



Review Article

An Updated Review On Dicliptera Bupleuroides: Its Phytochemicals, Medicinal Use, Traditional Use, Pharmacological Activity And Toxicity

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ABSTRACT

Dicliptera bupleuroides is a medicinal plant belonging to the family Acanthaceae and this is a perennial herb. Dicliptera bupleuroides also known as kaalu or kirch in local language. It is found in the planes of Pakistan and Afghanistan. Dicliptera bupleuroides possessed many kind of pharmacological properties like antioxidant, hepatoprotective, antimicrobial, antidiabetic and other biological activities. It contained phenols, flavonoids, ascorbic acid, lipids, starch, glycosides, and many other compounds. The purpose of this review article is to document the latest data about its medicinal use, traditional use, phytochemicals, and pharmacological activities and its toxicity of the plant.

INTRODUCTION

Medicinal plants or herbs are those plant which have medicinal value in them, these plants used since ancient times for their medicinal purpose and welfare of human beings. These plants mainly use to make Ayurveda medicine, as time passed different pharmaceutical industries also started to use these plants or herbs to manufacture herbal preparation. The preparation manufacture by these plants is based on established therapeutic efficacy explored from crude extract (1) (2). Now a day's herbal preparation is widely used by different communities for their therapeutic value and have less or fewer side effect with cheap cost. On other

hand medicine of synthetic origin has high cost and more side effect as they use different chemicals in them (3) (4). As the demand increase now a day's almost 30% of the pharmaceutical preparations are based on plants, and most of the developed countries which have less herbs growth they import raw materials of important plants from developing countries (5). Dicliptera bupleuroides Nees. Of the family Acanthaceae is a perennial herb. It is found in the planes of Pakistan and Afghanistan. The common name in Urdu is kaali boti (6). Dicliptera bupleuroides possessed antioxidant, hepatoprotective, antimicrobial, and other biological activities. It contained phenols,

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flavonoids, ascorbic acid, lipids, starch, glycosides, and many other compounds (7) (8).

Scientific Classification (35)

Botanical Name:	Dicliptera bupleuroides
Kingdom:	Plantae
Class	Equisetopsida
Family	Acanthaceae
Genus	Dicliptera
Species	Dicliptera bupleuroides

Plant Distribution

The plant *Dicliptera bupleuroides* is found in various regions like Afghanistan, Assam, Bangladesh, Cambodia, China South-Central, East Himalaya, India, Laos, Myanmar, Nepal, Pakistan, Thailand, Vietnam, and West Himalaya. It is an annual or subshrub and grows primarily in the subtropical biome (36).

Botanical Description

This herb is about 90 cm long and leaves are elliptic, 2-15 x 1-8cm, shortly acuminate at both ends and dark green in color and flowers are mainly pink with purplish tinge, which is (1.2-1.5) cm long. Flowering: November-June 15. Corolla pink with white tube (rarely entirely white), 15-22mm, lobes longer than tube. Capsule clavate, pubescent, 7-8mm.

Traditional Use

Local people use this plant in various ways like the decoction of whole plant used as a tonic and they use the pastes of leaves for the treatment of eczema and the extract of leaves use as ear drops. This herb also cures snakebite wounds, stomach disorder, and bone fractures. It has antimicrobial, antioxidant and hepatoprotective activity along with gastroprotective and insecticidal activities. The herb rich are flavonoids, phenolic compounds, lipids, glycosides and starch, etc (9).

Medicinal Uses

Dicliptera bupleuroides also cure the eye diseases and freshly crushed leaves when applied gently on the body three times in a day for a week in eczema. Fresh leaves of this plant can cure the diabetes and

juice of this plant used to cure stomach problems which is used by local people of kadyala (10).

Phytochemicals

Determination of primary metabolites of plant *Dicliptera bupleuroides* contains lots of active chemical constituents like

1. Carbohydrates content

Carbohydrates are determined by performing the method followed by (11) but have certain modifications.

2. Starch Content

Method followed to determine the starch content is of (12) where 100 mg powder sample was used to check the starch content in the plant.

3. Protein Content

Plant also content proteins which is determine by the method given in (13).

4. Amino acid content

Dicliptera bupleuroides contain amino acid which determine by using the method given in (14).

5. Total Lipid Content

Following the Jadid method to determine the lipid content in the plant which shows the presence of lipid in *Dicliptera bupleuroides* (15).

Secondary Metabolites

1. Glycoside

The presence of glycoside is determine using (16) Method.

After time Preliminary phytochemical analysis it revealed that *Dicliptera bupleuroides* contains different active chemical constituents like terpenoids, tannins, glycosides, flavonoids, alkaloids, proteins, carbohydrates, saponins, fats and fixed oils (17).

Pharmacological activities

Antibacterial Activity

The antibacterial activity of *Dicliptera bupleuroides* is performed with different fractions like n-hexane, CHCl₃, EtOAc and n-BuOH (18). Against two Gram-positive bacteria i.e. *B. subtilis* and *S. aureus* and also against two Gram-negative bacteria that is *E. coli* and *P. multocida* by using

disc diffusion method using streptomycin sulphate.

Table 1: Zones of inhibition (mm) and MIC values of various fractions of *Dicliptera bupleuroides* against Gram-positive and Gram-negative bacteria

Plant	Sample	Zones of Inhibition (mm)				MIC ($\mu\text{g/mL}$)			
		S. aureus	B. subtilis	P. multocida	E. coli	S. aureus	B. subtilis	P. multocida	E. coli
Dicliptera bupleuroides	n-Hexane fr.	-	-	14	12	-	-	251	276
	CHCl ₃ fr.	14	20 \pm	14	16	250	126	251	226
	EtOAc fr.	16	18	20 \pm	22 \pm	225	199	125	118
	n-BuOH fr.	12	14	12	14	278	251	278	251
	Aqueous fr.	16	14	18	14	225	226	202	252

The inhibition growth zones was measured in mm and MIC was also calculated (19). Antibacterial activity of *Dicliptera bupleuroides* is performed by using four different bacteria two of them are gram positive and two of them are gram negative. For the growth of bacteria nutrient agar is form and culturing of bacteria's were taking place on nutrient agar (20). The zone of inhibition is measure in mm and leaves and roots extract showed the activity against all tested bacteria. But the extract of stem not showed the effect against *B. subtilis*. On the other hand the highest zone of

inhibition of leaves against *B. subtilis* with diameter (17.0 \pm 0.5) mm. Stem have the max. Inhibition zone against *S. aureus* with diameter (19.0 \pm 0.6) mm. And roots have (18.3 \pm 1.2) mm inhibition zone was obtained by *S. aureus* (21).

Antifungal Activity

Antifungal activity of *D. bupleuroides* is carried out with different fractions like n-exane, CHCl₃, EtOAc and n-BuOH and checked against four different fungus like *A. alternate*, *A. niger*, *G. lucidum*, *A. flavus*.

Table 2: Zones of inhibition (mm) and MIC values of various fractions of, *Dicliptera bupleuroides* against Fungi

Plant	Sample	Zones of Inhibition (mm)				MIC ($\mu\text{g/mL}$)			
		A. niger	A. flavus	G. lucidum	A. alternata	A. niger	A. flavus	G. lucidum	A. alternata
Dicliptera bupleuroides	n-Hexane fr.	-	-	-	-	-	-	-	-
	CHCl ₃ fr.	14	12	16	-	251	278	224	-
	EtOAc fr.	12	12	20 \pm	14	276	274	126	251
	n-BuOH fr.	14	12	16	-	252	276	224	-
	Aqueous fr.	12	12	12	14	275	276	273	251

The growth inhibition zones of these fungus are measured in mm and also MIC was also calculated (19). To test the anti-fungal activity of *D. bupleuroides* two strains were used i.e. *A. oryza*, *A. niger*. For the growth of fungus PDA media is prepared for the culturing of fungus (22). To check its activity ethanolic extract of leaves, stem and

roots were used and the extract of leaves showed the antifungal activity against *A. oryza* along inhibition zone formation about 12.3 \pm 0.9 mm but against *A. niger* its showed no effect. On other hand stem shows activity against both fungi and the inhibiton zone is 9.7 \pm 0.8 mm and 10 \pm 0.6 mm respectively. Whereas root extract of the plant



have effect on *A. niger* and inhibition zone is (11.3±0.6) mm but no effect against *A. oryza* (21).

Hemolytic Activity

It's important to check the haemolytic activity of the herbal plants which have antioxidant, antimicrobial and other bioactivities, so that we can use them for pharmacological bioactivities. Before any drug discovery it's important to check its cytotoxic effect that's why now a days it's becoming new area of research in the drug lead discoveries(23). Different fractions of *D. bupleuroides* that is EtOAc, chloroform and n-butanol fraction found out that they have very less toxic effect only about 1-3 %, but in other hand the n-hexane fraction of *D. bupleuroides* showed 79% toxicity.

Table 3: Hemolytic activities of various fractions of *Dicliptera bupleuroides*.

Plant	Sample	Toxicity % ± S.E.M ^(a)
<i>Dicliptera bupleuroides</i>	n-Hexane fr.	79.3±1.1
	CHCl ₃ fr.	1.0±0.3
	EtOAc fr.	1.1±0.7
	n-BuOH fr.	3.1±0.1
	Aqueous fr.	1.1±0.1

The hemolytic activities of *D. bupleuroides* extracts were compared with the triton (19). The acute toxicity of the methanolic extract of the plant *Dicliptera bupleuroides* at the doses of 500, 1000, and 2000 mg/kg/p.o which showed 0 % toxicity in rats and and 100% mortality of animals (24). In vitro haemolytic activity is perform with fresh

human blood where Triton-X was used as positive control and PBS served as negative control. Hemolysis % was calculated by using the formula:

$$\% \text{Hemolysis} = \frac{\text{Abs (Sample absorbance)}}{\text{Abs (Control absorbance)}} \times 100$$

The extract of plant does not show or display any kind of toxic effect or mortality even in high doses. To check the DNA damage protection assay of *Dicliptera bupleuroides* where different fractions of plant extract is used to protect genomic DNA was determined using a method with slight modification (25) (26). In this the DNA damage was induced by hydroxyl radicals generated from Fenton's reagent. The DNA protection assay showed the safe use of animal as well as human (27). Toxicity study of plant *Dicliptera bupleuroides* is performed on 12 male mice in 3 different group and given them the dose of 1g/kg, 2g/kg and 5g/kg of ethanolic extract of leaves, root, and bark and down procedure is followed according to (28). As the result there is no mortality in mice and the behaviour is observed is normal (21).

Anthelmintic Activity

To check the anthelmintic activity of the plant *Dicliptera bupleuroides* the experiment is performed on earthworm which is collected from crops field of Sialkot. The activity is carried out according to the method of Ajaiyeoba (29).

Table 4: Anthelmintic activity of *Dicliptera bupleuroides* Nees.

Treatment	Concentration (mg/ml)	Paralysis Time (min)	Death Time (min)
+ve control	-	-	-
Standard (Albendazole)	25	48.2 ± 3.96	80 ± 7.90
	75	45.4 ± 3.64	70.2 ± 5.76
	100	37.4 ± 3.43	55.2 ± 6.37
Methanolic Extract	25	65.8 ± 2.58	133 ± 3.46
	75	62 ± 2.12	128.4 ± 5.94
	100	58 ± 4.30	112.6 ± 7.98
Hexane fraction	25	186 ± 8.39	514.4 ± 11.26
	75	175 ± 4.12	493.6 ± 11.61
	100	167.8 ± 1.92	473 ± 8.36
Ethyl acetate fraction	25	65 ± 2.23	142.6 ± 7.98
	75	60.4 ± 2.07	122.6 ± 7.98

	100	53 ± 2.91	92.4 ± 10.01
Chloroform fraction	25	84 ± 3.08	193.2 ± 8.34
	75	75 ± 4.12	184.8 ± 4.43
	100	66.2 ± 5.71	173.6 ± 3.04
n-Butanol fraction	25	62.8 ± 3.96	137.6 ± 5.59
	75	59.8 ± 2.86	126.6 ± 5.45
	100	54.6 ± 4.21	108.8 ± 7.39
Aqueous fraction	25	238.6 ± 12.03	676 ± 9.61
	75	223.6 ± 8.50	666.8 ± 7.12
	100	194.8 ± 10.13	617 ± 12.04

To check this properly different fractions of dicliptera bupleuroides i.e. methanol, hexane, ethyl acetate, chloroform, n-butanol, aqueous were use against adult Indian earthworm in three different concentrations i.e 25, 75, and 100mg/ml. Albendazole (20 mg/ml) is used as standard drug (24).

Insecticidal Activity

To check the insecticidal activity of the plant Dicliptera bupleuroides the experiment is performed on test insects which are Tribolium castaneum, Sitophilus oryzae, and Rhyzopertha dominica of same size and age of each species. Permethrin is use as standard insecticidal (30).

Table 5. Insecticidal activity of different fractions of Dicliptera bupleuroides Nees.

Extract/Fraction	% Mortality (Mean ± SD)		
	Tribolium castaneum	Sitophilus oryzae	Rhyzopertha 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

Different fraction of Plant Dicliptera bupleuroides have no insecticidal effect on any of the tested samples which Tribolium castaneum, Sitophilus oryzae, and Rhyzopertha dominica (24).

Antiulcer Activity

To check the antiulcer activity Ethanol-induced Gastric Ulcer Model is used in wistar rats followed the method of Mizui (31). To measure the ulcer index in rats all

stomach lesions were measured with the help of transparent millimetre scale and magnifying glass (32). Drugs like omeprazole, sucralfate, ranitidine used as reference drugs. Formula were used to calculate the inhibition percentage is:

% 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 (33).

Table 6. Effect of different fractions of Dicliptera on the ethanol-induced gastric ulcer.

Group Name	Ulcer No.	Ulcer score	Incidence ulcer (%)	Ulcer index	Inhibition of ulcer
Normal (10 ml/kg p.o)	0.00 ± 0.00***	0.00 ± 0.00***	0	0	0 %
Ethanol (10 ml/kg p.o)	7.83 ± 0.60	5.75 ± 0.57	100	11.36	0 %
Ranitidine	1.00 ± 0.52***	1.00 ± 0.47***	50	5.2	54.22 %



(50 mg/kg p.o)					
Omeprazole (20 mg/kg p.o)	0.67 ± 0.42***	0.58 ± 0.37***	33.33	3.56	69.56 %
Sucralfate (100 mg/kg p.o)	1.17 ± 0.54***	1.50 ± 0.67***	50	5.27	53.63 %
Methanol (500 mg/kg p.o)	1.33 ± 0.42***	0.83 ± 0.28***	83.33	8.55	24.73 %
Aqueous (200 mg/kg p.o)	1.17 ± 0.40***	0.83 ± 0.28***	83.33	8.53	24.88 %
Hexane (200 mg/kg p.o)	0.50 ± 0.22***	0.67 ± 0.33***	50	5.12	54.95 %
Chloroform (200 mg/kg p.o)	1.50 ± 0.50***	1.25 ± 0.48***	60.67	6.94	38.88 %
Ethyl acetate (200 mg/kg p.o)	1.83 ± 0.31***	1.83 ± 0.42***	100	10.37	8.73 %
Butanol (200 mg/kg p.o)	1.67 ± 0.31***	0.83 ± 0.21***	83.33	8.53	24.88 %

The n-hexane fraction showed the maximum %age inhibition of ulcer and after that chloroform also showed significant inhibition (24).

Hepatoprotective Activity

The hepatoprotective activity is carried out by using wistar rats using paracetamol-induced liver injury model according to the documented method (34).

Acute hepatoprotective study

The acute hepatoprotective study is performed in rats with different fraction of *D. bupleuroides* i.e. methanol, n-hexane, chloroform, ethyl acetate, aqueous, n-butanol. Silymarin is used as standard and paracetamol is use to induce the liver injury in rats.

Table 7: Acute Hepatotoxic effect on serum enzyme levels of different fractions of *Dicliptera bupleuroides* Nees.

Treatment	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Total Bilirubin (m/dL)
Control	57.83±5.58***	78.83±9.24***	109.7±1.76***	0.7±0.04
Paracetamol	1558.5±45.11	1606.67±42.74	301.0±43.38	0.73±0.03
Silymarin	83.33±8.21***	103.83±11.77***	126.67±20.95***	0.68±0.06
Methanol	111±15.56***	122.00±45.95***	145.33±13.59***	0.53±0.04*
Aqueous	129.67±14.36***	148.33±5.95***	179.67±30.30**	0.7±0.04
n-Hexane	91.5±10.03***	101.17±13.43***	135.5±11.70***	0.65±0.04
Chloroform	184.83±14.37***	204.67±22.79***	201.83±20.04*	0.6±0.04
Ethyl acetate	194.17±38.27***	201.5±28.56***	193.83±20.65*	0.67±0.06
n-Butanol	152.67±13.85***	171.17±27.70***	163.00±20.19***	0.72±0.05

Paracetamol is used for liver injury in rats which is responsible to raise the AST, TB, ALT, MDA and ALP and decrease GSH level. While n-hexane fraction of *D. bupleuroides* effectively restored all these changes (27).

Chronic hepatoprotective study

Chronic hepatoprotective study is perform with only one fraction of plant which is n-hexane for 14 days. The blood sample were collected to determine the enzyme serum level and liver were for histopathology.

Table 8: Effect of n-hexane fraction of *Dicliptera bupleuroides* Nees. Serum enzymes in paracetamol induced chronic hepatotoxicity (level of significance * p>0.001, ** p>0.01, * p>0.05)**

Treatment	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Total Bilirubin (mg/dL)
Control	57.83±5.58***	78.83±9.23***	109.7±1.76***	0.7±0.04



Paracetamol	275±64.33	224.67±27.96	251±15.32	0.73±0.03
Silymarin	115±15.05**	95±6.64***	126.67±20.95***	0.7±0.04
n-Hexane	88.17±7.85**	93.83±7.62***	136.33±8.46***	0.67±0.03

Paracetamol is used for liver injury in rats which is responsible to raise the AST, TB, ALT, MDA and ALP and decrease GSH level. While n-hexane fraction of *D. bupleuroides* effectively restored all these changes (27).

Anti-diabetic activity

Anti-diabetic activity is performed on mice where diabetes is induced on male mice by single intraperitoneal injection of alloxan (120 mg/kg) prepared in normal saline. Metformin is used as standard and ethanolic extract of *D. bupleuroides* leaves, stem and root was prepared and blood glucose levels were analysed using accucheck glucometer. As a result the ethanolic extract of leaves at 250mg/kg dose decreased the blood glucose level significantly and it again started increasing but did not reach its first level. The dose 250mg/kg of leaves showed 243.6±3.4 at 0 h, and after 24h it decreased to 189.4±9.1. Stem in other hand showed 213.6±4.8 at 0 hr and 218.6±9.4 at 24h it showed no significant. But ethanolic extract of roots showed no significant result at 0 h was 205.4±2.1 and value after 24 h was 193.6±9.6 (21).

CONCLUSION

Dicliptera bupleuroides is a very important medicinal plant which has no harmful effect to human and it has various pharmacological properties like antioxidant, hepatoprotective, antimicrobial, antidiabetic and other biological activities. The plant has lots of active chemical constituents like phenols, flavonoids, ascorbic acid, lipids, starch, glycosides, and many other compounds. This plant needs to be explored more to find out more benefits and medicinal use of *dicliptera bupleuroides*.

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