

Screening of Secondary Metabolites

Now a days, people are too much conscious regarding their health issue. They prefer herbal drug, wherever and whenever, this option is available to them. Herbal drug acts like any other drug, either direct as supplement that support the body system without side effect. The increasing demand of herbal drug develops the herbal industry. Naturally occurring substances used for drugs may be plants, animals and mineral origin. These Antibiotics or antimicrobial properties are because of different phyto-chemicals or phytoconstituent distributed in plants. *Loranthus parasiticus* (L)Merr. under Loranthaceae have potential medicinal activity history of Chinese tradition.

Materials and Methods

Plant specimen collection

The plant materials *Loranthus parasiticus*, *Macrosolen cochinchinensis* and *Viscum album* were collected from the different areas of south west Bengal. The plants were identified and authenticated by Prof. Amal Kumar Mondal (Associate professor of Botany, Vidyasagar University). The fresh plant leaves were air dried at room temperature until dried. The dried plant leaves were blended using a blender and stored in a clean glass ware container until needed for analysis. The extracts were filtered using Whatman filtered paper no.1.

Phytochemical screening

Chemical test were carried out on the aqueous extract and on the powdered specimen using standard procedure to identify the constituents as described by Somolenski, et al; (1974), Harborne, (1973), Sofowora, (1993) and Salehi, et al; (1992).

Test for tannins-1 g powdered + 20 ml boiled dist water (incubation for five minutes in water bath), filtered, while hot. 1 ml of cool filtrate was distilled to 5 ml with dist water + (2-3) of 10 % ferric chloride added. A bluish-black or brownish-green precipitate developed.

Test for saponins-1 g powdered + 10ml boiled dist water in a bottle bath (incubation for 10minutes), filtered.

2.5 ml filtrate + dist water and shake for 2 minutes (frothing observed).

Demonstration of emulsifying properties - 2.5 ml filtrate + 2 drops of olive oil + 10 ml dist water shake for few min. (formation of emulsion).

Test for phlobatannins- aqueous extract + 1 % aqueous boiled hydrochloric acid; red precipitation developed.

Test for terpenoids-5 ml extract + 2 ml chloroform + 3 ml of concentrated H_2SO_4 ; reddish brown precipitation formed.

Test for flavonoids-1 g powder + 10 ml of dist water; 5 minutes boiling and filtered. 1 ml cooled filtrate + drops of 20 % sodium hydroxide solution; yellow color developed, addition of acid changed to colorless solution.

Test for cardiac glycosides-5 ml extract + 2 ml of glacial acetic acid with 1 drop ferric chloride solution + 1ml concentrated H_2SO_4 . A brown ring developed. A violet ring

may appear below the ring while in the acetic acid layer, a greenish ring may be formed.

Test for combined anthraquinones- 1 g powdered + boiled 2 ml of 10 % hydrochloric acid for 5 mins. Cooled filtrate + equal volume of chloroform; Chloroform layer + Equal volume of 10 % ammonia solution; shake and allow separating; delicate rose pink color developed.

Test for free anthraquinones-0.5 g powder + 5 ml of chloroform; shake; filtrate + equal volume of 10 % ammonia solution; bright pink color developed.

Test for carotenoids-1 g powder + 10 ml chloroform; filtrate + 85 % sulphuric acid. A blue colour at the interface observed.

Test for reducing compounds- 1 g powder + 10 ml boiled dist water; cooled filtrate + 20 % sodium hydroxide solution + boiled with equal volume of Benedict qualitative solution; brick red precipitation observed.

Test for alkaloids-1 g powder + 10 ml boiled dist water + 10 ml hydrochloric acid. The pH of the filtrate was adjusted with ammonia to about 6-7. A very small quantity of the following reagents was added separately to about 0.5 ml of the filtrate in a different test tube and observed.

Picric acid solution.

10% tannic solution.

Mayer's reagent (Potassium mercuric iodide solution). The test tubes were observed for colored precipitates or turbidity.

XI.A.i - Results (*Loranthus parasiticus*)

The study indicated the presence of phytochemicals such as alkaloids, flavonoids, tannins, terpenoids, reducing sugars, carbohydrates and cardiac glycosides.

Phytochemical analysis of *Loranthus parasiticus* leaves extract showing presence of different active constituents (secondary metabolites) in different extracts. The leaf extract contained Tanin which is present in Aqueous, Acetone and Ethanolic extracts but in case of methanolic extract tanin is absent. Presence of saponins gives the positive result in Haemolysis test. Terpenoids is present in aqueous and methanolic extract and absent in acetone and ethanolic extract. Flavonoids are present in ethanolic and methanolic extract which is confirmed by different test but in case of aqueous and acetone extracts it is absent. Cardiac glycosides is presence in methanolic extract, which is confirmed by various test but it is absent in acetone and methanolic extract. Presence of Anthraquinone and Carotenoids are mostly in methanolic extract and absent in ethanolic extract. Alkalod is present which is confirmed by different reagents and mostly it is presence in aqueous, ethanol and methanol extract and it is absent in acetone extract. Reducing sugar is also present abundantly in aqueous, ethanol and methanolic extract but in case of acetone extract it is absent. Most of the result shows the presence of secondary metabolites which is abundant in methanolic extracts and absence in acetone extract.

Table - 34: Phytochemical screening of leaves powder of *Loranthus parasiticus*.

Phytochemical compounds	Name of the test	Aqueous	Acetone	Ethanol	Methanol
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Tanins	Goldbeater's skin test	+	+	+	-
	Ferric chloride test	+	+	+	-
	Phenazone test	+	+	+	-
	Gelatin test	+	+	+	-
	Test for catechin.	+	+	+	-
	Test for chlorogenic acid	+	+	+	-
Saponins	Forth formation test	+	+	+	-
	Haemolysis test	+	+	+	-
Terpenoids		+	-	-	+
Flavonoids	Shinoda test	-	-	+	+
	Alkaline reagent test	-	-	+	+
	Zinc hydrochloride test	-	-	+	+
Cardiac Glycosides	Kedde's glycosides test	+	-	-	+
	Keller-killiani test.	+	-	-	+
	Raymond's test	+	-	-	+
	Legal's test	+	-	-	+
	Baljet test	+	-	-	+
Anthraquinone		+	-	-	+
Carotenoids		-	+	-	+
Alkaloids	Dragendorff's Reagent	+	-	+	+
	Mayer's Reagent	+	-	+	+
	Wagner's Reagent	+	-	+	+
	Hager's Reagent	+	-	+	+
	Tannic Reagent	+	-	+	+
	Picrolonic acid test	+	-	+	+
Reducing sugar		+	-	+	+

XI.A.ii - Discussions

When a new drug to be discovered, qualitative phytochemical analysis is a very important step as it gives information about the presence of any particular primary or secondary metabolite in the extracts of the plant which is having a clinical significance. The different phytochemicals tests performed on the extracts of *Loranthus parasiticus* leave extracts show the presence of alkaloids, flavonoids, tannins, terpenoids, reducing sugars, carbohydrates and cardiac glycosides. Simultaneously, it is also to be observed that wheather this components have the capacity to suppress any microbes' growth or activity which is already proven by the experiment. The same result found by the Roy, et al; (2015), *current research*, Preliminary phytochemical analysis of leaf extract of *Loranthus parasiticus*(L) Merr. with reference to their anti-microbial activity.

XI.B.i - Results (*Macrosolen cochinchinensis*)

Phytochemical analysis of *Macrosolen cochinchinensis*(Lour.) Tiegh.leaves extract showing presence of different active constituents (secondary metabolites) in different extracts. The leaf extract contained tanin which is present in Aqueous, Acetone and Ethanolic extracts but in case of methanolic extract tanin is absent. Presence of saponins gives the positive result in Haemolysistest. Terpenoids is present in aqueous and methanolic extract and absent in acetone and ethanolic extract. Flavonoids are present in ethanolic and methanolic extract which is confirmed by different test but in case of aqueous and acetone extracts it is absent.Cardiac glycosides is presence in methanolic extract, which is confirmed by various test but it is absent in acetone and ethanolic extract.Presence of Anthraquinone and Carotenoids are mostly in acetone and methanolic extract and absent in ethanolic extract.Alkalod is present which is confirmed by different reagents and

mostly it is presence in aqueous, ethanol and methanol extract and it is absent in acetone extract. Reducing sugar is also present abundantly in aqueous, ethanol and methanolic extract but in case of acetone extract it is absent. Most of the result shows the presence of secondary metabolites which is abundant in methanolic extracts and absence in acetonic extract.

Table – 35: Phytochemical screening of leaves powder of *Macrosolen cochinchinensis*.

Phytochemical compounds	Name of the test	Aqueous	Acetone	Ethanol	Methanol
Tanins	Goldbeater's skin test	+	+	+	-
	Ferric chloride test	+	+	+	-
	Phenazone test	+	+	+	-
	Gelatin test	+	+	+	-
	Test for catechin.	+	+	+	-
	Test for chlorogenic acid	+	+	+	-
Saponins	Forth formation test	+	+	+	-
	Haemolysis test	+	+	+	-
Terpenoids		+	-	-	+
Flavonoids	Shinoda test	-	-	+	+
	Alkaline reagent test	-	-	+	+
	Zinc hydrochloride test	-	-	+	+
Cardiac Glycosides	Kedde's glycosides test	+	-	-	+
	Keller-killiani test.	+	-	-	+
	Raymond's test	+	-	-	+
	Legal's test	+	-	-	+
	Baljet test	+	-	-	+
Anthraquinone		+	-	-	+
Carotenoids		-	+	-	+
Alkaloids	Dragendorff's Reagent	+	-	+	+
	Mayer's Reagent	+	-	+	+
	Wagner's Reagent	+	-	+	+
	Hager's Reagent	+	-	+	+
	Tannic Reagent	+	-	+	+
	Picrolonic acid test	+	-	+	+
Reducing sugar		+	-	+	+

XI.B.ii – Discussions

Different extracts of *Macrosolen cochinchinensis* leaves were prepared and different tests of phytochemical constituent was performed using generally accepted laboratory technique for qualitative determinations. The different phytochemicals tests performed on the extracts of *Macrosolen cochinchinensis* leave extracts show the presence of alkaloids, flavonoids, tannins, terpenoids, reducing sugars, carbohydrates and cardiac glycosides.

XI.C.i - Results (*Viscum album*)

Phytochemical analysis of *Viscum album*. leaves extract showing presence of different active constituents (secondary metabolites) in different extracts. The leaf extract contained Tanin which is present in Aqueous, Acetone and Ethanolic extracts but in case of methanolic extract tanin is absent. Presence of saponins gives the positive result in Haemolysistest. Terpenoids is present in aqueous and methanolic extract and absent in acetone and ethanolic extract. Flavonoids are present in ethanolic and methanolic extract which is confirmed by different test but in case of aqueous and acetone extracts it is absent. Cardiac glycosides is presence in methanolic extract, which is confirmed by various test but it is absent in acetone and ethanolic extract. Presence of Anthraquinone and Carotenoids are mostly in acetone and methanolic extract and absent I ethanolic extract. Alkalod is present which is confirmed by different reagents and mostly it is presence in aqueous, ethanol and methanol extract and it is absent in acetone extract. Reducing sugar is also present abundantly in aqueous, ethanol and methanolic extract but in case of acetone extract it is absent. Most of the result shows the presence of secondary metabolites which is abundant in methanolic extracts and absence in acetonic extract.

Table – 36: Phytochemical screening of leaves powder of *Viscum album*.

Phytochemical compounds	Name of the test	Aqueous	Acetone	Ethanol	Methanol
Tanins	Goldbeater's skin test	+	+	+	-
	Ferric chloride test	+	+	+	-
	Phenazone test	+	+	+	-
	Gelatin test	+	+	+	-
	Test for catechin.	+	+	+	-
	Test for chlorogenic acid	+	+	+	-
Saponins	Forth formation test	+	+	+	-
	Haemolysis test	+	+	+	-
Terpenoids		+	-	-	+
Flavonoids	Shinoda test	-	-	+	+
	Alkaline reagent test	-	-	+	+
	Zinc hydrochloride test	-	-	+	+
Cardiac Glycosides	Kedde's glycosides test	+	-	-	+
	Keller-killiani test.	+	-	-	+
	Raymond's test	+	-	-	+
	Legal's test	+	-	-	+
	Baljet test	+	-	-	+
Anthraquinone		+	-	-	+
Carotenoids		-	+	-	+
Alkaloids	Dragendorff's Reagent	+	-	+	+
	Mayer's Reagent	+	-	+	+
	Wagner's Reagent	+	-	+	+
	Hager's Reagent	+	-	+	+
	Tannic Reagent	+	-	+	+
	Picrolonic acid test	+	-	+	+
Reducing sugar		+	-	+	+

XI.C.ii - Discussions

The aqueous extract of leaves of *Viscum album* were prepared and different tests of phytochemical constituent was performed using generally accepted laboratory technique for qualitative determinations. The study indicated the presence of phytochemicals such as alkaloids, flavonoids, tannins, terpenoids, reducing sugars, carbohydrates and cardiac glycosides.

The different phytochemicals tests performed on the extracts of *Viscum album* leave extracts show the presence of alkaloids, flavonoids, tannins, terpenoids, reducing sugars, carbohydrates and cardiac glycosides.