

Comparative Phytochemical Analysis of Hemi Parasite *Loranthus longiflorus* Desr; on Host *Azadirachta indica* by GCMS Analysis

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Abstract

Plant parasites are known to obtain primary and secondary compounds from their hosts. Mistletoes or parasitic plants are growing on various trees and belong to the Loranthaceae family. Numerous mistletoes don't synthesize or modify the secondary compounds taken up from their hosts. The hosts significantly influenced the phytoconstituents of hemi parasites. In traditional systems as well as in the modern system of medicine the plant *Azadirachta indica* is used as a medicine for various ailments and so the parasite *Loranthus longiflorus* grown on neem tree was chosen for the study. The present study is carried out to detect the phytochemical constituents of Host-*Azadirachta indica* and parasite *Loranthus longiflorus* from the Hyderabad region. Phytochemical analysis of methanolic leaf extracts revealed the presence of tannins, phenols, and Anthraquinones in both the host and parasite. GCMS analysis revealed the presence of five bioactive compounds in the host and parasite with varied compositions. The phytochemical and GCMS analysis indicated similarity in presence of phytoconstituents in both the host and parasite.

Keywords: GCMS, *Loranthus longiflorus*, Plant parasites, phytochemical constituents

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INTRODUCTION

Some plants acquire their food or water from other plants, which are known as parasites, and the source providing plants are known as hosts. The stem parasitic plants are commonly named mistletoes. Mistletoes are derived from the Anglo-Saxon word *mistelian* which means, "mist" for dung and "tan" for twig [1]. *Loranthus longiflorus* Desr. commonly known as Mistletoe, all-heal, devils fuge, belongs to *Loranthaceae* family with 76 Genera and 1000 species. The synonym for *Loranthus* is *Dendrophthoe falcata*. The semi-parasites possess leaves and synthesize the carbohydrates portion of their food. These parasites establish relations with host vascular elements to draw nutrients [2-3]. Hemi parasites have a parasitic relationship with more than 300 host plants [4]. Unlike other parasites, *Loranthus*

seeds do not require a germination stimulant from the living host [5]. Numerous hemi parasites do not synthesize or modify the secondary compounds taken up from their hosts [6], thus the presence of certain secondary compounds varies within and among the populations of hemi parasitic plants, depending on the host association of individual parasites [7], it has been used in traditional medicine and found to have antimicrobial, anti diabetic, antioxidant, anticancer, hypertensive and antiviral properties [8-10]. They are used as a health food for enhancing immunity and used as a pain reliever, aphrodisiac, narcotic and diuretic [11-12]. The aim of the study was comparative analysis of host-*Azadirachta indica* and parasite-*Loranthus longiflorus* for phytochemicals.

Preparation of plant material powder

The fresh leaves of parasite-*Loranthus longiflorus*

and host-*Azadirachta indica* were collected from Hyderabad. The plant material was washed thoroughly first with tap water and then distilled water to remove the surface contaminants. Later they are shade dried separately at room temperature ($30^{\circ}\text{C}\pm 5^{\circ}\text{C}$) for about two weeks to get constant weight and then pulverized into a dry powder using an electric blender. Until needed, the powdered leaf samples were kept in an airtight container.

Preparation of extract

The dried plant materials of *L. longiflorus* and *Azadirachta indica* leaves samples were extracted with Methanol in soxhlet apparatus for 3hrs. The extract was cooled, filtered, and concentrated using a vacuum flask evaporator. Finally, this extract was dissolved in 1ml methanol and centrifuged at 3000 rpm for 5min.

Phytochemical Analysis

Phytochemical analysis of methanol leaves extract was carried out following certain prescribed methods adopted by Srinivas R. *et al.*, 2014.

Test for Tannins

In 2ml of distilled water, a few drops of ferric chloride solution were added to roughly 2ml of extract. The appearance of green-tinted precipitate confirms the presence of tannins.

Test for Saponins

In a test tube, 3 ml of the extract was mixed with 3ml of distilled water and vigorously shaken for a few moments. The test tube was heated, and creating stable foam indicates the presence of saponins.

Test for Flavonoids

1ml of 10 percent lead acetate solution was added to 1 ml of extract in a test tube. The appearance of the yellow precipitate can determine the presence of flavonoids.

Test for Alkaloids

On a hot water bath, 3ml of the extract was mixed with 3 ml of 1% HCl. Then 1ml of the liquid was

split between two test tubes.

Dragendroff's Test

1 ml of Dragendroff's reagent was added to the first test tube. The presence of orange-red precipitate was regarded as a good sign.

Mayer's Test

1ml of Mayer's reagent was added to the second test tube. The formation of a buff-colored precipitate can confirm the presence of alkaloids

Test for Terpenoids

2 ml extract was dissolved in 2 ml CHCl_3 and evaporated to dryness. After that, 2 ml of concentrated sulphuric acid (H_2SO_4) was added and heated for about 2 minutes. The emergence of a grayish color indicates the presence of terpenoids.

Test for Steroids

Two tests were carried out to perceive the presence of steroids in the extract, and those were the Salkowski's test and the Liebermann test.

Salkowski's test: 2ml of the organic extract was dissolved in 2ml of chloroform. 2ml concentrated H_2SO_4 was added to this. The presence of steroids is shown by the appearance of red color in the chloroform portion.

Test for Phenols

5ml distilled water was added to 1gm of extract, and the solution was then treated with a 5% ferric chloride solution. The presence of phenols is confirmed by forming a dark green color.

GC-MS Analysis

The GC-MS analysis of the methanolic leaves extract was done at the Central Analytical Facility, University College of Technology, Osmania University using the standard GCMS model. The parasite plant *Loranthus longiflorus* and host *Azadirachta indica* leaves extracts were analyzed using a GCMS-QP2010 SHIMADZU, Japan, fused with an Optima 5 ms capillary column (30 0.25 mm) 0.25 m film thickness, as described by

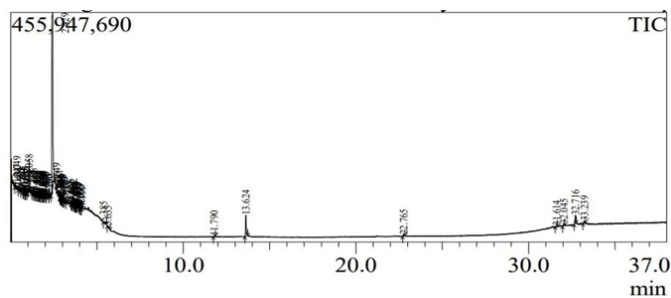


Fig 1: Chromatogram Neem Parasite E:/2022/OCT/10-uday-GCMS/Neem parasite.

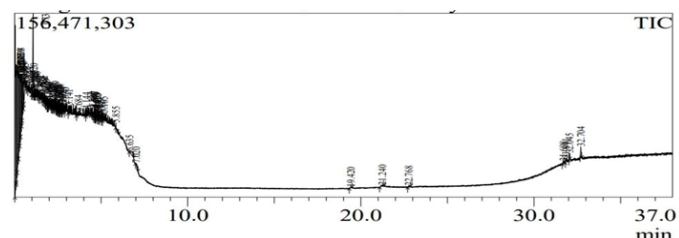


Fig 2: Chromatogram Neem Host E:/2022/OCT/10-uday-GCMS/Neem Host.

Table 1: Phytochemical analysis of parasite-*L. longiflorus* and Host- *Azadiracta indica*

S no	Constituents	Host- Azadiracta indica	Parsite- L.longiflorus
1	Test for Alkaloids	Absent	Absent
2	Test for tannins	Present	Present
3	Test for saponins	Absent	Absent
4	Test for flavonoids	Present	Absent
5	Test for terpenoids	Absent	Present
6	Test for steroids	Present	Absent
7	Test for Phenols	Present	Present
8	Test for Anthraquinones	Present	Present

Iheagwamet al (2019). The initial column oven temperature (60°C) is planned to climb to 160°C at a rate of 10°C/min, then to 250°C with hold duration of 2 min/increment. An injection volume of 1.0 L in the splitless mode with a split ratio of 1:1 and injector temperature set at 200° C with minor adjustments. The following were the mass spectrophotometer settings: 230°C for the ion source, 250°C for the interface, 4.5 minutes for the solvent delay, and 50–700 amu for the scan range. The multiplier voltage and electron ionization mode were modified to 70 eV and 1859 V. For compound identification, the retention time, fragmentation pattern, and mass spectral data of unknown components in the extracts equated to those in the Wiley and National Institute of Standards and Technology (NIST) libraries [14-24].

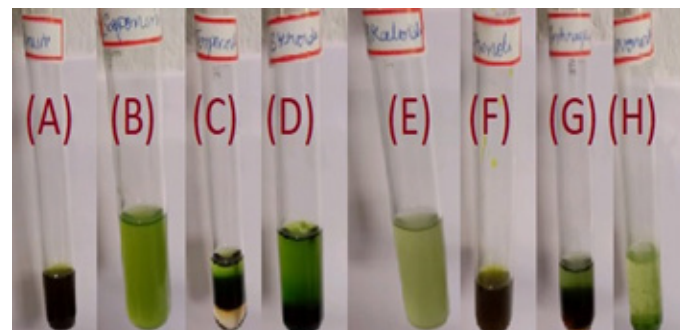
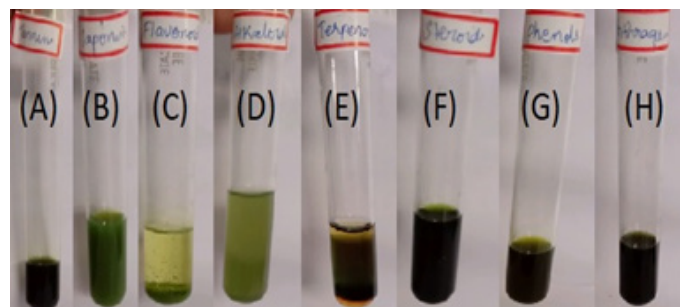


Fig 3: Phytochemical analysis of Host and Parasite plants.

(A) Tannins (B) Saponins (C) Flavonoids (D) Alkaloids (E) Terpenoids (F) Steroids (G) Phenol (H) Anthroquinones

RESULTS AND DISCUSSION

The phytochemical analysis of the methanol extract of two plant leaves *Loranthus longiflorus* –parasite and host-Neem revealed the presence of tannins, phenols, anthraquinones and the absence of saponins and alkaloids. Flavonoids and steroids absent in the parasite are present in host (Table-1 and Fig-3). The GC-MS chromatographic profile of the host and parasite plant leaves extracts showed various significant constituents like Difluorine monoxide; Nitrosyl chloride; 1-Propyne, 3-chloro; Pyridazine, 3-methoxy; N,N Dinitro 1,3,5,7 tetrazabicyclononane in diverse compositions. Host plant contains the compounds of 2-Nonynoic acid (15.26%), Methane, chloro Trinitron (13.04%) that were absent in parasite plant followed by Difluorine monoxide (11.28%), Nitrosyl chloride (2.11%), 1-Propyne, 3-chloro- (0.60%) N,N Dinitro 1,3,5,7 tetrazabicyclononane (0.41%), Pyridazine, 3-methoxy (0.28%). Parasitic plant contains highest level of Phenylethylene.3-methoxy-2,2-dinitro (62.40%) followed by Difluorine monoxide (0.40%), Nitrosyl chloride (0.38%), Pyridazine, 3-methoxy (0.13%), 1-Propyne, 3-chloro- (0.13%), N, N Dinitro 1, 3, 5, 7 tetrazabicyclononane (0.34%). 2-Nonynoic acid, Methane, chloro trinitron that were absent in host plants are absent in parasite plants.

Table 2: Phyto compounds identified by GC-MS analysis.

S. NO	COMPOUND NAME	RETENTION TIME		MOLICULAR WEIGHT	FORMULA	CAS NO	%AREA HOST	%AREA PARSITE
		(H.N)	(P.L)					
1	Difluorine monoxide	0.108	3.060	54	F ₂ O	7783-41-7	11.28%	0.40%
2	Nitrosyi chloride	0.595	3.875	65	CINO	2696-92-6	2.11%	0.38%
3	1-Propyne,3-chloro-	2.180	3.505	74	C ₃ H ₃ Cl	624-65-7	0.60%	0.13%
4	Pyridazine, 3-methoxy	2.180	3.505	110	C ₆ H ₆ N ₂ O	19064-65-4	0.28%	0.13%
5	N,N Dinitro 1,3,5,7 tetrazabicyclononane	2.545	3.584	218	C ₅ H ₁₀ N ₆ O	0-00-0	0.41%	0.34%
6	2-Nonynoic acid	0.059	0	154	C ₉ H ₁₄ O ₂	1846-70-4	15.26%	0
7	Phenylethylene,3-methoxy-2,2-dinitro	0	2.429	224	C ₉ H ₈ N ₂ O ₅	16990-0-2	0	62.40%
8	Methane, chloro trinitro	0.200	0	185	CCIN ₃ O ₆	1943-16-4	13.04%	0

CONCLUSION

Host and parasite plant leaves extracts depicts similar Bioactive compounds like Difluorine monoxide, Nitrosyi chloride, 1-Propyne,3-chloro, Pyridazine, 3-methoxy, N,N Dinitro 1,3,5,7 tetrazabicyclononane with varied composition. The analysis can be used as a useful tool to pharmacognacy. Finally, the present reports points towards the need of further investigations on the possible role of transferred secondary compounds either as mediators of host defense, host defense compounds utilize as cues of identification of host by the hemi parasite or compounds taken up by the parasite to support their defense against rejection by the host.

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