EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 12
383-388
Year: 2018

QUALITATIVE ANALYSIS OF *ACORUS COLATOLAMUS* AND *ACONITUM HETEROTHYLLUM*

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Article Received on 16/10/2018 **Article Revised on 06/11/2018** **Article Accepted on 27/11/2018**

ABSTRACT
Preliminary pharmacognostical standardization studies of the *Acorus Colatolamus* and *Aconitum Heterothyllum* other physical values and parameters will help to identify the species of plant. Photochemical screening refers to the extraction, screening and identification of the medicinally active substances found in plants. Some of the bioactive substances that can be derived from plants are carbohydrates, flavonoids, alkaloids, glycosides, steroids are present in *Acorus Colatolamus* and *Aconitum Heterothyllum* may carbohydrate contains fehling and bendicts test, protein with sulphur, precepetate test with 5% $HgCl_2$ and 5% lead acetate and cardiac glycoside in Beljet test are present. It is effective for digestive ailments such as flatulence, loss of appetite, abdominal dull pain and worms. *Aconitum heterophyllum* are cooling in potency and bitter in taste. They are used as expectorant, febrifuge, anthelmintic, anti-diarrhoeal, anti-emetic, and anti-inflammatory.

KEYWORD: *Acorus Colatolamus, Aconitum Heterothyllum.*

INTRODUCTION
Acorus calamus (also called **sweet flag** or **calamus**, among many common names is a species of flowering plant, a tall wetland monocot of the family Acoraceae, in the genus *Acorus*. Sweet flag (Acorus calamus) is commonly known drug in traditional system of medicine. It is a tall perennial wetland monocot plant from the Acoraceae family. The scented leaves and rhizomes of sweet flag have been traditionally used as a medicine and the dried and powdered rhizome has a spicy flavour and is used as a substitute for ginger, cinnamon and nutmeg for its odour.[1] Due to varied uses, there has been demand for the plant. The rhizomes are considered to possess anti-spasmodic, carminative, anthelmintic, aromatic, expectorant, nauseate, nervine, sedative, stimulant properties and also used for the treatment of epilepsy, mental ailments, chronic diarrhoea, dysentery, bronchial catarrh, intermittent fevers, glandular and abdominal tumors.

Taxonomical Classification
Kingdom: Plantae Subkingdom: Tracheobionta Super division: Spermatophyta Division: Magnoliophyta Class: Liliopsida Subclass: Arecida Order: Arales Family: Acoraceae Genus: Acorus Species: Calamus Vernacular Name Arabic: Vaj, Vash, Oudul Vaj; Sanskrit: Bhadra, Bhutanashini, Vacha; Hindi: Bach, Ghorbach, Safed bach; Gujarati: Gandhilovaj, Godavaj; Kashmir: Vachi, Vaigandar; Persian: Agar, Agarturki, Kannada: Baje, Vasa; English: Sweet flag, Calamus, Myrtle grass; Urdu: Bach, Vaj; Tamil: Vashambu, pullai-valathi; Nepali: Bojho; Ayurvedic: Vacha; Unani: Vaj turki, Bacch; Italy: Plant of Venus.

Traditional Uses
The rhizomes of sweet flag (Acorus calamus) are used for numerous medicinal purposes. The herb is used both internally as well as externally. In rheumatism, rheumatic fever and inflamed joints, the paste applied externally alleviates the pain and swelling. Internally sweet flag is valuable in a vast range of diseases. It is effective for digestive ailments such as flatulence, loss of appetite, abdominal dull pain and worms. The powder of sweet flag given with lukewarm salt-water, induces vomiting and relieves phlegm, while easing coughs and asthma.[2]

Aconitum Heterophyllum
Aconitum Heterophyllum (A. Heterophyllum) is an ayurvedic medicinal plant used as the main ingredient in many formulations mentioned in the Ayurvedic formulary of India. Aconitum species are also used as major components in Chinese and Bhutanese herbal medicines. This plant is also known in Indian English as atees and atis root, in Sanskrit as ativisha, shuklakanda, aruna, and vishada, in Urdu as atees, in Hindi as atis and atvika, in Bengali as ataish, in Telugu as ati vasa, in

Guajarati as ativakhani, in Marathi as ati vish, in Kannada as ati-vishsa, in Malayalam as ati-vidayam, and in Panjabi as atis.[3]

Kingdom: Plantae
Clade: Angiosperms
Clade: Eudicots
Order: Ranuculales
Family: Ranunculaceae
Subfamily: Ranuculoideae
Tribe: Dephinieae
Genus: Aconitum

Uses
Tubers of *Aconitum heterophyllum* are cooling in potency and bitter in taste. They are used as expectorant, febrifuge, anthelmintic, anti-diarrhoeal, anti-emetic, and anti-inflammatory. They are also used against poisoning due to scorpion or snake bite and to cure fever and contagious diseases. The aqueous extract of the root induces hypertension through action on the sympathetic nervous system and in higher doses, it becomes lethal.

Traditional Use
Aconite has long been used in Ayurveda and traditional Chinese medicine. Aconite was also described in Greek and Roman medicine by Theophrastus, Dioscorides and Pliny the Elder, who most likely prescribed the Alpine species *Aconitum lycoctonum*. Folk medicinal use of *Aconitum* species is still practiced in some parts of Slovenia.

Aconitum chasmanthum is listed as critically endangered. *Aconitum heterophyllum* as endangered and *Aconitum violaceum* as vulnerable due to over collection for Ayurvedic use.[4]

2. MATERIAL AND METHOD

2.1 Collection of Plant Material

The leaves of *Acorus Colatolamus* and *Aconitum Heterothyllum* collected from Botonical garden of RDS College of Pharmacy.

3. QUALITATIVE CHEMICAL INVESTIGATION OF EXTRACTS

3.1 Photochemical screening of extracts

Photochemical screening refers to the extraction, screening and identification of the medicinally active substances found in plants. Some of the bioactive substances that can be derived from plants are carbohydrates, flavonoids, alkaloids, glycosides, steroids, fats, tannins and phenolic compounds.

4. Tests for Carbohydrates

4.1. (Molish's test General test)
Took 2-3 ml aqueous extract, added few drops of naphthol solution in alcohol, shaken and added concentrated H_2SO_4 from sides of the test tube was observed for violet ring at the junction of two liquids.

4.1.1 Fehling's test: 1 ml Fehling's A and 1ml Fehling's B solutions was mixed and boiled for one minute. Added equal volume of test solution. Heated in boiling water bath for 5-10 min was observed for a yellow, then brick red precipitate.
4.1.2 Benedict's test: Equal volume of Benedict's reagent and test solution in test tube were mixed. Heated in boiling water bath for 5 min. Solution may appear green, yellow or red depending on amount of reducing sugar present in test solution.

4.2. Tests for Monosaccharides
4.2.1 Barfoed's test: Equal volume of Barfoed's reagent and test solution were added. Heated for 1-2 min, in boiling water bath and cooled. Red precipitates were observed.
4.2.2 Tests for Hexose Sugars
Cobalt-chloride test: 3 ml of test solution was mixed with 2ml cobalt chloride, boiled and cooled. Added $FeCl_3$ drops on NaOH solution. Solution observed for greenish blue (glucose), purplish (Fructose) or upper layer greenish blue and lower layer purplish (Mixture of glucose and fructose).
4.2.3 Tests for Non-Reducing Sugars
a) Test solution does not give response to Fehling's and Benedict's test.
b) Tannic acid test for starch: With 20% tannic acid, test solution was observed for precipitate.

4.3. Tests for Proteins
4.3.1. Biuret test (General test): Took 3 ml of T.S. added 4% NaOH and few drops of 1% $CuSO_4$ solution observed for violet or pink color.
4.3.2. Million's test (for proteins): Mixed 3 ml of T.S. with 5 ml Million's reagent, white precipitate. Precipitate warmed turns brick red or precipitate dissolves giving red colour was observed.
4.3.3. Xanthoprotein test (For protein containing tyrosine or tryptophan): Mixed 3 ml of T.S. with 1 ml concentrated H_2SO_4 observed for white precipitate.
4.3.4. Test for protein containing sulphur: Mixed 5 ml of T.S. with 2 ml 40% NaOH and 2 drops 10% lead acetate solution. Solution was boiled it turned black or brownish due to PbS formation was observed.
4.3.5. Precipitation test: The test solution gave white colloidal precipitate with following reagents:

- absolute alcohol
- 5% $HgCl_2$ solution
- 5% $CuSO_4$ solution
- 5% lead acetate
- 5% ammonium sulphate

4.4. Tests for Steroid
4.4.1. Salkowski Reaction: Took 2 ml of extract and 2 ml chloroform and 2 ml concentrated H_2SO_4 was added. Shacked well, whether chloroform layer appeared red and acid layer showed greenish yellow fluorescence was observed.
4.4.2. Liebermann-Burchard Reaction: Mixed 2ml extract with chloroform. Add 1-2 ml acetic anhydride

and 2 drops concentration H_2SO_4 from the side of test tube observed for first red, then blue and finally green colour.

4.4.3 Libermann's reaction: Mixed 3 ml extract with 3 ml acetic anhydride. Heated and cooled. Added few drops concentrated H_2SO_4 observed for blue colour.

4.5 Tests for Amino Acids

4.5.1 Ninhydrin test (General test):- 3 ml T.S. and 3 drops 5% Ninhydrin solution were heated in boiling water bath for 10 min. observed for purple or bluish colour.

4.5.2 Test for Tyrosine: Heated 3 ml T.S. and 3 drops Million's reagent. Solution observed for dark red colour.

4.5.3 Test for tryptophan: Take 3 ml T.S. added few drops glyco-oxalic acid and concentrated H_2SO_4 observed for reddish violet ring at junction of the two layers.

4.6. Tests for Glycosides

4.6.1 Tests for Cardiac Glycosides

4.6.1.1Baljet's test:- A test solution observed for yellow to orange colour with sodium picrate.

4.6.1.2 Legal's test (For cardenoloids):- Took aqueous or alcoholic test solution, added 1 ml pyridine and 1 ml sodium nitroprusside observed for pink to red colour.

4.6.1.3 Test for deoxysugars (Kellar Killani test):- Took 2 ml extract added glacial acetic acid, one drop of 5% $FeCl_3$ and concentrated H_2SO_4 observed for reddish brown colour at junction of the two liquid and upper layers bluish green.

4.6.1.4 Libermann's test (For bufadenolids):- Mixed 3 ml extract with 3 ml acetic anhydride. Heated and cooled. Added few drops concentrated H_2SO_4 observed for blue colour.

4.6.2. Tests for Saponin Glycosides

4.6.2.1 Foam test: The drug extract or dry powder was shaking vigorously with water. Persistent foam was observed.

4.6.2.2 Haemolytic test: Added test solution to one drop of blood placed on glass slide.

Hemolytic zone whether appeared was observed.

4.6.3. Tests for Coumarin Glycosides

Test solution when made alkaline, observed for blue or green fluorescence.

4.7. Tests for Flavonoids

4.7.1 Shinoda test: To dried powder or extract, added 5 ml 95% ethanol, few drops concentrated HCl and 0.5 g magnesium turnings. Pink colour was observed.

4.7.2 To small quantity of residue, added lead acetate solution observed for Yellow coloured Precipitate.

4.7.3 Addition of increasing amount of sodium hydroxide to the residue whether showed

Yellow coloration, which was decolorized after addition of acid, was observed.

4.7.4 Ferric chloride test: - Test solution, added few drops of ferric chloride solution observed for intense green colour.

4.8. Tests for Alkaloids

4.8.1 Dragendroff's test: Took 2-3 ml test solution added few drops Dragendroff's reagent observed for orange brown precipitate.

4.8.2 Mayer's test:- Took 2-3 ml test solution with few drops Mayer's reagent observed for precipitate.

4.8.3 Hager's test:- Took 2-3 ml test solution with Hagers reagent observed for yellow precipitate.

4.8.4 Wagner's test:- Took 2-3 ml test solution with few drops of Wagner's reagent observed reddish brown precipitate.[5,6]

4.9. Tests for Tannins and Phenolic Compounds

Took 2-3 ml test solution, added few drops of whether showed following was observed:-

a) 5% $FeCl_3$ solution : Deep blue-black coloured.
b) Lead acetate solution : White precipitate.
c) Gelatin solution : White precipitate.
d) Bromine water : Decoloration of bromine water.
e) Acetic acid solution : Red colour solution.
f) Potassium dichromate : Red precipitate.
g) Dilute iodine solution : Transient red colour.
h) Dilute HNO_3 :- Reddish to yellow colour.

RESULT

1. Drug - *Acorus Colatolamus*

S.No	Test	Positive & Negative
A-	**Test for carbohydrate**	
	Molish Test(General Test)	Negative
	For reducing Sugars	
a.	Fehlingh Test	Positive
b.	Bendicts Test	Positive
	Test for Monosaccharides	
	Barfoedf test	Negative
	Test for hexose sugars	
	Test non reducing sugars	
a.	Test solution does not gravitate response to fehlingh & venedicts Test	
b.	Tannic Acid Test for Starch:	
	With 20% Tannic Acid test solution was observe precipitate	Negative
B.	**Test for proteins**	
a.	Biuret test (General Test)	Negative
b.	Milliions Test (for Portions)	Negative
c.	Xanthoprotein Test (for protines contining, Try Ptophan)	Negative
d.	Test for Protines containing sulphur	Positive
e.	Precipipation Test	
i)	Absolute-Alcohal	Negative
ii)	5% Hgcl2 Solution	Positive
iii)	5% Cuso4 Solution	Negative
iv)	5% Lead Acetate	Positive
v)	5% Ammonium Sulphate	Negative
C.	**Test for steroid**	
a.	Salkowski Reaction	Negative
b.	Liebermann-Burchard Reaction	-
c.	Libermann's reaction	-
D	**Tests for Amino Acids**	
a.	Ninhydrin test (General test):	Negative
b.	Test for Tyrosine	Negative
c.	Test for tryptophan	-
E	**Tests for Glycosides:**	
I	Tests for Cardiac Glycosides	
a.	Baljet's test	Positive
b.	Legal's test (For cardenoloids)	Negative
c.	Test for deoxysugars (Kellar Killani test)	Negative
d.	Libermann's test (For bufadenolids)	-
II	Tests for Saponin Glycosides:-	
a.	Foam test	Positive
b.	Haemolytic test	-
III	Tests for Coumarin Glycosides:-	Negative
F	**Tests for Flavonoids**	
a.	Shinoda test	-
b.	To small quantity of residue, added lead acetate solution observed for Yellow coloured precipitate.	Positive
c.	Addition of increasing amount of sodium hydroxide to the residue whether showed yellow colouration, which was decolourised after addition of acid was observed.	Negative
d.	Ferric chloride test	Negative
G.	**Tests for Alkaloids**	
a.	Dragendroff's test:	Negative
b.	Mayer's test	Negative
c.	Hager's test	Negative
d.	Wagner's test	Negative

H.	**Tests for Tannins and Phenolic Compounds**	
a.	5% $FeCl_3$ solution	Negative
b.	Lead acetate solution	Negative
c.	Gelatin solution	-
d.	Bromine water	-
e.	Acetic acid solution	Negative
f.	Potassium dichromate	Negative
g.	Dilute iodine solution	Negative
h.	Dilute HNO_3	Negative

2. Drug - *Aconitum Heterothyllum*

S.No	Test	Positive & Negative
A-	**Test for carbohydrate**	
	Molish Test(General Test)	Positive
	For reducing Sugars	
a.	Fehlingh Test	Positive
b.	Bendicts Test	Positive
	Test for Monosaccharides	
	Barfoedf test	Negative
	Test for hexose sugars	
	Test non reducing sugars	
a.	Test solution does not gavetave response to fehlingh & venedicts Test	-
b.	Tannic Acid Test for Starch:	-
	With 20% Tannic Acid test solution was observe precipitate	Negative
B.	**Test for proteins**	
a.	Biuret test (General Test)	Negative
b.	Milliions Test (for Portions)	Negative
c.	Xanthoprotein Test (for protines contining, Try Ptophan)	Negative
d.	Test for Protines containing sulphur	Negative
e.	Precipipation Test	
i)	Absolute-Alcohal	-
ii)	5% Hgcl2 Solution	Negative
iii)	5% Cuso4 Solution	Negative
iv)	5% Lead Acetate	Negative
v)	5% Ammonium Sulphate	Negative
C.	**Test for steroid**	
a.	Salkowski Reaction	Negative
b.	Liebermann-Burchard Reaction	-
c.	Libermann's reaction	-
D.	**Tests for Amino Acids**	
a.	Ninhydrin test (General test):	Negative
b.	Test for Tyrosine	Negative
c.	Test for tryptophan	-
E.	**Tests for Glycosides:**	
	Tests for Cardiac Glycosides	
a.	Baljet's test	Positive
b.	Legal's test (For cardenoloids)	Negative
c.	Test for deoxysugars (Kellar Killani test)	Negative
d.	Libermann's test (For bufadenolids)	-
	Tests for Saponin Glycosides:-	
a.	Foam test	Negative
b.	Haemolytic test	-
	Tests for Coumarin Glycosides:-	Negative
F	**Tests for Flavonoids**	
a.	Shinoda test	-
b.	To small quantity of residue, added lead acetate solution observed for Yellow coloured precipitate.	Negative

c.	Addition of increasing amount of sodium hydroxide to the residue whether showed yellow colouration, which was decolourised after addition of acid was observed.	Negative
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c.	Hager's test	Negative
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a.	5% $FeCl_3$ solution	Negative
b.	Lead acetate solution	Negative
c.	Gelatin solution	-
d.	Bromine water	-
e.	Acetic acid solution	Negative
f.	Potassium dichromate	Negative
g.	Dilute iodine solution	Negative
h.	Dilute HNO_3	Negative

Photochemical screening refers to the extraction, screening and identification of the medicinally active substances found in plants. Some of the bioactive substances that can be derived from plants are carbohydrates, flavonoids, alkaloids, glycosides, steroids are present in *Acorus Colatolamus* and *Aconitum Heterothyllum* may carbohydrate contains fehling and bendicts test, protein with sulphur, precepetate test with 5% $HgCl_2$ and 5% lead acetate and cardiac glycoside in Beljet test are present. The most active extracts could be subjected for further pharmacological evaluation by isolation of the therapeutic anti inflammatory, anti emetic and further research on this plant can specify its pharmaceutical application.

CONCLUSION

Preliminary pharmacognostical standardization studies of the *Acorus Colatolamus* and *Aconitum Heterothyllum* other physical values and parameters will help to identify the species of plant. The most active extracts could be subjected for further pharmacological evaluation by isolation of the therapeutic antinflammetory and further research on this plant can specify its pharmaceutical application.

ACKNOWLEDGEMENT

The Department of pharmacognosy, R.D.S College of Pharmacy is acknowledged for their support in this study.

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