

Research Article

# Preliminary Phytochemical Analysis, Anthelmintic, Insecticidal and Protective Effect of *Dicliptera bupleuroides* Nees in Ethanol-induced Gastric Mucosal Damage Rats

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Abstract: Dicliptera bupleuroides Nees.is traditionally used as a general tonic, as a diuretic, in skin diseases, in snake bite, in fever, and in stomach troubles but limited literature found with the scientific ground. The objective of the present research is to prove the medicinal use of Dicliptera bupleuroides in anthelmintic, insecticidal, and antiulcer activity. Reference drug was albendazole (20 mg/ml) in anthelmintic, permethrin (239.5 µg/cm<sup>2</sup>) in insecticidal, ranitidine (50 mg/kg), omeprazole (20 mg/kg) and sucralfate (100 mg/kg) were used in antiulcer activity respectively. Time of paralysis and death was calculated in anthelmintic activity while the rate of % age mortality was calculated in insecticidal activity. Parameters such as mean ulcer indices and percentage ulcer inhibition, gastric volume, pH, mucous and protein contents were assessed in the ethanol-induced ulcer model. In the anthelmintic study ethyl acetate and n- butanol fraction showed the more significant results in comparison to reference drug (53/54 min paralysis and 92/108 min death time). No extracts showed insecticidal activity. N-hexane extract showed significantly (p < 0.05) reduced gastric lesion by 54.9% in rats at 50 mg/kg when compared to ranitidine at 54.2%, omegrazole at 69.5%, and sucralfate 53.6% respectively. Gastric volume, as well as total acidity, decreased when compared with positive control  $3.9 \pm 0.15$ ,  $98.1 \pm 3.8$  and chloroform fraction was  $1.8 \pm 0.2$ ,  $11.3 \pm 4.6$ . Gastric volume, pH, and total acidity of chloroform extract were  $1.8 \pm 0.2$ ,  $6.8 \pm 0.9$ , and  $11.3 \pm 4.6$ . Mucous and protein content of chloroform extract versus standard was  $505.1 \pm 16.9$ ,  $37.8 \pm 4.4439.0 \pm 11.9$ ,  $454.3 \pm 14.1$ , and  $432.6 \pm 14.8$  respectively. The above findings concluded that Dicliptera bupleuroides have medicinal importance in various pharmacological aspects.

Keywords: Anthelmintic, Anti-ulcer, Dicliptera bupleuroides, Gastric volume, Insecticidal, paralysis, % age mortality.

# 1. INTRODUCTION

*Dicliptera bupleuroides* Nees. of the family Acanthaceae is a perennial herb. It is found in the planes of Pakistan and Afghanistan. It is a flowering herb, length up to 90 cm, branched with hairy twigs, leaves ovate or acuminate, and with linear bracts [1]. It is used in traditional medicines for applying on the wound of snakebite, in fever, in stomach troubles, and also used in bone fracture [2]. The common name in Urdu is kaali boti [3]. *Dicliptera bupleuroides* possessed antioxidant, hepatoprotective, antimicrobial, and other biological activities. It contained phenols, flavonoids, ascorbic acid, lipids, starch, glycosides, and many other compounds [4 - 6]. Infection caused by helminths is the most common and more persistent form of infection. It is a degenerative disorder infecting a large proportion of the population around all over the world. Other contributing factors are malnutrition, pneumonia, eosinophilia, and anemia which pose a great threat to human health particularly in developing countries [7]. These helminths parasites and their larvae are passing through the human intestinal tract by eating contaminated food and also subsist into their body tissues [8]. Most of the helminths diseases are chronic, devastating in nature; they probably cause greater social and

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economic deprivation among different groups of living organisms, the ratio of morbidity may become also high. To control the overspread of worms by improving our management system coupled with the chemical control of helminths [9, 10]. In the treatment of helminths infection, the development of resistance is a foremost problem against conventional anthelmintics [11]. However, it is important to choose an alternative treatment against intestinal nematodes, which helps us to acquire knowledge of medicinal plants that have anthelmintic activity.

Several infections such as malaria, dengue fever, yellow fever, and many other infections can be transmitted from insects to human beings. These infections are symptoms of diseases. From the epidemiological point of view, dengue fever is the most serious disease based on its morbidity and mortality rates. A virus acquires about 10,000 deaths each year all around the world, in approximately 60 million people who are infected by viral infection [12, 13]. More focused on the prevention strategies to control insects' larvae because of lack of vaccines against a particular disease. So now a day's most common approach is the development of synthetic insecticides by using medicinal plants which has insecticidal potential [14]. Gastrointestinal infection is the major complication worldwide widely, peptic ulcer is most common among them remain the cause of significant morbidity and major burden for health care organization [15]. Even though various famous antiulcer drugs available in the market, having various toxicities and adverse effects. Thus there is a need to focus on searching for new alternative drugs [16]. The ulcer is a denegation of the digestive tract mucous membrane which is inflamed due to external factors [17]. There are various factors which are responsible for peptic ulcer [18]. Symptoms of ulcer are a pain in the stomach with a burning sensation, episodes of distress, pain after food intake or empty stomach, other common symptoms are vomiting, intolerance to fatty diet, and loss of appetite [19].

Physiologically, reactive oxygen species are the usual cause of various illnesses i.e peptic ulcer [20]. The safest treatment is the use of natural antioxidants which are responsible to react with free oxygen species to reduce the consequences of illness. *D. bupleuroides* has an antioxidant activity which is proved by literature [6] so it is used for gastroprotective activity has not been studied yet. The aim of the present study to evaluate the anthelmintic, insecticidal and antiulcer activity of *D. bupleuroides*.

#### 2. MATERIAL AND METHODS

### **2.1 Plant Material Collection and Extraction**

The plant was collected from Bhimber (Shamani), Kotli, Azad Kashmir and got authenticated by Dr. Uzma Hanif, Department of Botany, Government College University (GCU) Lahore, Pakistan. A specimen of the plant was deposited in the herbarium of GCU under voucher No: GC. Herb.Bot.3402. The plant was dried under shade, powdered whole herb. This powdered herb was dipped in commercial methanol for 7 days, filtered, and evaporated by using a rotary evaporator. After extraction fractionation was done by using different solvents according to polarity. The active fraction will be separated by using small column chromatography, preparative TLC [21].

#### 2.2 Helminths, Insects, and Animals

Earthworms collected from crops field of Sialkot, test insects (*Tribolium castaneum*, *Sitophilus oryzae*, and *Rhyzopertha dominica*), and Wistar rats  $(250 \pm 30g)$  were used. Use of animals following the rules set by the Institutional Ethical Committee for Animal Care and Experimentation under No. 416, College of Pharmacy, University of the Punjab, Lahore, Pakistan provided by Zoological Society of University of the Punjab [26].

### 2.3 Phytochemical Analysis

The whole herb was carried out according to the standard procedures for phytochemical analysis [22].

# 2.4 Total Phenolic Content

Estimation of total polyphenolic contents in plant samples was done according to the method described by Liaudanskas with little modifications [23]. Gallic acid was used as a standard.

#### 2.5 Total Flavonoid Content

Flavonoid determination was by the method

reported by Ejikeme *et al.* and Boham [24, 25]. The percentage of flavonoid was calculated.

# 2.6 Acute Toxicity

Preliminary experiments were carried out in mice (n=6). Methanolic extract of *D. bupleuroides* Nees. was administered in doses (500, 1000, and 2000 mg/kg/p.o) to find out toxicity which causes zero and 100 % mortality of animals.

### 2.7 Anthelmintic Activity

The anthelmintic activity was carried out according to the method of Ajaiyeoba [27]. The experimental procedure carried on adult Indian earthworm which is anatomically and physiologically resemble human intestinal worms. These earthworms were divided into groups of six and put into each petri dish containing three different concentrations (25, 75, 100 mg/ml) of all fractions of the extract of whole herb *D. bupleuroides* Nees. Albendazole (20 mg/ml) is used as standard drug. Then note the time of paralysis or any physiological change and mortality.

### 2.8 Insecticidal Activity

For insecticidal activity impregnated filter paper method was followed [28]. Filter paper placed in a petri dish, sample loaded over filter paper, left for 24 hrs for complete evaporation, put 10 healthy and active insects (*Tribolium castaneum*, *Sitophilus oryzae*, and *Rhyzopertha dominica*) of same size and age of each species. Incubate them at 27C for 24 hrs with 50% relative humidity in the growth chamber. Count the number of survival of each species and calculate the percentage of morality. Standard insecticide (permethrin) at a concentration of 239.5 µg/cm2 was used.

Percentage Mortality =100 - (No of insects alive in test) / No of insects alive in control)×100

# 2.9 Evaluation of Antiulcer Activity

### 2.9.1 Ethanol-induced Gastric Ulcer Model

Wistar albino rats were divided into 10 groups randomly (n=6), followed the method of Mizui [29]. After 24 hours of fasting, animals were

allowed free access to water for 2 hr before the experimental procedure [30]. (Group I served as normal (-ve control) received 5 ml/kg of distilled water, Group II (+ve control) was treated with absolute ethanol 1ml/animal. Groups III treated with reference drugs ranitidine; 50 mg/kg, omeprazole 20 mg/kg, and sucralfate 100 mg/kg followed by ethanol 1ml. Group IV (Extract treated) received methanolic extract; 500 mg/kg, followed by 1 ml ethanol, aqueous extract, n-hexane, chloroform, ethyl acetate, n-butanol 200 mg/kg, followed by 1 ml ethanol respectively [31 - 33].

## 2.9.2 Measurement of ulcer index

Stomachs were examined for a hemorrhagic lesion in the mucosal lining of the stomach. To remove blood contaminants, the stomach along the greater curvature was rinsed with cold saline. For determining ulcer index, all the stomach lesions were measured with the help of a transparent millimeter scale and magnifying glass [34]. Percentage inhibition was calculated by applying the formula:

% Gastro protection/Inhibition = (UIC – UIT)/UIC X 100

Where UIC is Ulcer Index in the control and UIT is ulcer index in the test rats [35].

#### 2.9.3 Determination of Total Acidity

Gastric acidity was determined by the method of shay [35]. For this gastric content was collected from the stomach into graduated tubes. These tubes were centrifuged for 15 min at 2000 rpm, the supernatant was used for gastric volume, pH, and total acidity by titrating with 0.01N NaOH.

# 2.9.4 Estimation of Gastric Mucus and Protein Content

Glandular portion of stomach weighed, dipped immediately into 0.1 % alcian blue solution and wash with 0.25 M sucrose solution after 15, 45 min interval respectively to remove the excess dye. This dye made a complex with gastric walls. Then this blue extract was shaken with diethyl ether, resulting emulsion centrifuged at 3000 rpm, and recorded its absorption at 580 nm. The alcian blue quantity extracted was determined per gram of glandular

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mucous from the standard curve of alcian blue [36]. The protein content of the stomach was estimated with the standard curve of bovine albumin solution (BSA standard curve) according to the Modified Lowery method [37].

# 2.9.5 Histological analysis of gastric ulcer

A small portion of the stomach from each group was fixed in preservative for histopathological studies. Thin sections of 5  $\mu$  were cut by using a microtome and stained with eosin and hematoxylin. These sections were studied for degenerative features [38].

# 2.10 Statistical analysis

The results were represented as Mean  $\pm$  SD. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Dunnett's t-test for multiple comparisons. The significance of the difference was accepted at p < 0.05. Graphical representation was made by using graph prism pad 6.

# 3. RESULTS

### 3.1 Phytochemical Analysis

Results are shown in Tables 1 and 2. Crude extract indicated the presence of all major classes of compounds carbohydrates, alkaloids, glycosides, tannins, flavonoids, triterpenoids, etc. all other fractions have different concentrations of polyphenols and flavonoids compared with Gallic acid and quercetin respectively. Standard curves are given in Figure 1 (A) and (B).

### **3.2** Anthelmintic Activity

Indian earthworms were divided into 22 groups, each extract divided into three concentrations 25, 75, and 100mg/ml, n=6. The time of paralysis and death in the control group is  $37.4 \pm 3.43$ ,  $55.2 \pm$ 6.37 respectively at 100 mg. While ethyl acetate and n- butanol fractions showed results comparable to the control group. These results are given in Table 3, paralysis time and time of death are given.

# 3.3 Insecticidal Activity

For insecticidal three kinds of insects (*Tribolium castaneum*, *Sitophilus oryzae*, and *Rhyzopertha dominica*) at 200 mg/3 ml, permethrin is standard insecticidal (conc. 239.5  $\mu$ g/cm<sup>2</sup>). The % age mortality is calculated. Results are given in Table 4.

### 3.4 Ethanol-induced Ulcer Model

Wistar albino rats were used for ethanol-induced ulcer model, n-hexane fraction showed maximum % age inhibition of ulcer after it chloroform showed significant inhibition. As result shown in Table 5. The graphical representation is given in figure 4 for all parameters. Gastric volume increased in the ethanol group while the decrease in standard drugs treated groups as well as extract treated groups. PH

Table 1. Preliminary phytochemical analysis of Dicliptera bupleuroides Nees.

515	J	
Phytochemical group	Test	Methanolic extract
Terpenoids	Salkowaski test	+ ++++
	Liebermann's test	+ +++
Tannins	Ferric Chloride test	+++
	Bromine water test	+ ++
Glycosides	Keller killani test	++
	Legal's test	+
Flavonoids	Alkaline reagent test	+ ++
	Lead acetate test	+ ++
A 11 - 1 - 1	Mayer 'test	+ +
	Wagner 'test	+ +
Alkalolds	Hager's test	+ +
	Dragendroff 's test	+ +
Proteins	Millon's test	++
	Ninhydrin test	+ +
Carbohydrates	Molisch 's test	+ ++
	Benedicts's test	+ + +
Saponins	Foam test	+
Fats and Fixed oil	Spot test	++

Parameters	%Total po	lyphenols	nols %Total flavonoids		
	Mean	SD	Mean	SD	
Methanol	0.947	$\pm 0.019$	0.074	$\pm 0.006$	
n-hexane	0.564	$\pm 0.021$	0.055	$\pm 0.003$	
Chloroform	0.906	$\pm 0.015$	0.171	$\pm 0.003$	
Ethyl acetate	0.886	$\pm 0.011$	0.185	$\pm 0.006$	
n-butanol	0.779	$\pm 0.004$	0.185	$\pm 0.004$	
Aqueous	0.55	$\pm 0.009$	0.046	$\pm 0.005$	

**Table 2.** Determination of Polyphenols and Flavonoids content.



Fig. 1. (A) Graph of estimation of total phenolic content. (B) Graph of estimation of total phenolic content.

Treatment	Concentration (mg/ml)	Paralysis Time (min)	Death time (min)
+ve control	-	-	-
Standard (Albendazole)	25	$48.2\pm3.96$	$80\pm7.90$
	75	$45.4\pm3.64$	$70.2 \pm 5.76$
	100	$37.4 \pm 3.43$	$55.2\pm6.37$
Methanolic Extract	25	$65.8\pm2.58$	$133\pm3.46$
	75	$62 \pm 2.12$	$128.4 \pm 5.94$
	100	$58 \pm 4.30$	$112.6 \pm 7.98$
Hexane fraction	25	$186\pm8.39$	$514.4 \pm 11.26$
	75	$175 \pm 4.12$	$493.6 \pm 11.61$
	100	$167.8\pm1.92$	$473\pm8.36$
Ethyl acetate fraction	25	$65 \pm 2.23$	$142.6\pm7.98$
	75	$60.4\pm2.07$	$122.6\pm7.98$
	100	$53 \pm 2.91$	$92.4\pm10.01$
Chloroform fraction	25	$84 \pm 3.08$	$193.2\pm8.34$
	75	$75 \pm 4.12$	$184.8\pm4.43$
	100	$66.2 \pm 5.71$	$173.6\pm3.04$
n-Butanol fraction	25	$62.8\pm3.96$	$137.6\pm5.59$
	75	$59.8\pm2.86$	$126.6\pm5.45$
	100	$54.6 \pm 4.21$	$108.8\pm7.39$
Aquous fraction	25	$238.6\pm12.03$	$676\pm9.61$
	75	$223.6\pm8.50$	$666.8\pm7.12$
	100	$194.8 \pm 10.13$	$617 \pm 12.04$

Table 3. Anthelminthic activity of Dicliptera bupleuroides Nees.

Results are shown as Mean $\pm$  SEM. Significant at P < 0.05, P < 0.01, P < 0.001, ns=not significant

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	% Mortality (Mean ± SD)			
Extract/Fraction	Tribolium castaneum	Sitophylus oryzae	Rhzopertha dominica	
-ve Control	0	0	0	
+ve Control (Permethrin) (20 mg / 3 ml)	100	100	100	
Methanol (200 mg / 3 ml)	0	0	0	
n-Hexane (100 mg / 3 ml)	0	0	0	
Ethyl acetate (100 mg / 3 ml)	0	0	0	
Chloroform (100 mg / 3 ml)	0	0	0	
Butanol (100 mg / 3 ml)	10	0	0	
Aqueous (100 mg / 3 ml)	0	0	0	

Table 4. Insecticidal activity of different fractions of Dicliptera bupleuroides Nees.

Table 5. Effect of different fractions of Dicliptera on the ethanol-induced gastric ulcer.

Group name	Ulcer no.	Ulcer score	Incidence of ulcer (%)	Ulcer index	Inhibition of ulcer (%)
Normal (10 ml/kg p.o)	$0.00 \pm 0.00^{\textit{***}}$	$0.00 \pm 0.00$ ***	0	0	0
Ethanol (10 ml/kg p.o)	$7.83\pm0.60$	$5.75 \pm 0.57$	100	11.36	0
Ranitidine (50 mg/kg p.o)	$1.00 \pm 0.52^{***}$	$1.00\pm0.47^{\boldsymbol{\ast\ast\ast\ast}}$	50	5.2	54.22
Omeprazole (20 mg/kg p.o)	$0.67 \pm 0.42^{***}$	$0.58\pm0.37^{\boldsymbol{\ast\ast\ast\ast}}$	33.33	3.46	69.56
Sucralfate (100 mg/kg p.o)	$1.17\pm0.54^{\boldsymbol{\ast\ast\ast\ast}}$	$1.50 \pm 0.67 \textit{***}$	50	5.27	53.63
Methanol (500 mg/kg p.o)	$1.33 \pm 0.42^{***}$	$0.83 \pm 0.28^{***}$	83.33	8.55	24.73
Aqueous (200 mg/kg p.o)	$1.17 \pm 0.40^{***}$	$0.83 \pm 0.28 \textit{***}$	83.33	8.53	24.88
Hexane (200 mg/kg p.o)	$0.50 \pm 0.22^{***}$	$0.67 \pm 0.33 \textit{***}$	50	5.12	54.95
Chloroform (200 mg/kg p.o)	$1.50 \pm 0.50^{***}$	$1.25 \pm 0.48^{***}$	60.67	6.94	38.88
Ethyl acetate (200 mg/kg p.o)	$1.83 \pm 0.31^{***}$	$1.83\pm0.42^{\boldsymbol{\ast\ast\ast\ast}}$	100	10.37	8.73
Butanol (200 mg/kg p.o)	$1.67 \pm 0.31^{***}$	$0.83 \pm 0.21 \textit{***}$	83.33	8.53	24.88

Results are shown as Mean± SEM. Significant at P < 0.05, P < 0.01, P < 0.001, ns=not significant

decreased in the ethanol-treated group, increased in all other groups.

Total acidity increased in the ethanol-treated group, all groups showed a decrease in acidity, given in Table 6. Mucous content decreased and protein content increased in the ethanol-treated group and vice versa in all other treated groups, given in Table 6.

### 3.5 Macroscopic Examination

With the help of magnifying glass, all stomachs

were examined for measurement of ulcer index, streak, spot, hemorrhage, and lesions in all treated groups, results given in Figure 2.

### 3.6 Histopathological Studies

As shown in Figure 3, the stomach in the group received chloroform and butanol similar to the group treated with standard drugs ranitidine, omeprazole, and sucralfate in Figure 2. The results indicate that the dose of chloroform has maximum protection of gastric mucosa of the stomach in the ethanol-induced model as compared to all other

Group name	Gastric volume (ml)	рН	Total acidity (mEq/L)
Normal (10 ml/kg p.o)	$1.35 \pm 0.06$ ***	$4.00 \pm 0.11*$	28.33 ± 2.03***
Ethanol (10 ml/kg p.o)	$3.95\pm0.15$	$2.45\pm0.17$	$98.17\pm3.82$
Ranitidine (50 mg/kg p.o)	$2.13 \pm 0.23$ ***	$5.77 \pm 0.39$ ***	38.17 ± 2.95***
Omeprazole (20 mg/kg p.o)	$1.80 \pm 0.29$ ***	$5.96 \pm 0.49$ ***	$32.33 \pm 2.80$ ***
Sucralfate (100 mg/kg p.o)	$1.77 \pm 0.20$ ***	$5.45 \pm 0.33$ ***	$39.17 \pm 3.44 ***$
Methanol (500 mg/kg p.o)	$1.72 \pm 0.22$ ***	$4.90 \pm 0.25$ ***	53.67 ± 5.13***
Aqueous (200 mg/kg p.o)	$2.02 \pm 0.19$ ***	$4.60 \pm 0.27$ **	$44.42 \pm 4.80$ ***
Hexane (200 mg/kg p.o)	$2.28 \pm 0.25$ ***	$4.68 \pm 0.27$ ***	$46.42 \pm 6.57$ ***
Chloroform (200 mg/kg p.o)	$1.85 \pm 0.21$ ***	$6.88 \pm 0.91 \textit{***}$	$11.33 \pm 4.62$ ***
Ethyl acetate (200 mg/kg p.o)	$2.52 \pm 0.24$ ***	$5.80 \pm 0.41$ ***	$40.42 \pm 4.24 \textit{***}$
Butanol (200mg/kg p.o)	$2.03 \pm 0.31$ ***	$6.65 \pm 0.57$ ***	$08.83 \pm 3.77$ ***

Table 6. Gastric juice parameters in ethanol induced acute gastric ulcer.

Results are shown as Mean $\pm$  SEM. Significant at P < 0.05, P < 0.01, P < 0.001, ns=not significant



**Fig. 2.** Macroscopic examination of stomachs in different groups e.g., A) Normal, B) Ethanol, C) Ranitidine, D) Omeprazole, E) Sucralfate, F) Methanolic, G) Aqueous, H) n-Hexane, I) Chloroform, J) Ethyl Acetate, K) Butanol.



**Fig.3.** Sections stained with hematoxylin and cosin (H&E) displaying the regenerated glandular epithelium width in stomachs of rats treated with ranitidine, omeprazole, and sucralfate, methanolic, aqueous, n-hexane, chloroform, ethyl acetate, and butanol extract of *Dicliptera bupleuroides* in ethanol-induced ulcer model.

fractions, these results are comparable to standard treated groups

standard gallic acid.

## 4. **DISCUSSION**

Nature is the ultimate source of human health, we can say that human being entirely dependent upon natural resources to fulfill their requirements [39]. For this purpose, the demand for medicinal plants and phytochemicals is increased by overcoming the increasing demand for comfort and the beneficial needs of society [40]. Our present project in this aspect to the development of alternative therapies for the treatment of various ailments. In this study, we evaluate the anthelmintic, insecticidal, and antiulcer activity of a natural herb found in the region of Pakistan [41]. The preliminary phytochemical screening of Dicliptera bupleuroides Nees. shown in Table 1. The extract showed the presence of polyphenolic compounds, saponins, flavonoids, and alkaloids. The content of total phenolic and flavonoids were expressed as follows:

Y= 1.025X + 0.195, R2=0.9984 for polyphenol

Y=0.0008X + 0.0483, R2=0.9969 for flavonoid standard is quercetin.

Results were shown in Table 2 and Figure 1(A) and (B).

In plants, polyphenolics and flavonoids are a major class of compounds that naturally possess the antioxidant potential to show therapeutic action in numerous biological ailments. Antifungal, antiviral, anti-inflammatory, anti-allergic, anticarcinogenic, antithrombic hepatoprotective, and cytotoxic effects. So that plants have flavonoids showed the greatest interest for researchers. It exerts beneficial effects on lipid peroxidation, which is a major cause of various diseases. In pharmacological profile, flavonoids have free radical scavenging activity, antioxidants, and also interact with protein phosphorylation [31].

The data revealed that the ethyl acetate and butanol fraction showed the maximum mortality/

paralysis time at 100mg/ml in comparison to other fractions. Helminths are parasitic, causing severe effects in the animal as well as in men. Human infections by helminths exist throughout the world and it may increase day by day due to travel and immigration from developing countries. However various advances have occurred in the development of new synthetic drugs but serious side effects and development of resistance are still a major hurdle in the treatment of these parasitic infections. These factors paved the focus on the development of new alternative herbal remedies for helminths [42]. Nowadays medicinal plants are screened for their major constituents which are responsible for the anthelmintic property. The use of Dicliptera bupleuroides Nees. crude extract and its fractions have shown significant anthelmintic activity, it would be used against intestinal nematodes. Ethyl acetate and n-butanol fraction showed the maximum anthelmintic property.

The fractions of *Dicliptera bupleuroides* Nees. were also screened for their insecticidal effects against Tribolium castaneum, Sitophilus oryzea, and Rhyzopertha dominica using permethrin as a standard drug [42]. There was no insecticidal effect on all tested samples against Tribolium castaneum, Sitophilus oryzae, and Rhyzopertha dominica (Table 3).

Antiulcer activity of Dicliptera bupleuroides Nees was investigated in the ethanol-induced ulcer model. The ulcer can be induced by different factors, the most commonly involved factors are the administration of non-steroidal anti-inflammatory drugs, H-pylori, environmental factors, intake of ethanol, and lifestyle factors [17]. Daniel and his colleagues induced ulcer 1st time in rats by using sucralfate as a protective drug [32]. Continuous use of alcohol also causes gastric mucosal damage by stimulating parietal cells which increases the level of cAMP and histamine. This may lead to an increase in gastric and mucosal secretion which made the grounds for stomach lesions, hemorrhage, and inflammation and blood congestion [43, 44] Standard drugs were ranitidine, omeprazole, and sucralfate, they belong to different classes and their significant results are agreed with the work of other authors [31, 32, 33].

In the present study, the *Dicliptera bupleuroides* Nees of all fractions were evaluated for anti-ulcer activity by the ethanol-induced ulcer model. When results of %age of ulcer protection compared to +ve control group (0%) showed following indices i.e methanolic extract (24.73%), aqueous (24.88%), n-hexane (54.95%), chloroform (38.88%), ethyl acetate (8.73%) and butanol fraction (24.88%) given in Table 1. The Gastric volume and total acidity of reference drugs and extract/fractions treated groups decrease, these values given in Table 2. Gastric volume and total acidity of the +ve group were 3.95  $\pm$  0.15 and 98.17  $\pm$  3.82. The standard group showed values of gastric volume  $(2.13 \pm 0.23, 1.80 \pm 0.29, 1.77 \pm 0.20)$  and total acidity was (38.17±2.95, 32.33±2.80, 39.17±3.44) respectively. Mucous and protein content increased in standard and extract-treated groups in comparison to the ethanol group (Table 3). The mechanism of gastro protection of Dicliptera bupleuroides Nees. maybe due to the cytoprotective, antisecretory, and antioxidant potential of phytoconstituents present in the extract [44, 45].

## 5. CONCLUSION

All the extracts/fractions of *Dicliptera bupleuroides* Nees. were used for evaluating various biological activities such as anthelmintic, insecticidal, and anti-ulcer activity. Ethyl acetate and butanol fraction showed good results in comparison with the reference drug-treated group in the anthelmintic study. No significant results showed in the insecticidal study. n-hexane and chloroform extract showed a good protective effect in antiulcer activity when compared to the ethanol-treated group.

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## 7. CONFLICT OF INTEREST

The authors declare no conflict of interest.

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