INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA):IJPS00]
Journal Homepage: https://www.ijpsjournal.com

OPEN ACCESS

Research Article

Pharmacognostic Studies, Phytochemical Screening And Physicochemical Analysis Of Dicliptera Bupleuroide

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ARTICLE INFO

Received: 23 April 2024
Accepted: 27 April 2024
Published: 28 April 2024
Keywords:
Phytochemical, Analysis, Dicliptera Bupleuroide
DOI:
10.5281/zenodo.11080874

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. The purpose of this study is to perform its pharmacognostical study, its phytochemical screening and also its physicochemical analysis to understand the plant more.

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). Ischemic heart disease where acute myocardial infarction (AMI), cause deaths in various countries ether they developed or developing countries. Heart disease problem cause various deaths, according to WHO Cardiovascular diseases (CVDs) are the leading cause of death globally, taking an estimated 17.9 million lives each year (5). Myocardial infarction (MI) is the acute condition that occurs when there is imbalance between coronary blood supplies and does not full fill the myocardial oxygen demand (6). As the study goes on and different experiments

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

done which provide the clinical evidences which reveals the involvement of reactive oxygen species in cardiovascular diseases (7)(8). More specifically in pathological or disease conditions, such as MI, diabetes, or stroke, the production of free radicals may override the scavenging effects of antioxidants leading to oxidative stress (9)(10). Dicliptera bupleuroides possessed antioxidant, hepatoprotective, antimicrobial and other biological activities. It contains phenols, flavonoids, ascorbic acid, lipids, starch, glycosides and many other compounds (11)(12)(13). Thus, in this study, aim to perform a phytochemical investigation of leaves of Dicliptera bupleuroides plant to isolate its major compounds. Preliminary Qualitative Analysis is performed to determine different chemicals like alkaloids, carbohydrates, glycosides, proteins, flavonoids, terpinoides, etc.

MATERIAL AND METHOD

Plant material collection and extraction

The plant was collected from local fields of mandi, Himachal Pradesh. The plant were washed and leaves were separated and dried under shade and then the leaves was powdered. This powdered herb was then extracted by using a soxhlet extraction method and ethanol is used as solvent. 100gm of powder was extracted with 700 ml of ethanol for 6hr. The ethanolic extract of Dicliptera bupleuroides was concentrated with distillation method and evaporate excessive solvent.

Pharmacognostic Studies

Morphological Evaluation

The macroscopic evaluation of the plant Dicliptera bupleuroides is carried out to check its taste, odor, colorm shapem apex, nature, length, width, thickness, etc. The taxonomical description was made according to the related articles and the data given in books (14)(15).

Microscopical characters

Microscopic examination is employed for the quantitative evaluation and for this purpose some cell size measured by using micrometer (16)(17) and specific histological features including, stomatal index, vein-islet and vein termination number and palisade ratio were noted (18)(19).

Phytochemical analysis

Phytochemical analysis is perform to identify different phytochemical present in the leaf of Dicliptera bupleuroides by using different tests (20).

1. Test for Alkaloids

a. Mayer's test

Take few ml of plant sample extract, two drops of Mayer's reagent are added along the sides of test tube. Appearance of white creamy precipitate indicates the presence of alkaloids (21).

b. Wagner's test

A few drops of Wagner's reagent are added to few ml of plant extract along the sides of test tube. A reddish- Brown precipitate confirms the test as positive (22).

2. Test for Carbohydrates

a. Molish's test

To 2 ml of plant sample extract, two drops of alcoholic solution of α- naphthol are added. The mixture is shaken well and few drops of concentrated sulphuric acid is added slowly along the sides of test tube. A violet ring indicates the presence of carbohydrates.

b. Benedict's test

To 0.5 ml of filtrate, 0.5 ml of Benedict‟s reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic coloured precipitate indicates the presence of sugar.

3. Test for Fixed oils and Fats

a. Spot test

A small quantity of extract is pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oils.

b. Saponification test

A few drops of 0.5 N alcoholic potassium hydroxide solution is added to a small quantity of extract along with a drop of phenolphthalein. The mixture is heated on a water bath for 2 hours.

Formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats (23).

4. Test for Glycosides

For 50 mg of extract is hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate is subjected to the following tests.

a. Borntrager's test

To 2 ml of filtered hydrolysate, 3 ml of choloroform is added and shaken, choloroform layer is separated and 10% ammomia solution is added to it. Pink colour indicates presence of glycosides (21).

b. Legal's test

50 mg of extract is dissolved in pyridine, sodium nitroprusside solution is added and made alkaline using 10% NaOH. Presence of glycoside is indicated by pink colour.

5. Test for Phenolic compounds and Tannins

a. Ferric Chloride test

The extract (50 mg) is dissolved in 5 ml of distilled water. To this few drops of neutral 5% ferric chloride solution are added. A dark green colour indicates the presence of phenolic compound (24).

b. Gelatin test

The extract (50 mg) is dissolved in 5 ml of distilled water and 2 ml of 1% solution of Gelatin containing 10% NaCl is added to it. White precipitate indicates the presence of phenolic compounds (21).

c. Lead acetate test

The extract (50 mg) is dissolved in of distilled water and to this 3 ml of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of phenolic compounds.

d. Alkaline reagent test

An aqueous solution of the extract is treated with 10% ammonium hydroxide solution. Yellow fluorescence indicates the presence of flavonoids.

e. Magnesium and Hydrochloric acid reduction

The extract (50 mg) is dissolved in 5 ml of alcohol and few fragments of magnesium ribbon and concentrated hydrochloric acid (drop wise) are added. If any pink to crimson colour develops, presence of flavonol glucosides is inferred (25).

6. Test for Proteins

The extract (100 mg) is dissolved in 10 ml of distilled water and filtered through Whatmann No. 1 filter paper and the filtrate is subjected to test for proteins.

a. Millon's test

To 2 ml of filtrate few drops of Millon‟s reagent are added. A white precipitate indicates the presence of proteins (26).

b. Biuret test

2 ml of filtrate is treated with 1 drop of 2% copper sulphate solution. To this 1 ml of ethanol (95%) is added, followed by excess of potassium hydroxide pellets. Pink colour ethanolic layer indicates the presence of protein (31).

7. Test for Saponins

The extract (50 mg) is diluted with distilled water and made up to 20 ml. The suspension is shaken in a graduated cylinder for 15 minutes. A two cm layer of foam indicates the presence of saponins (23).

Physicochemical analysis

This analysis included the total ash, water soluble ash, acid insoluble ash and sulphated as value and these test were carried out according to standard procedure (27)(17).

Total Ash:

Accurately weighted 2 to 3 gm of air-dried leaves and bark in a tarred platinum or silica dish and incinerated at a temperature not exceeding 450oc until free from carbon, cooled and weighted. If the carbon free were not obtained, exhausted the charred mass with hot water. Collected the residue on an ashles filter paper incinerated the residue and filter paper until the ash turned white or nearly so, added the filterate, evaporate to dryness and ignited at a temperature not exceeding 450oc.

Percentage of ash with reference to the air dried drug was calculated (28).

Acid insoluble ash:

Ash was boiled with the 25ml of 2M hydrochlodric acid for 5 min, insoluble matter was collected in a Gooch crucible or on an ash less filter paper, washed with hot water, ignited, cooled in a desiccator and weighed. Percentage of acid insoluble ash with reference to their air-dried drug was calculated (29).

Water-soluble ash:

Boiled the ash for 5 min with 25ml of water, collected the insoluble matter in a Gooch crucible or on as ash less filter paper, wash with hot water, and ignited for 15min at a temperature not exceeding 450oc. Subtract the weight of insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash. Percentage of water-soluble ash with reference to the air-dried drug was calculated (30).

RESULT

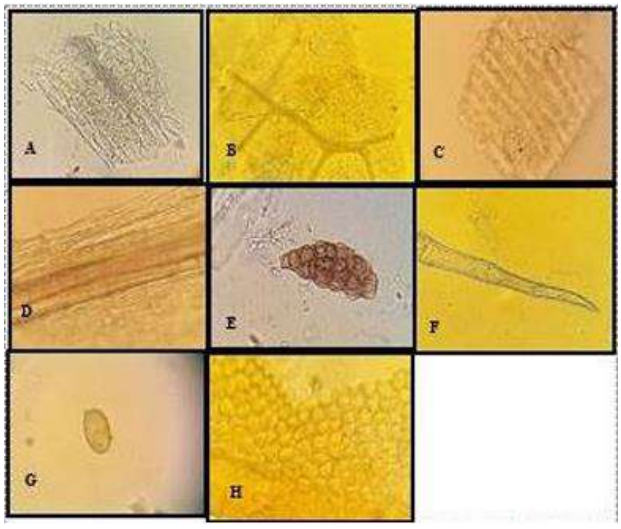
Morphological Evaluation

The plant Dicliptera bupleuroides is also commonly known by the local people krech and kali booti, which is 1-2 feet in height and leaves are ovate and 2-6cm long. Flowers are purple or pink in color.

Table 1. Morphological Study of leaf of D. bupleuroides.

Parameters	Leaf
Taste	Characteristic
Odor	Pungent
Color	Dark Green
Texture of powder	Fine
Shape	Ovate
Margin	Linear
Apex	Obtuse
Length	5-17 ± 2.68 mm
Width	1.2-2.2 ± 0.216 cm
Thickness	0.1-0.4 ± 0.06 mm
Surface	Smooth

Microscopical characters



D. bupleuroides leaf A: epidermal tissue, B: epidermis with trichome base, C: epidermis with stomata, D: vessels in leaf lamina, E: head of glandular trichome, F: glandular trichome, G: pollen grain, H: palisade tissue.

Phytochemical Analysis

The qualitative phytochemical investigation of ethanolic extract of leaves extract of the Dicliptera bupleuroides showed the presence of different secondary metabolites like Terpenoids, flavonoids, tannins, alkaloids, glycosides, proteins, carbohydrates, saponins and also fats and fixed oils.

Table 2. Preliminary phytochemical analysis of leaves of D.bupleuroides

Phytochemical Groups	Name of test	Crude Extract
Terpenoids	Salkowaski test	+ + + +
	Liebermann's test	+ + + +
Tannins	Ferric Chloride test	+ + + +
	Bromine water test	+ + + +
Glycosides	Bromine water test	+
	Keller killani test	+
	Legal 's test	+ +
Flavonoids	Alkaline reagent test	+ + +
	Lead acetate test	+ + +
Alkaloids	Mayer 'test	+ +
	Wagner 'test	+ +
	Hager 's test	+ +
Proteins	Millon 's test	+ +
	Ninhydrin test	+ +
Carbohydrates	Molisch 's test	+ + +
	Benedicts 's test	+ + +
Saponins	Foam test	+
Fats and fixed oil	Spot test	+

Physicochemical analysis

The ash value of the herb showed high content of total ash and water soluble ash followed by acid insoluble ash

Table 3. Ash Values of D. bupleuroides

Parameter	Value % (w/w)
Total ash	35.55 ± 0.336
Acid insoluble ash	16.28 ± 0.171
Water soluble ash	1.936 ± 0.028
Sulphated ash	39.806 ± 0.057

CONCLUSION

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

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HOW TO CITE: Munish Choudhary, Dev Prakash Dahiya, Chinu Kumari, Bhopesh Kumar, Anita, Dinesh Kumar Thakur, Pharmacognostic Studies, Phytochemical Screening And Physicochemical Analysis Of Dicliptera Bupleuroide, Int. J. of Pharm. Sci., 2024, Vol 2, Issue 4, 1241-1253. https://doi.org/10.5281/zenodo.11080874