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QUALITATIVE ANALYSIS OF *HOLOPTELAE INTEGREFOLIA* AND *PLUMBAGO ZEYLANICA*

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ABSTRACT
Holoptelae Integrefolia has been known to be protease inhibitor. Mucilage and juice obtained from boiled bark has been reported to be useful in rheumatism, intestinal tumour when applied externally. 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 *Holoptelae Integrefolia* are carbohydrates, monosaccharide, proteins, precipitated test, steroid amino acids, cardiac glycoside, tannin and phenolic compound may absent but saponin glycoside, flavonoids with NaOH, alkaloids in wagner's test may contains present and *Plumbago Zeylanica* may carbohydrate in Bendicts test, protein containing sulphar and Alkaloid contains wagner's test may contains present.

KEYWORD: *Holoptelae Integrefolia, Plumbago Zeylanica.*

INTRODUCTION
HOLOPTELAE INTEGREFOLIA
Nature has blessed mankind with a treasure of medicinal plants. Natural products have always remained a profile source for the discovery of new drugs and are used since Vedic period.[1] Holoptelea integrifolia is a medium-sized large glaborous tree about 15-25 m in height with whitish or yellowish grey bark exfoliating in irregular flakes and possesses an offensive smell when cut freshly.[2] It belongs to family Ulmaceae and is having 15 genera and 200 species. Vernacular Names: Hindi-Chirmil, Chilbil, Chilla, Dhamna, Kandru, Kanju, Karanji, Kumba, Kunjanali, Kunj; Gujarati- Charel; Marathi-Papara; Sanskrit- Chirbilva; Tamil-Ayi[5] Malayalam- Aval; Punjabi- Arjan, Kacham, Khulen, Papri; Telugu- Nemali, Nevili, Pedanevili; Uriya-Dharango.[3, 4, 5, 6]

Holoptelea integrifolia

CHEMICAL CONSTITUENTS

The plant has been reported to possess chemical constituents like terpenoids, sterols, saponins, tannins, proteins, carbohydrates and alkaloids[3], flavonoids. The phytoconstituents isolated from stem bark are holoptelin-A and holoptelin-B,2-aminonaphthoquinone, Friedlin, epifredlin,β-sitosterol, β-D-glucose, β-amyrin, hederagenin (heart wood), hexacosanol. 1, 4-naphthalenedione has been isolated from leaves of Holoptelea integrifolia and is reported to possess antibacterial activity against Staphylocopccus aureus.[7]

TRADITIONAL USES

Plant is useful in treatment of obesity, edema, and bronchitis. It has been known to be protease inhibitor. Mucilage and juice obtained from boiled bark has been reported to be useful in rheumatism, intestinal tumour when applied externally. Bark juice is applied to rheumatic swellings.[6]

PLUMBAGO ZEYLANICA

Plumbago zeylanica L, commonly known as chitrak or lead wort-white flowered is innate to South Asia. It is dispersed in tropical and subtropical countries of the world. Budding in deciduous woodland, savannahs, scrublands from sea level up to 2000 m altitude.[7,8] In India it is sprinkled in central India to West Bengal, Maharashtra, and Uttar Pradesh to some parts of South India. The plant also enjoys regional names in different state Gujarati: Agni / vahini, Kannada:chitramula, Malayalam: chitrakmula/ bilichitramula, Punjabi: Veellakeduveli, Bangali: chitra, Tamil: chita, Telugu: kodiveli/ chitramoolam, Hindi: chitraka/chitramol, Sanskrit: chitra.[9] But commonly used name persisted to be chitraka.[10,11] Plumbago is from Plumbaginaceae family comprises of 10 genera and 280 species. The genus Plumbago take account of 3species that is Plumbago indica L. (P. rosea L.) P. capensis L., and P. zeylanica L., in all these 3 species Plumbago zeylanica is most cultivated because of its high therapeutic uses.

Plumbago zeylanica

Therapeutic uses

P. zeylanica is a widespread curative herb all over Africa and Asia. It has been cast-off as a cure for skin sicknesses, infections and intestinal worm's viz. leprosy, scabies, ringworm, hookworm, dermatitis, acne, sores and ulcers subsequently ancient times. The old systems of medicine in diverse parts of the landmasses have been employing all amounts of P. zeylanica for a diversity of treatments.

2. MATERIAL AND METHOD

2.1 Collection of Plant Material

The leaves of *Holoptelae Integrefolia* and *Plumbago Zeylanica* 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 and 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.[12,13]

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. HOLOPTELAE INTEGREFOLIA

S.No	Test	Positive & Negative
A-	**Test for carbohydrate**	
I	Molish Test(General Test)	Negative
	For reducing Sugars	
a.	Fehlingh Test	Negative
b.	Bendicts Test	Negative
B.	**Test for Monosaccharides**	
a.	Barfoedf test	Negative
C.	**Test for hexose sugars**	-
I	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
D.	**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	Negative
ii)	5% Hgcl2 Solution	Negative
iii)	5% Cuso4 Solution	Negative
iv)	5% Lead Acetate	Negative
v)	5% Ammonium Sulphate	Negative
F.	**Test for steroid**	
a.	Salkowski Reaction	Negative
b.	Liebermann-Burchard Reaction	-
c.	Libermann's reaction	-
G.	**Tests for Amino Acids**	
a.	Ninhydrin test (General test):	Negative
b.	Test for Tyrosine	Negative
c.	Test for tryptophan	-
H.	**Tests for Glycosides:**	
I	Tests for Cardiac Glycosides	-
a.	Baljet's test	Negative
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.	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.	Positive
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	Positive

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

2. Drug - *PLUMBOGO ZEYLANICA*

S.No	Test	Positive & Negative
A-	**Test for carbohydrate**	
i	Molish Test(General Test)	Negative
ii	For reducing Sugars	
a.	Fehlingh Test	Negative
b.	Bendicts Test	Positive
B.	**Test for Monosaccharides**	
i	Barfoedf test	Negative
ii	Test for hexose sugars	-
iii	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
C.	**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	-
ii)	5% Hgcl2 Solution	Negative
iii)	5% Cuso4 Solution	Negative
iv)	5% Lead Acetate	Negative
v)	5% Ammonium Sulphate	Negative
F.	**Test for steroid**	
a.	Salkowski Reaction	Negative
b.	Liebermann-Burchard Reaction	-
c.	Libermann's reaction	-
G.	**Tests for Amino Acids**	
a.	Ninhydrin test (General test):	Negative
b.	Test for Tyrosine	Negative
c.	Test for tryptophan	-
H.	**Tests for Glycosides:**	
I	Tests for Cardiac Glycosides	
a.	Baljet's test	Negative
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	Negative
b.	Haemolytic test	-
III	Tests for Coumarin Glycosides:-	Negative
I.	**Tests for Flavonoids**	
a.	Shinoda test	Negative
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
d.	Ferric chloride test	Negative

J.	**Tests for Alkaloids**	
a.	Dragendroff's test:	Negative
b.	Mayer's test	Negative
c.	Hager's test	Negative
d.	Wagner's test	Positive
K.	**Tests for Tannins and Phenolic Compounds**	
a.	5% $FeCl_3$ solution	Negative
b.	Lead acetate solution	Negative
c.	Gelatin solution	Negative
d.	Bromine water	Negative
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 *Holoptelae Integrefolia* are carbohydrates, monosaccharide, proteins, precipitated test, steroid amino acids, cardiac glycoside, tannin and phenolic compound may absent but saponin glycoside, flavonoids with NaOH, alkaloids in wagner's test may contains present and Plumbago Zeylanica may carbohydrate in Bendicts test, protein containing sulphar and Alkaloid contains wagner's test may contains present. The most active extracts could be subjected for further pharmacological evaluation by isolation of the osteoarthritis, skin infection, Anti-inflammatory, Anthelmintic and further research on this plant can specify its pharmaceutical application.

CONCLUSION

Preliminary pharmacognostical standardization studies of the *Holoptelae Integrefolia* and *Plumbago Zeylanica* 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 antimicrobials and further research on this plant can specify its pharmaceutical application.

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