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Antimicrobial and Antidiabetic evaluation of *Dicliptera bupleuroides* Nees

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In present study phytochemical, antibacterial, antifungal and antidiabetic activities of leaves, stem and roots of *Dicliptera bupleuroides* Nees of family Acanthaceae were investigated. Saponin were present in leaves and stem but absent in roots while lipids were present in leaves but absent in stem and roots whereas al others tested phytochemicals were present in root, stem and leaves. For antibacterial activity *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* bacteria were used and it was observed that ethanolic extract of leaves and roots showed effective potential against all tested bacteria whereas the extract of stem does not show any activity against *B. subtilis.* All results were compared with antibiotics, tetracycline, cefoperazone and erythromycin. *Aspergillus niger* and *Aspergillus oryza* fungi were used to determine antifungal effects. Stem and roots extract showed activity against *A. niger* whereas the extract of leaves does not show any activity. Leaf and stem crude extracts were antifungal against *A. oryza* but not effective against root crude extract. All the results were compared with antimycotics, ampicillin and terbinafine. Antidiabetic activity of ethanolic extract of leaves, stem and roots of *D. bupleuroides* N*ees* were administered to normal and alloxan induce male mice. The metformin (250 and 500 mg/kg) was used as standard drug whereas the control group only administered with distilled water. At 0 hour the glucose level of leaves, stem and root crude extracts were 243.6 ± 3.4, 213.6 ± 4.8 and 205.4 ± 2.1 respectively. After 24 hours the glucose level of leaves extract administered mice lowered down to 189.4 ± 9.1. The ethanolic extract at 500 mg/kg of concentration and showed blood glucose level at 0hour of leaves, stem and roots, i.e. 227.8 ± 17.7, 222.2 ± 8.4 and 206.8 ± 2.8 respectively while after after 24 hours leaves extract showed best results in lowering blood glucose level which was 123.0 ± 5.3. Standard drug metformin at both concentrations (250 and 500 mg / kg) showing significant results. In case of control group, the blood glucose level remains almost same. After the comparison of all groups the results showed that ethanolic extract of *D. bupleuroides* Nees play a vital role in treatment of diabetes. Acute toxicity study showed efficient results at 500 mg/kg concentration animals with in that variety not showed any fatal or mortal were experimented.

Keywords: Phytochemical, Antibacterial, Antifungal, Anti-diabetic, Ethnomedicinal plant, *Dicliptera bupleuroides*

INTRODUCTION

In ancient times, plant parts such as barks, leaves, flowers, roots, fruits and seeds have been used as medicines by developing different products from them (Criagg and David, 2001). Plants have affected humans for logical and

profitable resource particularly in the form of medicinal plants because of their huge potential towards curing of ailments (Ajaib et al. 2014).

Phytochemistry essentially deals with the vast different types of plant based organic substances (secondary metabolites) and these were used in drug development (Ajaib et al. 2020). Phytochemicals are reliable source for the treatment of different health hazards (Kirtikar and Basu, 1995). Different metabolites were known in this universe in which few of them were used in our daily life such as flavonoids, proteins, lycopene and many of them need to explored (Singh, 2008). Most of the secondary metabolites or phytochemical groups are act as expectorant and emulsifying mediator (Maxwell et al. 1995). The phytochemicals or plant natural products provide vast facility for pharmacists to produce diverse compounds that helps in curing of different ailments effectively (Soobrattee et al. 2013).

Since antiquity, the plant kingdom has provided a variety of compounds of known therapeutic properties, like analgesics, anti-inflammatories, antidiabetic, antipyretic, anticancerous and others. In recent years, antimicrobial properties of plant extracts have been reported with increasing frequency from different parts of the world (Cowan, 1999). At first time incidentally a material gained by the plants which were called the antimicrobial compound by Osborn (1943) and after that research on this field successfully initiated (Austin et al. 1999).

Diabetes is derived from a Greek word ‘Dia’ means ‘through’ and ‘betes’ means ‘to pass’. Diabetes can be expediently classified into two types’ one is Diabetes mellitus (DM) and the other one is Diabetes insipidus. The clinical condition in which the emission of insulin is leading to hyperglycemia and glycosuria is Diabetes mellitus (Kamran et al. 2016). The most frequent sign of DM includes increased urine output (polyuria), increased thirst due to water loss (polydipsia), loss of body weight (due to protein loss), and increase in appetite (hyperhagia). Whereas due to the lack of ADH secretion diabetes insipidus is caused (Ajaib et al. 2016; Patil et al.2014). An additional type of diabetes known as Gestational diabetes occurs during pregnancy this type of diabetes caused high blood glucose levels and goes away after the birth (Maruthupandian et al. 2011).

Dicliptera bupleuroides Nees commonly known as the Kaalu or Kirch by local people and belongs to a dicot plants family known as Acanthaceae It is about 90 cm, long much branched herb with hairy twigs. Flowers are mainly pink with purplish tinge; 1.2-1.5 cm long (Malik and Ghafoor, 1988). According to Shah et al. (2013) decoction of whole plant of *D. bupleuroides* is used as a tonic while leaves pastes is used for treatment of eczema and earache. Local people of Kadyala (Sahib Ditta and Rubina Bibi) demonstrated that they used the fresh leaves of this plant to cure the diabetes and juice of this plant used to cure stomach troubles.

Fig 1: *Dicliptera bupleuroides* Nees

Family Acanthaceae contains approximately 220 genera and nearly 4000 species and belongs to the taxon dicotyledonous flowering plants. Plants of this family are predominant in tropical and subtropical regions with few species in the temperate regions. The Acanthaceae family contains almost herbs or shrubs, apart from few are trees or vines (Kirtikar and Basu, 1995). Many plants in this family reported to have various ethanomedicinal importance like anticancer, antidiabetic, antimicrobial, hepatoprotective and anti-inflammatory (Komalavalli et al. 2014).

MATERIALS AND METHODS

Plant Material

The various parts of *D. bupleuroides, i.e*. leaves, stem, and roots were collected from Kadyala District Bhimber Azad Jammu & Kashmir The collected plant dried and mounted on an herbarium sheet and then authenticated from herbarium Department of Botany, MUST with a voucher specimen no. MUST.BOT.5352. The collected plant material, *i.e.* leaves, stem, and roots were shade dried for about a week and grinded in the form of powder.

Preparation of Ethanolic Extract of Plant

About 250g of finally powdered plant material was taken in a glass container and dip in ethanol. The glass container was then sealed and remained airtight for 7 days with occasionally shaking and stirring. The mixture was filtered with the help of Whatman filter paper (Bibby RE200, Sterilin Ltd., UK). The ethanolic extract of *D. bupleuroides* was evaporated on a rotary evaporator and a sticky concentrate was obtained.

Phytochemical analysis

Following tests which were done are given in detail below as described by Sofowara (1993) and Raman (2006).

1. Test for Triterpenoids

Salkowaski test

About 2 ml plant extract added in 1 ml of $CHCl_3$ then added 1 ml of concentrated H_2So_4 by the side of walls of test tube. Yellowish shade layer developed which point out occurrence of triterpenoid.

Liebermann's experiment

About 1 ml extract in it add 2ml chloroform ($CHCl_3$) , then 1 ml glacial acetic acid (CH_3COOH) by the side of walls of test tube at last 2-3 ml of conc. H_2SO_4. Deep reddish color indicated the presence of triterpenoid.

2. Test for Sterols

Salkowaski test

1ml of extract added with 1ml chloroform then by addition of 1ml of concentrated H_2So_4 along with walls red tint indicated the presence of sterol.

Liebermann's test

About 2 ml of extract added in 2 ml of chloroform ($CHCl_3$), 1ml of glacial acetic acid (CH_3COOH) with the length of walls after that added 2 ml conc. H_2So_4. Green shade layer indicated occurrence of sterol.

3. Test for Glycosides

i. Test of Bromine water

About 1ml extract and in it added 2-3ml bromine**'s** water, yellowish coloured precipitates showed glycosides existence.

Test of Keller-killani

1ml of extract and added 2-3 ml of glacial acetic acid (CH_3COOH), put in 2ml solution of ferric chloride then further 2 ml conc. H_2So_4 along wall side. Blue shade on top whereas glowing chocolate tint in inferior indicated the presence of glycoside.

upper layer in bark and root extract of *D. bupleuroides*

4. Test for Flavonoids

Test of Ferric chloride

1ml extract, added 1ml suspension of ferric chloride ($FeCl_{3)}$. With presence of deep green precipitates flavonoids are pointed out.

Alkaline reagent test

About 2 ml of extract, in it added 2-3 ml of sodium hydroxide, intense yellow color formed than added 2 ml of dilute hydrochloric acid which turned to colorless.

Zinc-hydrochloride Acid-Reduction test:

About 2 ml of extract was added in zinc dust and then added 2 ml of hydrochloric acid (HCl). Red magenta precipitate formed that showed existence of Flavonoids.

Test of Lead acetate

1 ml of extract was supplemented with 2 ml lead acetate suspension. Yellowish color precipitate showed presence of flavonoids.

5. Test for Alkaloide

Mayer's experiment

In about 2 ml plant extract put in 3 ml of Mayer's reagent, Cream colored impulsive produced that designated alkaloid existence.

Wagner's experiment

2 ml of extract added 2 ml Wagner's reagent, chocolate shade precipitates formed which showing alkaloid existence.

Hager's experiment

1 ml extracted material when 2 ml of Hager's reagent added, brown color precipitate indicated presence of alkaloids.

Dragendroff's test

1 ml plant concentrate when added in 1 ml of Dragendroff's reagent, red chocolate impulsive indicated existence of alkaloids.

6. Test for Proteins

Millon's analysis

About 1 ml extract added with 2 ml of Millon's reagent and heated on water bath until boiling, reddish color showed the presence of protein.

Biuret test

2 ml of extract, added 2 ml 40 % sodium hydroxide diluted by $CuSO_4$ solution. Blue color pointed out proteins existence.

i. Ninhydrin's analysis

In 1 ml extract added 2ml Ninhydrin**'s** reagent, indigo color designated protein existence.

ii. Xanthoproteic test

2 ml of extract added 2 ml of Conc. H_2So_4, after heating on water bath blue color precipitates created which showed protein existence.

7. Test for Carbohydrate

Barfoed's experiment

2 ml of extract and in it added 2 ml Barfoed reagent in addition heated it so that it boils, on water bath reddish color impulsive showed carbohydrates occurrence.

Molisch's experiment

About 1 ml extract, added 2ml of alpha-naphthol ($C_{10}H_8O$) and then added 2 ml of Conc. H_2So_4 beside walls in tubes. Purple color ring formed in intersecting point which indicated carbohydrates existence.

Benedict experiment

About 2 ml plant concentrate, added in it 2 ml Benedict reagent heat it till boiling into water bath. Brick reddish precipitates formed which indicated carbohydrates existence.

8. Test for Saponin

Foam examination

1 ml extract and added 2-3 ml of water, on strongly shaking foam formation occurred. If foam remains stable for 10 minutes it points out saponin occurrence.

Bromine water experiment

In 2 ml of ethanolic extract in it add some bromine**'s** water drop, golden precipitate point out saponin occurrence.

Legal experiment

3 ml extract, added 1g of Pyridine (C_5H_5N) after that add nitroprusside suspension to make it alkaline in nature. Cherry reddish tint showed saponins existence.

9. Test for Lipids

Soap formation experiment

2 ml of extract, by adding some amount of 0.5 n alcoholic KOH in it then add small amount of phenolphthalein ($C_{20}H_{14}O_4$), after the formation of mixture heat it for half an hour into water. Lipids Indicate by soap development.

10. Test for Tannin

Ferric chloride experiment

1 ml extract, added 1-2 drops $FeCl_3$solution, formation of profound bluish or greenish brown shade pointed out the presence of tannin.

Antimicrobial activity

Antimicrobial activity of *D. bupleuroides* comprises of antibacterial and antifungal activity. For the antibacterial analysis different bacteria used of which gram positive bacteria include *Staphylococcus aureus* and *Bacillus subtilis* while gram negative includes *Escherichia coli* and *Pseudomonas aeruginosa*. For antifungal action two strains used which were *Aspergillus niger* and *Aspergillus oryza*.

Preparation Nutrient Agar

For investigation of antibacterial activity of *D. bupleuroides* the culturing of bacteria was taking place on nutrient agar. 14g nutrient agar was accurately weighed and dissolved in 500 ml distilled water in conical flask. With cotton close the mouth of flask and autoclave it at 121 ^{o} C for about 15 minutes and the temperature was 15 lb pressure (Cruick-Shank et al. 1975).

PDA Media Preparation

For investigation of antifungal activity of *D. bupleuroides* the fungal specimen was cultured on PDA. 19.5g of PDA media accurately weighing then dissolved in 500 ml distilled water in conical flask. Cover the flask with cotton and autoclave it (Johansen, 1940).

Sterilization process

The petriplates were autoclave at 121^{o} C for 15-16 minutes and temperature was 15 lb pressure.

Preparation of bacterial strains

Inoculation of strains of bacteria takes place within laminar flow. With the help of alcohol

sterilize the surface of laminar flow. Turn on the UV light for 10 minutes before using the hood. After that turn sprit lamp turned on to avoid contamination and nutrient agar transfer to petriplates carefully. Let nutrient agar medium to solidify for some minutes. The bacterial loop which was used for inoculation was red hot on sprit lamp to remove contamination from its surface. Then open the petri dishes by finger of left hand and shift strains of bacteria on prepared medium into petridishe. For transferring bacteria bacterial loop was used. To avoid all the petriplates containing the bacterial strain from contamination covered the plates with stick film and placed them into incubator at 37^{o} C for 24 hours.

Preparation of fungal strains

To avoid the contamination, the surface and walls of laminar flow cleaned properly with the help of alcohol. Sprit lamp turns on and the prepared PDA poured in petriplates near the sprit lamp to avoid any type of contamination. Let the medium to solidify for some minutes. Inoculum loop heated on sprit lamp until it become brick red to remove contamination from its surface. Then open the petridishes by finger of left hand and shift fungal strains on prepared medium with the help of inoculums loop. To avoid all petridishes having fungal strain from contamination wrapped the plates with stick film and placed them into incubator at 27 ^{o}C for 48 hours.

Agar well diffusion method

For test of antibacterial and antifungal activities of different parts of *D. bupleuroides* first of all pour the prepared Nutrient agar and PDA media respectively in petriplates. Plates were swabbed with the help of sterile cotton swabs and wells were prepared by means of sterile cork borer. The extract was poured in wells with sterile micropipet and allowed to diffuse in room temperature. Wrapped the plates with the help of stick film and placed them into incubator. In antibacterial activity zone of inhibition formed after 24 hours and incubator was set at 37 ^{o}C while in antifungal activity zone of inhibition formed after 48 hours and incubator was set at 27 ^{o}C.

Measurement of Zone of inhibition

After 24 hours of incubation in case of antibacterial activity and 48 hours in antifungal activity the zone of inhibition noted. Readings were noted with the help of ruler in mm. The zone at first observed with unaided eye. If the zone around the disc not formed, it is considered 0 mm and if around the disk zone formed measure it from one edge to other edge with ruler and note the reading.

Anti-diabetic activity

Animals

Mice were housed in the animal house of the Punjab University College of Pharmacy, University of the Punjab at 25±5°C. The animals were fed on standard diet and water. All the protocols of animal treatment were approved by animal ethical committee on the college.

Acute toxicity evaluation

Twelve Balb/c male mice (n = 4), were divided into three groups. First group administered a dose of 1g/kg, second group administered the dose of 2g/kg and third group administered the dose of 5g/kg of ethanolic extract of leaves, bark and roots, after 16 h fasting, following up and down procedure according to (Ahmed et al. 2012). The animals were observed at 1 h after administration of extracts, and also at 6 h and up to 24 h for any sign of toxicity viz., weakness or aggressiveness, food refusal, loss of weight, diarrhea, discharge from eyes and ears, noisy breathing and mortality (Hamid et al. 2008; Umar et al. 2010).

Induction of Diabetes

Diabetes was induced in the Balb/c male mice after single intraperitoneal injection of alloxan (120mg/kg) prepared in normal saline. The solution was prepared and immediately injected to the mice. 5% glucose solution was administered four hours after injection through oral gavage. Treatment with plant extracts, *i.e. D. bupleuroides* was started 72 h after alloxan injection.

Study Protocol

An acute study of ethanolic extract of *D. bupleuriodes* leaves, stem and root was designed. Blood glucose levels were analyzed at 0 hr interval with Accucheck glucometer and then relative treatments were given to the groups. Each group contained five animals. The blood glucose levels were analyzed at 2, 4, 6, 8 and 24hrs. after treatment. All the drugs were dissolved in water and administered once. The diabetic mice were divided into following groups.

1-Control diabetic group: This group was administered distilled water at the time when other groups were given treatments.

2-Metformin treated group: Metformin 250 and 500mg/kg was administered.
3-Ethanolic extract of leaves 250 and 500mg/kg
4-Ethanolic extract of stem 250 and 500mg/kg
5-Ethanolic extract of root 250 and 500mg/kg

RESULTS AND DISCUSSION

Qualitative phytochemical investigation of ethanolic extract of Leaves, Stem and Root extract of *D. bupleuriodes* revealed the presence of different secondary metabolites. Results were present in (Table: 1).

Antimicrobial activity

Leaves and roots crude extracts showed good effects against all tested bacteria whereas the extracts of stem not showed any activity against *B. subtilis.* In leaves crude extracts the highest zone of inhibition showed in *B. subtilis* with diameter 17.0 ± 0.5 mm while the least zone of inhibition showed by *E. coli* with diameter 9.6 ± 0.8 mm. In stem the maximum zone of inhibition was obtained by *S. aureus* with diameter 19.0 ± 0.6 mm while the minimum zone of inhibition was obtained by *E. coli* with diameter 14.8 ± 1.0 mm. In roots the maximum zone of inhibition was obtained by *S. aureus* with diameter 18.3 ± 1.2 mm while the minimum zone of inhibition was obtained by *B. subtilis* with diameter 9.0 ± 0.5 mm. The results obtained were compared with standard antibiotic discs. Tetracycline the maximum zone was obtained by *S. aureus* with diameter 19 ± 0.5 mm while the minimum zone was obtained by *E. coli* with diameter 15 ± 1.1 mm. In cefoperazone the maximum inhibition zone was obtained by *S. aureus* with diameter 20.3 ± 0.8 mm and minimum zone was obtained by *B. subtilis* with diameter 16.0 ± 0.5 mm. In Erthromycin the maximum zone was obtained by *B. subtilis* with diameter 20.0 ± 0.5 mm and minimum inhibition zone was obtained by *P. aeruginosa* with diameter 11.3 ± 0.8 mm. Comparison of all the results showed in (Table 2).

In antifungal activity ethanolic extract of leaves, stem and root were used. Extract of leaves showed activity against *A. oryza* and formed zone of inhibition 12.3 ± 0.9 mm but does not showed any activity against *A. niger.* Extract of stem showed activity against both *A. niger* and *A. oryza* and zone of inhibition formed against these fungi was 9.7 ± 0.8 mm and 10 ± 0.6 mm respectively. Extract of roots showed activity against *A. niger* and formed zone of inhibition 11.3 ± 0.6 mm but does not showed any activity against *A. oryza*. The effects of these parts compared showed that the highest zone formed by leaves (12.3 ± 0.9 mm) against *A. oryza* while the minimum zone of inhibition formed by extract of stem (9.7 ± 0.8 mm) against *A. niger.* The standard antimycotics, zone of inhibition formed by ampicillin and terbinafine against *A. niger* were 18.3 ± 0.9 mm and 20 ± 0.6 mm respectively. Against *A. oryza* ampicillin formed 16 ± 0.6 mm zone of inhibition whereas terbinafine formed 15.7 ± 0.3 mm zone of inhibition. Comparison of all the results showed in (Table 3).

Determination of non-toxic concentrations

Acute toxicity study revealed about the safe nature of *D. bupleuriodes* plant. Experiment was conducted on normal healthy mice. No mortality in mice was observed and their behaviour also appeared normal in all the animals. Neither lethality nor any toxic reaction among animals was found at any dose selected until the conclusion of study.

Determination of anti-diabetic activity

Considerable reduction in glucose concentration was observed into ethanolic extract of leaves at 250 mg/kg dose. After two hours of dose administration the blood glucose level falls down significantly it again starts increasing but not reaches its first level and after 24 hours it remains less which showed that 250mg/kg leaves ethanolic extract appreciably lowered the sugar concentration when evaluate against control diabetic group and equivalent efficacy as Metformin taken as standard drug was observed. Non-significant reduction in sugar concentration was examined with ethanolic extract of stem. Ethanolic extract of roots has shown fewer efficacies when compared with metformin and leaves extract results. The standard drug metformin also showed the significant results. Control group showed not good results. Comparison of all the results showed that the lowering of blood glucose of ethanolic extracts of leaves at dose 250mg/kg was at 0 hr 243.6±3.4 and after 24 hrs its decrease to 189.4±9.1. The blood glucose of ethanolic extract of stem at 0 hr was 213.6±4.8 and at 24 hrs was 218.6±9.4 it shown no significant results. The ethanolic extract of roots has also no significant results in decreasing the glucose levels, at 0 hr 205.4±2.1 and value after 24 hrs 193.6±9.6. These outcomes judge against standard and control group. Onset of anti-diabetic action of metformin was at 0 hr 219.4± 5.1 and at 24 hrs was 182.0±10.4 (Table 5).

Table I: Phytochemical Evaluation of Ethanolic extract of *Dicliptera bupleuriodes*

Group	Name of Test	Ethanolic extract of Leaf	Ethanolic extract of stem	Ethanolic extract of root
Triterpenoids	Salkowaski test	+++	++	++
	Liebermann's Test	++	+++	+
Sterols	Salkowaski test	+++	++	++
	Liebermann's Test	++	+++	+++
	Sulphur test	++	+	+
Glycosides	Bromine water test	+	_	_
	Keller-killani test	+++	+++	++
Flavonoids	Ferric chloride test	++	++	++
	Alkaline reagent test	++	+	++
	Zinc-hydrochloride test	_	_	_
	Lead acetate test	+++	+	+
Alkaloids	Mayer's test	_	_	+
	Wagner's test	_	++	++
	Hager's test	+++	++	++
	Dragendroff's test	+	+++	+
Proteins	Millon's test	+++	++	++
	Ninhydrin test	_	_	_
	Biuret test	++	+++	++
	Xanthoprotic test	+	_	+++
Carbohydrates	Barfoed's test	+++	_	_
	Molisch's test	+++	+++	+++
	Benedict's test	+++	_	_
Saponin	Foam test	+++	+++	_
	Bromine water test	+	_	_
	Legal's test	_	_	_
Lipids	Soap formation test	+++	_	_
Tannins	Ferric chloride test	+++	++	+++

Key: -Absent +Present ++Moderately present +++ Strongly present

Table 2: Zone of inhibition produced by Leaves, Stem and Roots of *D. bupleuriodes* and Antibiotics against Bacterial strains (mm)

List of Bacteria	Leaves	Stem	Roots	Tetracycline 15µg	Cefoperazone 20 µg	Erthromycin 15µg
E. coli	9.6 ± 0.8	14.8±1.0	9.7 ± 0.3	15 ± 1.1	17.3 ± 0.8	14.0 ± 1.1
B. subtilis	17.0 ±0.5	_	9.0 ± 0.5	17.3 ± 0.8	16.0 ± 0.5	20.0 ± 0.5
S. aures	14.6 ±0.3	19.0±0.6	18.3 ±1.2	19 ± 0.5	20.3 ± 0.8	13.3 ± 0.8
P. aeruginosa	12.3 ±0.8	18.6±0.5	17 ± 1.1	16.3 ± 0.6	17.6 ± 0.6	11.3 ± 0.8

***** (SEM ±)

Table 3: Zone of inhibition produced by Leaves, Stem and Roots of *D. bupleuriodes* and antimycotics against fungal strains (mm)

List of Fungi	Leaves	Stem	Roots	Ampicillin 20 µg	Terbinafine 20 µg
A.niger	_	9.7 ± 0.8	11.3 ± 0.6	18.3 ± 0.9	20 ± 0.6
A. oryza	12.3 ± 0.9	10 ± 0.6	_	16 ± 0.6	15.7 ± 0.3

***** (SEM ±)

Table 4: Anti-diabetic effect of *D. bupleuriodes* ethanolic extract 250 mg / kg of different parts in alloxan treated diabetic mice at different time intervals

Time interval	Control diabetic group	Metformin	Eth ext Leaves	Eth ext Stem	Eth ext roots
O hr	257.8 ± 7.2	219.4 ± 5.1	243.6 ± 3.4	213.6 ± 4.8	205.4 ± 2.1
2 hr	225.4 ± 9.4	215.0 ± 5.2	177.4 ± 2.7***	197.4± 22.3***	180.0 ± 8.2***
4 hr	226.8 ± 6.4	179.8 ± 6.6***	206.7 ± 7.1	169.6± 12.5***	206.8 ± 16.1
6 hr	232.6 ± 10.4	154.2 ± 5.3***	178.6 ± 1.6***	192.0 ± 12.6	202.0 ± 19.5
8 hr	240.4 ± 12.1	126.4 ± 2.3***	168.4± 10.2***	205.6 ± 5	187.4±22.3***
24 hr	256.6 ± 10.6	182 ± 10.4***	189.4 ± 9.1***	218.6 ± 9.4	193.6 ± 9.6

All values are presented as SEM (n=5). Two ways ANOVA was applied using Bonferroni post-hoc test and all the groups are compared with Control group at all-time intervals. *** represents p<0.001

Table 5: Anti-diabetic results of *D. bupleuriodes* ethanolic extract 500 mg / kg of different parts in alloxan treated diabetic mice at different time intervals

Time interval	Control diabetic Group	Metformin	Eth ext Leaves	Eth ext stem	Eth ext roots
O hr	243.8 ± 5.9	221.4 ± 6.8	227.8 ± 17.7	222.2 ± 8.4	206.8 ± 2.8
2 hr	227.4 ± 7.1	180.0 ± 7.9***	183.8 ± 10.3***	179.4 ± 13.6***	159.2 ± 6.9***
4 hr	226.8 ± 6.4	158.4 ± 6.2***	152.4 ± 12.5***	164.2 ± 5.8***	166.4 ± 7.4***
6 hr	229 ± 11.4	169.0 ± 8.6***	146.4 ± 2.4***	171.2 ± 13.2***	169.6±10.7***
8 hr	234.6 ± 10.2	149.0 ± 3.4***	165.6 ± 21.2***	188.0 ± 4.9***	181.6 ± 6.9***
24 hr	245.0 ± 5.9	178.4 ± 3.5***	123.0 ± 5.3***	206.8 ± 2.8	207.8 ± 4.1

All values are presented as SEM (n=5). Two ways ANOVA was applied using Bonferroni post-hoc test and all the groups are compared with Control group at all-time intervals. *** represents p<0.001

For further studies the dose concentration increased from 250mg/kg to 500mg/kg. At this concentration the leaves extract showed the best results. A graded dose response relationship was observed with leaves extract. 500mg/kg leaves ethanolic extract considerably lowered the glucose concentration when judge against control diabetic group and equivalent value as Metformin which is taken as standard drug was observed. The stem and root extract at this concentration 500mg/kg never showed any considerable decrease in sugar. The lowering of blood glucose of ethanolic extracts of leaves at dose 500mg/kg was at 0 hr 227.8±17.7 and after 24 hrs its decrease to 123.0±5.3 which is one of the best results. The blood glucose of ethanolic extract of stem at 0 hr was 222.2±8.4 and at 24 hrs was 213.6±6.5 it shown no significant results at this increased concentration. The ethanolic extract of roots has also no significant results in decreasing the glucose levels, at 0 hr 206.8±2.8 and value after 24 hrs 207.8±4.1. Onset of anti-diabetic action of metformin was at 0 hr 221.4± 6.8 and at 24 hrs was 178.4±3.5 this also showed the best results. Comparison of all the results is depicted in (Table 5).

Phytochemicals now a day used as medicines due to drug resistance of pathogens and hence, current investigation was considered to estimate and confirm phytochemical activity of ethanolic extracts of various parts of *D. bupleuriodes* Nees. Most of the secondary metabolites such as triterpenoids, sterols, glycosides, flavonoids, alkaloids, proteins, carbohydrates and tannins were occurred in different parts of *D. bupleuriodes*. These metabolites were medicinally very important such as the terpenoids used against various diseases the results were compared with Mahato and Sen (1997) work. In case of alkaloids the results were compared with Herrera et al. (2001) work and demonstrated that it was used as analgesic mediators and are establish in medicinal plants. In the work of Edeoga et al. (2001) it was found that alkaloids were very significant in medication and comprised of the precious drugs. According to the work of Maxwell et al. (1995) tannins were strong bioactive compounds found in medicinal plant often meet in food artifacts of plant parts that can be used for beneficial reason. Flavanoids have antioxidant properties and used in case of irritation, against microorganisms, compared with

Barakat et al. (1993). In case of glycosides results were compared with Trease and Evans (1998) it was used as stimulant in case of cardiac collapse. Sterols that were present in different parts of *D. bupleuriodes* have been stated to use as antibacterial activities with important compound as sex hormones as described by Okwu (2001). Saponin that was present in leaves and stem was used to prevent blood losing and in curing injuries as discussed by Okwu and Josiah (2006). These phytoconstituents form defense as antibiotics, which facilitate the body to struggle against diseases and microbial attack as reported by Sodipo et al. (2000).

Different parts of *D. bupleuroides* showed different inhibitory effect against bacteria and fungi. In the antibacterial analysis different bacteria used of which gram positive bacteria include *Staphylococcus aureus* and *Bacillus subtilis* while gram negative includes *Escherichia coli* and *Pseudomonas aeruginosa*. This work associated with the study of earlier researchers who found that plants have compounds that are antimicrobial as studied by Olukoya et al. (1986). These bacteria cause various diseases so these strains were used in studies. For example, the gram positive bacteria such as *S. aureus* are a main reason of nosocomial diseases universally. *S. aureus* diseases are frequently hard to cure due to the huge heterogeneity, phenotypic changing, intra-strain variety, hypermutability and mainly the little settlement variations. It is very significant to highlight that congregation resistant reactions against constant diseases by *S. aureus* is inadequate resulting usually into chronic diseases, which in turn can direct to life aggressive conditions as documented by Costa et al. (2013).

Gram negative bacteria such as *P. aeruginosa* generally cause the cystic fibrosis and bronchiectasis. It creates numerous poisons that deliberate ciliary thump, rouse mucus creation and injure epithelium. It sticks on to epithelial cells, damaged mucosa and mucus. It causes various rigorous diseases, such as pneumonia or bacteraemia, and is linked with elevated death speeds as documented by Tsang et al. (1994) and *E. coli* can reason of diarrhea or hemorrhagic colitis in human beings. Hemorrhagic colitis infrequently developed the hemolytic uremic syndrome (HUS) which is a vital origin of severe renal breakdown in kids and death in adults. Other symptoms include abdominal cramps, nausea and vomiting compared with Ahn et al. (2009).

In leaves the highest zone formed in *B. subtilis* with diameter 17.0 ± 0.5 mm while the least zone formed showed in *E. coli* with diameter 9.6 ± 0.8 mm. In stem the highest zone obtained with *S. aureus* with diameter 19.0 ± 0.6 mm while the least zone obtained with *E. coli* with diameter 14.8 ± 1.0mm. In roots the greatest zone formed in *S. aures* with diameter 18.3±1.2mm while the least zone formed in *B. subtilis* with diameter 9.0 ± 0.5 mm. Leaves and roots extract showed activity against all tested bacteria whereas the extract of stem does not show any activity against *B. subtilis.* When these results were compared it was found that *D. bupleuroides* illustrate significant adjacent to them similar studies were also reported by Ajaib et al. (2020) during the evaluation of antimicrobial activity of *Strobilanthes glutinous*.

In antifungal activity two fungal strains *A. niger* and *A. oryza* were used. *A. niger* caused spergillosis whereas *A. oryza* caused various allergic reactions and produce diversity of mycotoxins. In present study stem and roots extract showed activity against *A. niger* whereas the extract of leaves does not show any activity. Extract of leaves and stem showed activity against *A. oryza* but extract of roots showed no activity. When the activity of these parts compared it was found that highest inhibitory concentration formed by leaves 12.3 ± 0.9 mm against *A. oryza* while the minimum zone of inhibition formed by extract of stem 9.7 ± 0.8 mm against *A. niger.* This recommends that the ethanolic extract of this plant showed wide range actions a somewhat similar finding was also discussed by Ajaib et al. (2017) while evaluation antifungal potential in *Cocculus laurifolius.*

Diabetes causes many secondary problems which are polyuria, polyphasia, ketosis, retinopathy and also considering the cardiovascular disorder. It affects the carbohydrate, protein and lipid metabolism, effecting in chronic hyperglycemia and irregularity of lipid shape (Kumar and Clark, 2002). Instead of the progress in field of science the extensive utilization of hypoglycemic agents, diabetes and related complications continue to be a major health problem world widely (Burke *et al.,* 2003). In anti-diabetic activity the ethanolic extract of leaves at dose 250 mg/kg reduced the blood glucose levels significantly the lowering of blood glucose was observed at 2 h which was 177.4±2.7*** and significant lowering was also observed at 24 h which was 189.4±9.1***. But the other plant parts such as stem and roots ethanolic

extract showed no significant decrease in blood glucose level. So the dose concentration increased to 500 mg/kg. At this concentration leaves extract as usually showed best results the lowering of blood glucose at 2 h was 183.8±10.3***and at 24 h was123.0±5.3*** depicting the potential of the extract to be used in the treatment of diabetes. The results are comparable with metformin suggesting that the extract decreases the processes of gluconeogenesis in liver and increases the utilization of the glucose in the glycolysis pathway. The extracts of leaves contain triterpenoids and flavonoids that might be responsible for increasing the anti-oxidative status of the body thus enhancing the body's immunity to combat the disease. Acute toxicity study revealed the safe nature of plant extract the results were compared with Ahmed et al. (2012) during investigation of antidiabetic effects of *Tetracera indica*.

CONCLUSION

This research was an effort that concludes ethno-medicinal values about *Dicliptera bupleuroides* Nees a wild plant belonging to family Acanthaceae. Qualitative phytochemical investigation detected existence of triterpenoids, sterols, glycosides, alkaloids, flavonoids proteins, carbohydrates and tannins in leaves, stem and roots extract. Except some metabolites such as saponin that was present in leaves and stem but absent in roots. Lipid was present in leaves but absent in stem and roots. It was concluded from results that most of metabolites were present in this plant. For the antimicrobial investigation ethanolic extract of leaves, stem and roots was used. It was concluded from the study that *D. bupleuroides* have antibacterial and antifungal activities and can be used to treat various diseases. All the results compared with standard antimicrobial drugs (Tetracycline, Cefoperazone, Erythromycin, Ampicillin and Terbinafine).

In anti-diabetic activity leaves extract of *D. bupleuroides* given significant results as compared to stem and root extract. All the results were compared with standard drug (Metformin) and control group. Studies showed that extracts of leaves contain triterpenoids and flavonoids that might be responsible for increasing the anti-oxidative status of the body thus enhancing the body's immunity to combat the diseases. It was concluded that leaves crude extract was as efficient as compared standard drug but further investigations such isolation and clinical trials are suggested before this plants used as medicine.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

AUTHOR CONTRIBUTIONS

MA designed and PR performleed the experiments and both also wrote the manuscript. SHK, SA, FS and KHB help experiments and reviewed the manuscript. All authors read and approved the final version.

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