic activity was carried out by acetic acid induced writhing method [32]. Swiss albino mice were categorized in 4 groups with 6 animals in each groups and proper marking was done individually as test group I and II, standard group and control group. The test groups were received with MEAV at a dose of 200 and 400 mg/kg b. wt. respectively. Similarly, indomethacin of dose 10 mg/kg b. wt. used as a standard drug and normal saline as control were given orally with the help of a gavage 30 min before the administered of 0.7% (v/v) acetic acid (0.01 ml/g) intraperitoneal injection to induce the writhing. The writhes count (number of abdominal constrictions) were observed and counted for 30 min for every single animal in each group.

Percentage of protection was expressed using formula:

Percentage of protection =  $(A - B)/A \times 100$ 

Where, A = No. of writhes in control, B = No. of writhes in test.

**Table 1** Phytochemical screening of the methanol extract of Artemisia vulgaris

SI. No.	Phytoconstitutents	Test results		
1	Proteins (Xanthoproteic test)	Present		
2	Carbohydrates (Benedict's test)	Absent		
3	Alkaloids (Mayer's test)	Absent		
4	Flavanoids	Present		
5	Triterpenoids	Present		
6	Saponins (frothing test)	Present		
7	Tannins	Absent		
8	Glycosides (Keller-Kilani test)	Present		

# Tail immersion methods

Tail immersion methods in mice were employed to detect the analgesic activity [33, 34]. The experiment was carried out on the basis of observation that the pain relieve drugs was capable of lasting the time taken for the reflex withdrawal of tail by mice when immersed in warm water at a temperature of 55 °C.

According to divided groups, the standard group received the drug indomethacin at the dose 10 mg/kg b. wt., the test group treated with MEAV at the dose of 200 and 400 mg/kg b. wt. respectively and normal saline in control were administered orally. After the administration of the test and standard drug, the lower part of the tail was marked up to1-2 cm and dipped into the water bath at 55 °C temperature. The reaction time of the deflexion of tail by the mice were observed and recorded at 0, 10, 15, 30 and 60 min respectively.

# Statistical analysis

The data obtained in in vitro antioxidant activity were expressed as mean ± standard deviation of mean (SD) and the statistical significance of in-vivo analgesic activity was analyzed by one way analysis of variance (ANOVA) followed by post hoc Duncan multiple comparison tests.

# Results

# Phytochemical screening

The phytochemical screening test of the methanol extract of *A. vulgaris* (MEAV) leaves was presented in Table 1. The Test results showed the presence of saponins, glycosides, flavanoids, protein, triterpenoids.

# In-vitro antioxidants activity

Antioxidant properties of the MEAV was measured using DPPH radical scavenging and reducing power properties. The antioxidant ability of the leaves extract was analysed based on their ability to scavenging free radicals. In present study, the MEAV showed high scavenge activity of DPPH radical, which may attribute to its hydrogen donating ability. The antioxidant activity of both MEAV extract and standard (ascorbic acid) comparatively tested at different concentrations (10, 20, 30, 40, 50, 60 µg/ml). The DPPH radical scavenging properties were found to be concentration dependent shown in Fig. 1. The percentage inhibition of MEAV extract was found in the range of 12.23-68.06% which was lower than ascorbic acid (14.37-93.53%). This means the inhibition percentage was also increase with increase in concentration of MEAV. The IC<sub>50</sub> value obtained for ascorbic acid and Artemisia vulgaris is 2.9 and 4.3 respectively (Table 2).

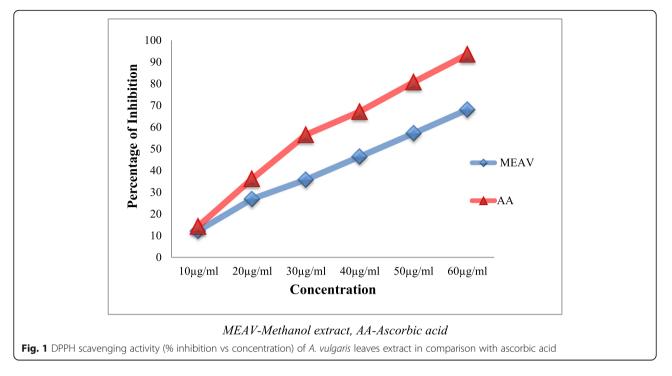
In order to confirm the antioxidant activities, the plant extract was further analyzed by reducing power assay. Here, it was observed that the absorbance at 700 nm was increased with the increase in the concentration of the MEAV which was mainly due to the conversion of ferric to ferrous ions shown in Fig. 2. The absorbance of the MEAV was observed as  $0.13 \pm 0.02$ ,  $0.27 \pm 0.03$ ,  $0.42 \pm 0.03$ ,  $0.62 \pm 0.05$ ,  $0.79 \pm 0.02$  and  $0.95 \pm 0.05$  in comparison with the standard (ascorbic acid) with  $0.18 \pm 0.03$ ,  $0.35 \pm 0.04$ ,  $0.53 \pm 0.05$ ,  $0.71 \pm 0.01$ ,  $0.85 \pm 0.05$  and  $1.04 \pm 0.05$  at concentration of 5, 10, 15, 30, 60 and 100 µg/mL respectively (Table 3).

# In-vivo analgesic activity

The peripheral analgesic effect of MEAV was evaluated by acetic acid induced writhing test in mice. The

Table 2 Percentage inhibition of methanol extract of A. vulgaris leaves and standard (ascorbic acid)

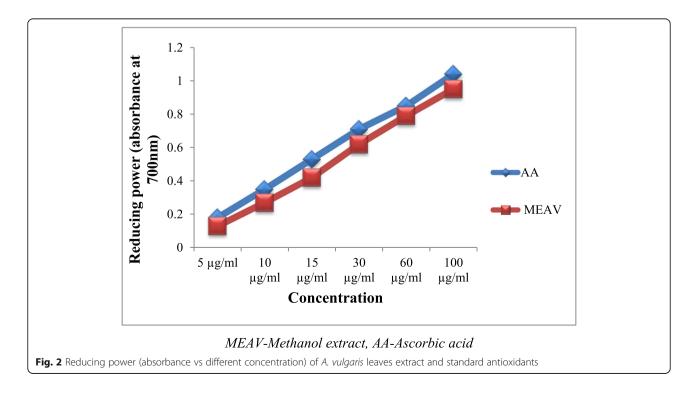
Concentration	Percentage inhibition (MEAV)	Percentage inhibition (AA)		
10 µg/ml	12.35	14.37		
20 µg/ml 26.94		36.23		
30 µg/ml	35.82	56.50		
40 µg/ml	46.46	67.17		
50 μg/ml	57.16	80.75		
60 µg/ml 68.06		93.53		
IC <sub>50</sub> µg/ml	4.3	2.9		



analgesic effect was tested at selected dose of MEAV at 200 and 400 mg/kg b. wt. The given doses of MEAV were showed an inhibition effect on the writhing reaction induced by acetic acid in comparison with the standard drug indomethacin. The doses of MEAV at 200 and 400 mg/kg b.wt. has significantly inhibited the writhing response induced by acetic acid at 8.60% and 32.03%

respectively whereas indomethacin at a dose of 10 mg/kg b. wt. exhibited 56.87% inhibition (Table 4).

This study was accordance with test of tail immersion methods. Here, the control group showed faster tail flicks which increases at different intervals of time. The MEAV exhibited potent analgesic activity at the dose levels of 200 and 400 mg/kg b. wt. The analgesic activity of higher dose



Concentration	Absorbance of AA (Mean ± SD)	Absorbance of MEAV (Mean ± SD)		
5 µg/ml	0.18 ± 0.03	0.13 ± 0.02		
10 µg/ml	$0.35 \pm 0.04$	$0.27 \pm 0.03$		
15 µg/ml	0.53 ± 0.05	$0.42 \pm 0.03$		
30 µg/ml	0.71 ± 0.01	$0.62 \pm 0.05$		
60 µg/ml	$0.85 \pm 0.05$	0.79 ± 0.02		
100 µg/ml	$1.04 \pm 0.05$	$0.95 \pm 0.05$		

Table 3 Absorbance at different concentration of methanol extract of *A. vulgaris* leaves and standard ascorbic acid in reducing power

(400 mg/kg b. wt.) was moderate at the maximum time of the studies (60 min) at a value of  $3.40 \pm 0.300$ in comparison with the activity of the standard drug indomethacin (10 mg/kg body wt.) at  $5.134 \pm 0.351$ .Whereas, the lower dose at200mg/kg b. wt. was resulted least activity and showed the value  $2.90 \pm 0.200$  (Table 5). Therefore, the intensity and the period of analgesic effect of MEAV were considered as dose dependent.

# Discussion

In this study, the presence of saponins, glycosides, flavanoids, protein, triterpenoids were reported in the phytochemicals screening. The phytochemicals in most of the plants extract had diverse biological properties, such as analgesic, anti-carcinogenic, anti-inflammatory and antioxidant activities and their presence also ensured the medicinal potential and their therapeutic activities [35]. The previous literature also supported that the analysis of Artemisia species showed the presence of coumarin, saponins, glycosides, flavanoids, proteins, and triterpenoids [6]. There was also report that terpenoids is one of the most commonly found metabolites in most of the genus of Artemisia [9]. A similar phytochemical study in the aerial parts of Artemisia vulgaris had shown the presence of certain secondary metabolites like amino acid, carbohydrate, flavonoids, phenolic compounds, phytosterol, proteins, saponins and tannin [36].

Subsequently, *A. vulgaris* leaves extract has shown a reasonable potential of antioxidants activity with the percentage inhibition of 68.06% at  $60 \mu g/ml$  concentration and ferric reducing power of  $0.95 \pm 0.05$  at  $100 \mu g/ml$ 

ml. The resulted antioxidant properties might be considered due to the high contents of flavonoid compound which act as a scavenging agents for different oxidizing species such as hydroxyl radical and super oxide anion (O2-•) [1]. Iqbal et al. reported that the methanol extract of A. annua leaves showed the higher scavenging activities and reducing power comparatively with the other solvents. His finding further resulted that the constituent having antioxidant properties were extracted efficiently with the polar solvents in the extract of Artemisia species [7]. The  $IC_{50}$  value interprets the results of the DPPH assay, which defies the minimum concentration of methanol extract, requires to scavenging 50% of free radicals [37]. The scavenging properties may be due to chelation and transfer of electron or the ability of hydroxyl groups to donate the hydrogen to free radical [38, 39]; similarly the present finding was showed resemblance with these previous studies. Hence it can say that the tested extract have ability to transform the ferric ion (Fe3+) to ferrous ion (Fe2+) as reducing power with the increase of concentration of the extract.

In addition to in-vivo study, the methanol extract of *A. vulgaris* showed a noteworthy analgesic effect in the acetic acid-induced writhing test and tail immersion activity. And it was known that induced acetic acid to observe the abdominal constriction and tail immersion to see the deflexion of the tail were the suitable technique to assess the centrally acting analgesics activity [40]. Since, the pain that causes by administering of acetic acid is due to the endogenous substances such as serotonin histamine,

Table 4 Effect of methanol extract of A. vulgaris leaves extracts on acetic acid-induced writhing behavior in mice

Groups	Treatment	Dose (mg/kg b.wt.)	No.of Writhes in 30 mins (mean $\pm$ sem)	Inhibition %	
Control	Normal Saline		42.67 ± 3.756		
Std. Drugs	Indomethacin	10	18.33 ± 0.882*	56.87	
Test 1	MEAV	200	39.00 ± 1.155	8.60	
Test 2	MEAV	400	29.00 ± 1.155*	32.03	
	One way ANOVA	F value	27.213		
		P value	< 0.0001		

Each value is the mean  $\pm$  SEM for 6 mice, \* $P \le 0.05$ , compared with control. Data were analyzed by using One-way ANOVA followed by Duncan's multiple comparison tests

Table 5 Protective effect of methanol extract of A. vulgaris leaves extract on tail withdrawal reflexes induc	ed by tail immersion
method in mice	

Groups	Treatment	Dose (mg/ kg b. wt)	Reaction time in seconds at time (minutes) (mean $\pm$ sem)				
			basal time	10	15	30	60
Control	Normal saline		1.10 ± 0.577	$1.40 \pm 0.115$	$1.60 \pm 0.264$	1.834 ± 0.230	2.734 ± 0.757
Std. Drugs	Indomethacin	10	1.47 ± 0.272	$2.534 \pm 145^{*}$	$3.334 \pm 0.230^{*}$	$4.10 \pm 0.200^{*}$	$5.134 \pm 0.351^{*}$
Test 1	MEAV	200	$1.067 \pm 0.881$	$1.50 \pm 0.300$	1.734 ± 0.152	$2.367 \pm 0.208^{*}$	$2.90 \pm 0.200^{*}$
Test 2	MEAV	400	$1.234 \pm 0.120$	$2.067 \pm 0.208^{*}$	2.834 ± 0.251 <sup>*</sup>	$3.034 \pm 0.152^{*}$	$3.40 \pm 0.300^{*}$
One way ANOVA		F value	1.319	14.136	40.778	71.556	17.509
		P value	0.334	< 0.001	< 0.0001	< 0.0001	< 0.001

Each value was the mean  $\pm$  SEM for 6mice, \*P  $\leq$  0.05, compared with control. Data were analyzed by using One-way ANOVA followed by Duncan's multiple comparison tests

prostaglandins (PGs), bradykinins and substance P at the nerve ending [41]. Thus, the analgesic activities of the extract may be presence of saponins and that too pharmacological history of these compounds in analgesic and antispasmodic activity [42].

Moreover, the finding of the present study had shown 8.60% and 32.03% at 200 and 400 mg/kg b.wt. in acetic induced writhing effect and in tail withdrawal reflexes time with  $3.40 \pm 0.300$  and  $2.90 \pm 0.200$  at the same dose. Tatiya et al. reported that ethyl acetate extract of B. retusa inhibit the writhing effect induced by the acetic acid at dose dependent with strong inhibition of 65.62% at 400 mg/kg and suggested that the analgesic effect of the extract was due to the inhibition of the synthesis of arachidonic acid metabolite [43]. Recently, Bhattacharjee et al. evaluated the analgesic activity of methanolic extract of Lathyrus sativus seeds at the dose 200 and 300 mg/kg b. wt. and found a very significant inhibition (87.09% and 80.65%) of writhes induced by acetic acid in mice in comparison to indomethacin (70.97%) [44]. Consequently, a similar kind of the finding was made by the Islam et al. with extract of Campsis radicans, where he took the doses of 200 and 400 mg/kg b.wt., and proved high analgesic activity [45]. Overall, the finding of present study was also fully supported by these above previous researches.

# Conclusion

According to the present finding, the studies conclude that methanol extract of *Artemisia vulgaris* has good antioxidant property and it could be attributed to the presence of flavonoids, triterpenoids, saponin, glycosides and proteins. The constituents of this plant can be used as an accessible source of natural antioxidants in cosmetics, food supplements as well as in pharmaceuticals. The estimated results also provide useful information for using *A. vulguris* as a source of analgesic drugs. A further study is required to find out the novel bioactive compound of *A. vulgaris* which can lead more effective in various biological activities.

# Abbreviations

g: gram; CPCSEA: The Committee for the Purpose of Control and Supervision of Experiments on Animals; FeCl3: Ferric chloride; H<sub>2</sub>SO<sub>4</sub>: Sulphuric acid; HNO<sub>3</sub>: Nitric acid; DPPH: 2,2-diphenyl-1-picrylhydrazyl; MEAV: methanolic extract of *Artemisia vulgaris*; OECD: Organization for Economic Co-operation and Development; ANOVA: Analysis of variance

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# Authors' contributions

NMT, JT and AK\* carried out the collection of plant. NMT and JT conducted the extraction process and the research work. NMT wrote the manuscript. NMT and AK\* design the study, statistical analysis and data interpretation of data. NMT and AK\* revised the manuscript. All authors read and approved the final manuscript.

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# Availability of data and materials

Not applicable.

# Ethics approval and consent to participate

This study was approved by ethical research committee of Mizoram University, Aizawl, Mizoram, India. (Approval No. MZU-IAEC/2018/10).

#### Consent for publication

Not applicable.

# **Competing interests**

The authors declare that they have no competing interests.

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