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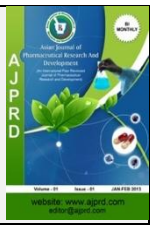

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Review Article

An Overview of Phytochemical Identification of Secondary Metabolites of Medicinal Plant Dactylorhiza Hatagirea

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A B S T R A C T

Plants are a valuable source of new natural products. Despite the availability of different approaches for the discovery of therapeutically, natural products still remain as one of the best reservoirs of new structural types. The standardized extracts of plants, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity. According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. HPLC is a versatile, robust, and widely used technique for the isolation of natural products, HPLC is a chromatographic technique that can separate a mixture of compounds and is used in phytochemical and analytical chemistry to identify, quantify and purify the individual components of the mixture. Currently, this technique is gaining popularity among various analytical techniques as the main choice for fingerprinting study for the quality control of herbal plants. Natural products are frequently isolated following the evaluation of a relatively crude extract in a biological assay in order to fully characterize its properties. Early studies showed that *Dactylorhiza hatagirea* root are rich source of phytochemicals including flavonoids, phenolics, alkaloids. Therefore this study was conducted with the aim to investigate phytoconstituents present in *Dactylorhiz ahatagirea* root and assessment of their antioxidant characteristics.

Key words: Investigate, Phytoconstituents, Antioxidant, and Characteristics.

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INTRODUCTION

Dactylorhiza hatagirea (D.Don) Soo belongs to the family Orchidaceae. The plant is native and near endemic to Indian Himalayan region [26]. Its distribution extends to Pakistan, Afghanistan, Nepal, Tibet and Bhutan. In India, it is reported from Jammu and Kashmir, Sikkim, Arunachal Pradesh, Uttarakhand, and Himachal Pradesh [1]. Generally, it is widely and narrowly distributed at an altitudinal ranges between 2500 to 5000 m amsl in open grassy slopes and alpine meadows [2]. It is commonly known as panja, salam-panja, hath-panja or hatajari in Uttarakhand; salempanja in Kashmir and wanglak or angulagpa in various parts of Ladakh. Generally, the plant is a perennial herb, up to 60 to 70 cm in height, having palmately lobed, divided root tubers with broadly lanceolate leaves arranged more or less along the stem and purple flowers, but some time white.

Scientific classification

Kingdom: Plantae
(Unranked): Angiosperms
(Unranked): Monocots
Order: Asparagales
Family: Orchidaceae
Subfamily: Orchidoideae
Genus: Dactylorhiza
Species: D. incarnate

Figure 1: Different parts of *Dactylorhiza hatagirea*

Habitat

As it is highly traded in the name of 'Panchaule' or 'Salampanja' and found in wild, is being unscientifically collected for its commercial importance. *Dactylorhiza hatagirea* is native of the Himalaya. It is found throughout from west to east at temperate to subalpine biocliates within 2800 – 4000 m altitude. Flowers spotted rosy-purple in a terminal spike, borne on a robust leafy stem. It has palmately lobed root tubers, grows well in moist places, open areas, shrub land and open meadows.

Uses

The Juice extracted from tuber is used as tonic and also used for the treatment of pyorrhea (inflammation of the gum & teeth). Root paste is externally applied as poultice on cuts and wounds and extract is given in intestinal disorders. The term HattaHaddi is probably coined because it is used for treating bone fractures.

Chemistry

The plant tubers of *D. hatagirea* contain a glucoside, abitter substance, starch, mucilage, albumen, a trace of volatile oil and ash [3]. Chemically, dactylorhins A to E, dactyloses A and B and lipids, etc., are found as major constituents.

Pharmacological activity

According to Ranpal,[4] 2009 rhizomatous part of *D. hatagirea* has shown resistance against all Gram positive and Gram negative bacteria, but its aerial part has shown limited resistance against some bacteria. Zonation of inhibitions (ZOIs) between the two parts of *D. hatagirea*, the rhizome part is more effective than the aerial part against all tested organisms, except Escherichia coli. Further, it is interesting to note that *E. coli*, one of the very resistant bacteria to synthetic drugs, was found to be very susceptible to the extract of this plant. This finding is distinctive from the folkloric uses *D. hatagirea*. Hence, this plant can be a potential source for evolving newer antimicrobial compounds for treating dysentery caused by *E. coli* As per Thakur and Dixit, 2007)[5], the herb shows the effectiveness in improving and preventing the functionality of sexual organ and may be helpful in improving the sexual behavior and performance also. The results also corroborate the hype that the plant is capable of being nominated as herbal cure for sexual dysfunction. There is also, sufficient evidence that the plant increase testosterone level in adult male rats. Clinical data on testosterone also suggest that a slightly increased level of testosterone in adult males results in an increased sexual desire and arousability[6].

Materials and methods

Phytochemical Screening:-[7, 8,9]

pH Determination:-

1% solution: 1 gm of the accurately weighed drug is dissolved in water and filtered pH of filtrate is checked with a standardized glass electrode.

pH 10% solution:10gm of the accurately weighed drug is dissolved in water and filtered. pH of filtrate is checked with standardized glass electrode.

Table: 1 pH of powder drug:

pH	1 % solution	10 % solution
	7.55	7.32

Fluorescence analysis

XRF is employed for the qualitative and quantitative analysis of liquids and solids in order to determine their chemical composition. It is frequently used in the metal industry for examining glass, ceramics, building materials as well as for analysinglubricants and mineral oil products. Practical detection limits are around a few mg/kg.

Table 2: Fluorescence analysis

Chemical treatment	Fluorescence Observed	
	Under visible light	Under uv light
Powder as such	Green	Light green
Powder + 1N NaOH in methanol	Yellowish green	Pale green
Powder + 1N NaOH in water	Light red	Dark red
Powder + 50%HCL	Light yellow	Pale green
Powder + 50%HNO3	Light yellow	Pale green
Powder + 50%H2SO4	Dark green	Green
Powder + Petroleum ether	Dark green	Dark green
Powder + chloroform	Black	Dark green
Powder + picric acid	Green	Dark green
Powder + 5%Ferric chloride solution	Black	Light green
Powder + 5% Iodine solution	Brown	Light brown
Powder + Methanol	Dark brown	Black

Preliminary Phytochemical Screening

Detection of Carbohydrates

Molish test - To about 2 ml of a alcoholic extract few drops of a napthol (20% in ethyl alcohol)was added .then about 1 ml of conc.H2so4 was added along the side of the test tube. Reddish violet ring at the junction of the two layers appered in the presence of carbohydrates.

Fehling test:-The extract 0.5 g is heated with dilute HCL to hydrolyse polysaccharide. The reaction mixture is neutralized by adding Na0H solution and then Fehling's solution 1 and 2 are added. Red precipitate is formed in case of reducing sugars or carbohydrates.

Benedicts test: - To 2 ml of benedicts reagents, add 1ml of the sugar solution and keep it in the boiling water bath for minute, formation of green, yellow or red ppt in notised carbohydrates presents.

Barfoed test: - 1 ml of test solution is heated with 1 ml of barfoed's reagent on water bath, if red cupric oxide is formed, monosaccharide is present. Disaccharides on prolong heating (about 10 min.) May also cause reduction owing to partial hydrolysis to monosaccharide.

Test for Starch

Test for Gums and Mucilages:-

A) Treat the test solution with ruthenium red solution, pink color observed

B) Treat test solution with chine ink, transparent, spherical dilated fragments on Black back Grounds are observed.

C) Treat the test solution with thionic solution .after 15 min wash with alcohol Mucilage turns violet red.

Test for Proteins and Amino Acid

A) Ninhydrin test: - To the test solution add ninhydrin solution, boil, violet color indicates presence of amino acid.

B) Biuret test: - To the test solution (2) ml add biuret reagent (2ml),violet colour indicates presence of proteins.

C) Millions test: - To the test solution add about 2 ml of millions reagent ,whiteppt indicate presence of amino acid.

D) Xanthoproteictest :-T o the test solution (5ml) add 1 ml of conc. Nitric acid and boil ,yellow ppt is formed, after cooling it, add 40% sodium hydroxide solution, orange color is formed.

Test for Fixed Oils and Fats

Spot test: -Press drug in between filter paper and left few second, if stain

Persist, Then fixed oils are present.

Saponification test :- Add few drop of .5 n alcoholic potassium hydroxide to a small quantity of various extract along with a drop of phenolpthlein separately and with a drop of phenolpthlein separately and heat on a water bath for 1-2 hour.the formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

Test for Alkaloids

Dragendroff's reagent: - Alkaloids gives reddish brown precipitate with dragendroff's reagents (potassium bismuth iodide)

Mayer's reagent test: - Alkaloids gives reddish brown precipitate with wagnar's reagent. (Iodine in potassium iodide)

Wagners reagent test:-Alkaloids gives reddish brown precipitate with wagnar'sreagent.

Hager's reagent test: - Alkaloids gives yellow precipitate with hager's reagent. (Saturated solution of picric acid)

Tannic acid test: - Alkaloids gives buff colour precipitate with tannic acid solution.

Test for Glycocides

Legal's test: - Treat the test solution with pyridine and alkaline sodium nitroprusside solution,blood red colour appears.

Baljet test: - Treat the test solution with picric acid or sodium picrate orange colour is formed.

Bontrager's test:- Boil the test materials with 1 ml of sulphuric acid in a test tube for 5 min filter while hot. cool the filterate and shake with equll volume of dichloromethane or chloform separate the lower layer of dichlomethene or chloform and shake it with half of its volume of dilutes ammonia. a rose pink to red colour is produced in the ammonical layers.

Test for cardiac glycocides (keller –killiani test) :- Extract the drug with chloform and evaporate it to dryness .add 0.4 ml of glacial acetic acid containing trace amount of ferric chloride.transfer to a small test tube,add carefully 0.5 ml of conc. H2so4 by side of the test tube .acetic acid layers show blue colour.

Test for cyanogeneticglycocides: - Prepare a solution of guaiacum resin in absolute alcohol and allow it to dry on paper.Treat it with copper sulphate solution .the paper turns blue due to prussic acid with HCN.

Test for Phytosterols

Liebermann's test: - The residue was dissolved in conc.sulphuric acid and a few drops of aq. Sodium nitrate solution were added,Appearance of red blue when made alkaline with aq. Sodium hydroxide indicating.

Liebermann-Burchard's test: - Treat the extract with few drops of acetic anhydride,boil and cool,then add conc. H2so4 from the side of the test tube,brown ring is formed at the junction two layers and upper layers turns green which shows presence of steroids and formation of deep red colour indicates presence of triterpennoids.

Salkowski's test: - Treat the extract with few drops of conc. H2so4,red color at lower layer indicate presence of steroids and formation of yellow coloured lower indicate presence of triterpenoids.

Test for Flavonoids

Shinodatest : Treat dry powder and extract with 5 ml of 95% ethanol and few drops conc. Hydrochloric acid and 0.5 gm magnesium turning pink colour observed.

Lead acetate test : Add small quantity of lead acetate in the residue, yellow colourpricipitation formed.

Addition of increasing amount of sodium hydroxide to the residue shows colouration, which decolorize after addition of acids.

Test for Tannins and Phenolic Compounds:-

Ferric chloride test – Treat the extract with ferric chloridesolution ,bluecolourapperes if hydrolysable tannins are present and green colour appears if condensed tannins are presents.

Phenazone test :- Add aboutn0.5 gm of sodium acid phosphate to 5 ml of aqueous extract .warm it and filter ,to the filtrate add 2% phenazone solution bulky ppt are formed ,which often coloured.

Test for chlorogenic acid: - treat the test solution with aqueous ammonia and expose to air gradually, green colour is developed.

Test for Saponins:

Foam test: - Place 2 ml solution of in water in a test tube, shake well, stable froth (foam) is formed.

Heamolysis test:- Add 0.2 ml of solution of saponin (prepared in 1% normal saline)to 0.2 ml of blood in normal saline and mix well .centrifuge and note the red supernatant compare with controls tube containing 0.2 ml of 10 % blood in normal saline diluted with 0.2 ml of normal saline.

Test for Volatile Oils:

A. To a thin section of drug add sudan 3rd solution, red colour obtained by globules indicates presence of volatile oil.

B. To a thin section of drug add few drops of tincture alkana; red colour indicates presence of volatile oils.

RESULT AND DISCUSSION

Table 3: Phytochemical screening of plant extract

S.no.	Test		Powder drug	Pet ether	Methanol
1	**Carbohydrates**	Molish test Fehling test Benedicts test Iodine test	+ve +ve +ve +ve	-ve +ve -ve -ve	+ve +ve +ve +ve
2	**Gum and Mucilage**	Rhedanium red Swelling property	+ve +ve	-ve -ve	+ve -ve
3	**Protein and Amino acid**	Biuret test Ninhydrine test Heavy metal test Xanthoproteic test	+ve +ve +ve +ve	-ve -ve +ve -ve	+ve +ve +ve +ve
4	**Fixed oils and Fats**	Spot test Saponification test	+ve +ve	-ve -ve	-ve -ve
5	**Alkaloids**	Mayers test Dragendroff test Hager's test Wagnar test	-ve +ve +ve +ve	-ve -ve -ve +ve	-ve -ve -ve +ve
6	**Glycocides**	Legal test Bontrager test Killer killiani test Baljet test	+ve -ve +ve +ve	+ve -ve +ve -ve	+ve -ve -ve -ve
7	**Phytosterol**	Salkowaski test Libermannsbuchard Libermann test	+ve -ve +ve	+ve -ve Slightly +ve	+ve -ve +ve
8	**Flavanoids**	Ferric chloride test Shinoda test Lead acetate Reaction with acid and base	+ve -ve +ve -ve	-ve -ve +ve -ve	-ve -ve +ve -ve
9	**Tannin and Phenolic compound**	Ferric chloride test Lead acetate test Pot.dichromate test Copper sulphate	+ve +ve +ve +ve	+ve -ve +ve +ve	+ve -ve -ve Slightly +ve
10	**Saponins**	Foam test	+ve	-ve	+ve
11	**Volatile oils**	H Hydrodistillation	-ve	-ve	-ve

CONCLUSION

Phytochemical studieswere focused on preparation of petroleum ether and methano extract of **Dactylorhiza Hatagirea.** Both extracts and **Dactylorhiza Hatagirea** leaf powder were studied to identify the presence of specific phytoconstituents in them. It was identified that **Dactylorhiza Atagirea** contains carbohydrates, flavanoids, glycocides, steroids, tannins, proteins and amino acid, alkaloids, saponins, etc. Whereas petroleum ether extract contains steroids, antraquinoneglycocides, reducing sugar, flavanoids, amino acid and tannins . And methanol extract contains carbohydrates, proteins, amino acid, steroids, tannins, phenolic compound, flavonoids, saponins.

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