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Loranthus longiflorous Desr: A wide host range parasite harbored on a few new hosts on Sri Devaraj URS academy of higher education and research campus and Phytochemical comparison of host and Parasite

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ABSTRACT

The range of parasitic plants displays a significant variation in specificity for the hosts. The multiple factors that influence host specificity are pollination, birds, richness of host plants and their compatibility and the environmental conditions. Our research work describes about the distribution of the parasite, *Loratnhus longiflorus* on diversified hosts observed in a Medical college campus. The entire campus is about 100 acres and the field observation was made to find out the spread of *Loranthus*. The recognized host plants are actually well distributed in Kolar district of Karnataka. The selected campus is having nearly 50 different indigenous and exotic varieties of tree species. After careful scrutiny, the parasitic plants identified on *Albizia amara, Albizia saman, Azadirachta indica, Ixora singaporensis, Millingtonia hortensis, Lagerstroemia flos-reginae, Muntingia calabura, Pongamia pinnata, Punica granatum, Peltophorum pterocarpum, Tabebuia rosea, Tabebuia argentea* and *Tabernaemontana divaricata.* Among those 13, 07 plants have been identified as new hosts and 04 plants belong to Fabaceae. The pattern of haustoria was primary, epicortical and cortical on the identified hosts. Among the studied phytochemicals, only gums and mucilages; resins and saponins are present in both *T. divaricata* and *Loranthus*. Glycosides are the small estexisting phytochemicals in all the studied hosts and parasite. Majorly occupied phytochemicals were phenolics, flavonoids, gums and mucilage, resins and saponins.

Key words: *Loranthus longiflorus, Phytochemical compounds, Haustoria, Epicortical, Cortical.*

Introduction

All the plants synthesize food material by photosynthesis by using water, minerals, sunlight and carbon dioxide. Some plants acquire their food or water from other plants, which are known as parasites and the source providing plants are known as hosts. These may be differentiated as complete or partial parasites of stem or root depending on the utility of the parasite. Partial parasites may be either root or stem parasites. Partial parasites will have either root, no green leaves or have leaves but not the

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roots. In both the instances, parasite plant depends on host plants for either water or food. The relationship will be strong as long as there is no harm from either side. Parasitic plants have haustoria, similar to root, that penetrate the xylem of host for water and phloem for food. There are two haustorial types depending on the hosts. (a) Solitary and (b) Epicortical are the types and it is not clearly known what factors influence the development of type of haustoria.

The stem parasitic plants commonly named as mistletoes. Mistletoes are derived from the Anglo-Saxon word *misteltan* which means, "mist" for dung and "tan" for twig (Calder and Bernhardt, 1983). The name is suitably set as the birds defecates the seeds on the branches of other trees. The abundance of mistletoes is more in places where there is more access to sunlight. These plants are otherwise known as epiphytic angiosperm parasites that live as semi-parasites on trees.

The two words, parasite and host are host range and host preference. Host range is the total number of different species that can be parasitized. Host preference is the choice of parasite for the desirable host to get optimum growth. The selection of the host is based on nastic movements, i.e., response to host stimulus that permit them to move towards the host (Runyon *et al*., 2006). Except the coldest regions, parasitic plants are distributed everywhere on earth. The distribution of Mistletoes is found to be high in the canopy of the forest trees, where there is abundant sunlight.

Loranthus longiflorus Desr. Var. falcata (L.f.) Kurz, commonly known as Mistletoe, all heal, devil's fuge, belongs to Loranthaceae family with 76 Genera and 1000 Species. The synonym for *Loranthus* is *Dendrophthoe falcata*. The plant is classified as below

Kingdom: Plantae
Phylum: Magnoliophyta
Class: Magnoliopsida
Order: Santalales
Family: Loranthaceae
Genus: *Loranthus*
Species: *longiflorus*

Loranthus spp are widely distributed in tropical region. The parasite is almost seen throughout India. There are two species of *Dendrophthoe*, *D. falcata* var. coccinea (Red honey suckled mistletoe) and *D. falcata*var, falcata (Honey suckled mistletoe). Red honey suckled is classified by pink to red coloured flowers and honey suckled mistletoe is by white coloured flowers. On our campus, the white coloured plant is detected, which is a perennial shrub with drooping clusters of twigs from the host plant from the attachment, and easily acknowledged on the host plant. The location where the parasite spears its haustoria shows swelling and forms tumors. The measure of the outgrowths depends on the age of the parasite. Though *Loranthus* randomly selects the hosts, its establishment depends on the relationship between the two plants in terms of osmotic pressure or merely unknown (Sampath Kumar and Selvaraj, 1980).

Plant description

It is a branchless pendulous, glabrous. The leaves range from alternate to sub opposite, lanceolate to ovate-oblanceolate, margins are entire, obtuse at apex. The petiole is up to 1.0 cm long, flowers are tubular with approximately 0.8 mm long, five lobed, stamens are five and 4.0 mm long, globose ovary with one each of locule, ovule, filiform style, capitate stigma, fruits are coloured red in drupes, are glabrous textured; seeds are two in number with oblong shape and approximately 09 mm in size (Sambandan *et al*., 2015).

Most of mistletoes are shrubs with woody natured, brittle stem and elongated leaves. Many species of *Loranthus* genera are having large, tubular, showy flowers in clusters. Because of their attractive colour, nectar and drawn-out nature, flowers are attracted by birds. Birds eat the berry fruits and the seeds are surrounded by a tasteless mucilaginous sap i.e., viscin. Birds transmit the seeds within a short period of time. The seeds are stuck to the young branches of the host plant, once the seed is positioned on the host plant branch; they germinate and penetrate the host tissue. The cellulosic strands which are bounded by mucilaginous pectic substance aid the seed to attach to the host plant. Unlike other parasites, *Loranthus* seeds do not require a germination stimulant from the living host (Nickrent and Musselman, 2004).

Although *Loranthus* has leaves to photosynthize, they cause damage to the host plant, sometimes leads to death of the host plant. An advantage of *Loranthus* is host specific and this gives advantage to mistletoes in heterogeneous communities (Vijayan *et al.*, 2015).

Medicinal properties of *Loranthus*

Although it is a hemi-parasite, it has medicinal

properties like aphrodiasic, cooling, diuretic, astringent, bitter, and valuable in controlling asthma, renal and vesical calculi, pulmonary tuberculosis, menstrual disorders, and swelling wounds. For women, the decoction made out of the plant is useful as an antifertility agent; anticancer activity, antioxidant, and neuroprotective against oxidative stress (The Ayurvedic Formulary of India 2000; Daniel *et al*., 2011).

In spite of its parasitic nature, this plant has got many Ayurvedic advantages. Antibiofilm activity of *Loranthus* leaf methanolic extract was tested against 17 bacterial strains and it was noted by Karthikeyan *et al*. (2012) that biofilm activity was reduced upto 70-90%. The Ag nanoparticles of aqueous leaf extract showed cytotoxicity when assessed on carcinoma cell lines (MCF-7) (Sathishkumar *et al*., 2014). The ethanolic stem extracts of *Loranthus* contain proteins, carbohydrates, glycosides, steroids, triterpenes, flavonoids, tannins and phenolic compounds. The extracts significantly inhibited seizures induced by maximal electroshock, (MES) also observed dropping in motor coordination and reduced the duration of hind limb tonic extensor phase in mice tested (Sinoriya *et al*., 2011). The methanolic and aqueous (water) extracts of leaves showed *in vivo* anti-inflammatory activity and *in vitro* antioxidant activity (Patil *et al.,* 2011). The hydro-alcoholic extract of the aerial parts of the plant was tested on female Wistar rats for studying the antifertility efficacy and it was established from the study that the extract has antifertility activity (Pattanayak and Mazumder, 2009). An experiment with ethanolic extracts of the aerial parts on rats with excision and incision wound models, showed potential would heal property. The same extracts showed antioxidant activity by increasing levels of catalase activity and inhibiting superoxide dismutase levels, lipid peroxidation, and reduced glutathione (Pattanayak and Sunita, 2008).

Materials and Methods

Study area

Sri Devaraj Urs Academy of Higher Education and Research (SDUAHER) campus is situated at Tamaka (abutting the National Highway No.4 – between Bangalore and Chennai), 5 km East from Kolar city and 75 km from Bangalore in Karnataka, India (13° 7′ 57.2484′′ N 78° 10′ 21.5148′′ E), spread over almost 100 acres. Soil of this luxurious green campus is healthy and the soil is red loamy, red sandy and mixed red in texture. The climate is tropical. The average yearly temperature is 24.2 °C and arun-of-the-mill twelve-month rainfall is 650 mm. The temperature in May averages 27.9 °C and recorded as the warmest month of the year. At 20.5 °C on an average, December is the coolest month of the year. The annual temperature variation on an average is around 7.4 °C. With 1.0 mm rainfall, January is considered as the driest month. In October, the rainfall reaches its peak, with an average of 141 mm. Between the driest and wettest months, the difference in precipitation is around 140 mm. The campus is rich with both indigenous and exotic varieties of plants ranging from herbs, shrubs, twines to trees. To get to know the distribution summary of the parasite and host plants in our campus, field study was carried out.

The host and parasites were identified based on the characteristic features given in the flora of Madras Presidency (Gamble, 1967) Flora of Karnataka (Sankara Rao *et al*., 2014), Flora of Karnataka (Cecil, 1996). In Kolar district, the parasite is distributed in Ittikladurga S.F.II; Narasimhadevarabetta S.F. V; Chickballapur-Bagepalli Road 17th km; Rayalpad S.F.I. The photographs of both host and parasite were taken. Also the live hosts and parasite were identified by Dr. Balakrishna Gowda, Professor, Department of Forestry & Environmental Science, University of Agricultural Sciences, GKVK Campus, Bengaluru, India.

Phytochemical analysis

Sample preparation

During morning hours, the leaf materials of both host and parasite were collected. Under running tap water, the leaves were scrubbed to take out all the dust and dirt. Shade dried, made fine powder and stored at room temperature in brown bottles.

Test for Saponins

Uniform measure of crude powders of both the plants was mixed with 5 mL of water in a clean tube, mixed vigorously for about 2 min. The appearance of 2 cm foam clearly shows the presence of saponins.

Tests for Flavonoids (Shinoda Test)

Four parts of magnesium ribbon were added to

equal measure of the leaf powders, trailed by the adding of a few drops of concentrated hydrochloric acid. Reddish colour appearance in the tube confirms flavonoids.

Tests for Glycosides (Liebermann's Test)

To a clean tube, added same measure of the plant powders, 2.0 mL of each acetic acid and chloroform and heated up for 5 min, then cooled and H_2SO_4 was added to it. The presence of glycosides was noted when the solution turned green colour.

Test for Steroids

The variation in colour from violet to green/blue confirms the presence of steroids after adding 2 mL each of acetic anhydride and leaf powder to 2 mL of H_2SO_4.

Test for Phenolics (Ferric Chloride test)

A few drops of 5% ferric chloride solution (neutral) and equal measures of leaf powders were mixed in a glass tube and presence of phenolic compounds was confirmed by the appearance of dark green colour.

Test for phytosterols (Libermann Burchard's test)

Mixed equal measure of the leaf fine particles in a glass tube with 2 mL acetic anhydride, added 1 or 2 drops of concentrated sulphuric acid slowly to the mixture from the sides of the test tube.

Test for Proteins (Biuret test)

Plant material (2 measures) was taken in a tube, followed by addition of 1 drop of 2% copper sulphate solution. To this 1 mL of ethanol (95%) was added, followed by excess of potassium hydroxide pellets. Pink colour ethanoic layer indicates the presence of proteins.

Test for gum and mucilage

Leaf powder of five measures was taken and 2 mL of absolute alcohol was added to it and kept for constant stirring for 10 min. White/ Cloudy precipitate confirms the presence of gums and mucilage.

Test for resins

The powders were treated with a few drops of acetic anhydride solution followed by addition of 1 mL of concentrated sulphuric acid. The appearance of colour differing from orange to yellow specifies the presence of resins.

Test for fixed oils

The dry powders were pressed between the filter paper; the appearance of oil stain on the paper confirms the presence of fixed oils.

Results

The study area and master plan map of SDUAHER is shown in figure 1. On Sri Devaraj Academy of Higher Education and Research campus, *Loranthus* was identified on 13 tree species. Most of the plants are trees, except *Ixora* and *T. divaricata* which are ornamental plants. The varieties of families the hosts belong are Fabaceae (04), Meliaceae (01), Rubiaceae (01), Bignoniaceae (03), Lythraceae (02) Apocynaceae (01) and Muntingiaceae (01). The new and existing hosts for the parasite are also mentioned in the table (Table 1).

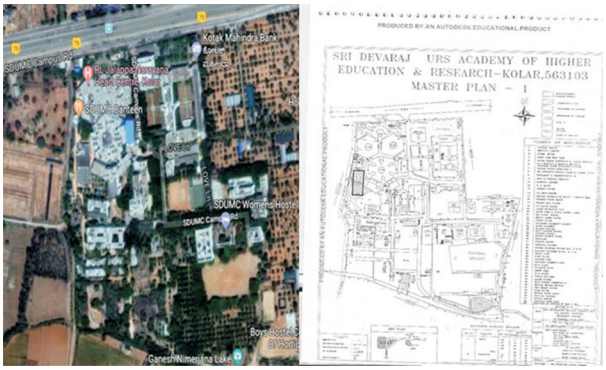

Fig. 1. Study area and map of Sri Devaraj Urs Academy of Higher Education and Research

The hosts include *Albizia amara, Albizia saman, Azadirachta indica, Ixora singaporensis, Millingtonia hortensis, Lagerstroemia flos-reginae, Muntingia calabura, Pongamia pinnata, Punica granatum, Peltophorum pterocarpum, Tabebuia rosea, Tabebuia argentea* and *Tabernaemontana divaricata* and among 13 hosts, 07 host plants have been identified as new hosts. Out of 13 hosts, 04 plants belong to Fabacea. The pattern of haustoria was primary, epicortical and cortical on the hosts identified (Figure 2).

The campus has about 50 different varieties of indigenous and exotic trees species. But host plant is restrictedly occupied only on 13 tree species. This indicates that *Loranthus* has high host specificity. The definition for the host range is defined as the total number of host plants that can inhabit the parasite plants in a given inhabitants and is to be

Table 1. Host plants of *L. longiflorous* identified on medical college campus. Highest number of *Loranthus* has been observed on *Pongamia pinnata*. Flowering status was different for both the parasite and host plants in some. In *Peltophorum* the parasite was lost after certain period of time.

S. No.	Host plant	Common name	Family	Flowering state of the host/parasite	No. of parasitic plants on one host	Host status name the host/ parasite on one host
1.	*Albizia amara* (Roxb.) B.Boivin	Bitter Albizia	Fabaceae	Both	02	New
2.	*Albizia saman* F.Muell.	Rain tree	Fabaceae	Both	02	Known
3.	*Azadirachta indica* A. Juss	Neem	Meliaceae	Both	02	Known
4.	*Ixora singaporensis* L.	West Indian Jasmine	Rubiaceae	Both	02	New
5.	*Millingtonia hortensis* Linn.	Tree Jasmine	Bignoniaceae	Parasite	02	Known
6.	*Lagerstroemia flos-reginae* (L.) Pers.	Pride of India	Lythraceae	Parasite	02	New
7.	*Muntingia calabura* L.	Jamaica Cherry	Muntingiaceae	Both	02	Known
8.	*Pongamia pinnata* (L.) Pierre	Hongay oil tree	Fabaceae	Both	>08	Known
9.	*Punica granatum* L.	Pomegranate	Lythraceae	Both	02	Known
10.	*Tabebuia rosea* DC.	Rosy trumpet tree	Bignoniaceae	Both	01	New
11.	*Peltophorum pterocarpum* K. Heyne	Yellow flamboyant	Fabaceae	None	01	New
12.	*Tabebuia argentea* (Bureau & K. Schum.) Britton	Golden Bell	Bignoniaceae	Both	02	New
13.	*Tabernaemontana divaricata* R.Br. ex Roem. & Schult.	Crape Jasmine	Apocynaceae	Both	01	New

determined by the resistance of the host plant and host-parasite interaction.

On observation, we noticed that *Loranthus* did not establish its space on the host, *P. pterocarpum*. So, we performed phytochemical analysis of the host plant but not the parasite in this set of plants.

Among the studied phytochemicals, only gums and mucilages; resins and saponins are present in both *T. divaricata* and *Loranthus*. Glycosides are the smallest amount phytochemicals in all the plants studied. Majorly, the phytochemicals in all the plants studied were phenolics, flavonoids, gums and mucilage, resins and saponins. As these phytochemicals have major biological properties towards conventional medicine, the identification of these phytochemicals from novel sources will be of great importance.

Discussion

In general, if more number of parasites grow on one particular host plant, the host plant will exhaust and sometimes, leads to decease of the host plant. But to our surprise, none of the host plants express any noticeable signs, like necrosis, chlorosis or death of the region where the parasite hosts. Any parasite will not kill its host as the parasite nurtures itself by taking up nutrients and other essential compounds for its survival. In spite of *Loranthus* being an autotroph or photosynthetic, parasitic plant's complete dependency on the host is for carbon and nitrogen contents and competes with its hosts. If not the host plant is susceptible, there will not be any host-parasite interaction (Downey, 2004) can also be interpreted that if the haustoria is not accurately established, leads to dropping of the parasites, but once it is established, they prosper on the hosts. Hence, they pick host plants having more canopies, where they take more sunlight for its food preparation.

The survival of *Loranthus* on the host plant is established by two factors, the rate of communication of the parasite with the host tree by the discrete seeds on the host by birds and the dwelling of location of the seed on the tree (Carlo and Aukema, 2005). It is understood that plant parasitism is evolved in the environment where water and nutrient are in scarce (Bowie and Ward,

Fig. 2. *Loranthus* harboring on varieties of host plants on the medical college campus.
A. *Albizia amara*, B. *Albizia saman*, C. *Azadirachta indica*, D. *Ixora singaporensis*, E. *Millingtoniahortensis*, F. *Muntingia calabura*, G. *Lagerstroemia flos-reginae*, H. *Pongamia pinnata*, I. *Punicagranatum*, J. *Tabebuia rosea*, K. *Peltophorum pterocarpum*, L.*Tabebuia argentea* , M.*Tabernaemontana divaricata*. N. Fruits of *Loranthus* on *Pongamia* a. Primary, b. Epicortical and c. cortical haustoria of *Loranthus*

2004). Literature has proven that nitrogen is one of the limiting factors for the parasite's selection of the host. Our observation is also coinciding with this statement; at more locations and profuse growth of *Loranthus* on *P.pinnata*, belongs to Fabaceae, has been noted and it is in agreement with Kelly (Kelly, 1992); Matthies (1996) and Seel and Press (Seel, 1993).

Pokhriyal *et al*., (1990) grew 27 tree species to know their fast growing nature for nitrogen fixation study. One among them is *Peltophorum* species. Out of 27, 11 trees are non-nitrogen fixing trees belong to Caesalpinioideae. We take this point into consideration and might be the possible intention for non-setting up of *Loranthus* on *P. peltophorum* as this could be its non-nitrogen fixing capacity.

Some host plants are susceptible or resilient to haustorium permeation at altered stages. In this example, haustorium might have experienced a kind of resistance pressure by *Peltophorum*. Mistletoe's establishment might have blocked and non-host plant, *Peltophorum* species, developed wound periderm which blocks access to vascular tissue, particlularly, xylem. This might curtail the establishment (.

When considering the distribution of parasite in Karnataka, zone 2 includes Tumkur Bengaluru rural, and arid Kolar province, showed highest distribution of *Pongamia pinnata* (Hosalli *et al.*, 2015). Based on Environmental impact assessment report submitted to Karnataka Industrial Areas Development Board (KIADB), Bengaluru, the listed host plants in the present study for *Loranthus* parasite are predominantly grown. This makes the parasite to spread among the plant population. Conversely, current outcomes of our study determine that *Loranthus* is not a damage causing parasite to its host as the host plants are not showing any damage symptoms.

The question arises here is how did the mistletoe

Table 2. Phytochemical analysis of *Loranthus* and its hosts

Plant	Phytochemicals									
	Glycoside	Phenolic	Flavonoid	Phyto- sterols	Protein	Gum	Resin and mucilage	Saponin	Fixed oil	Steroid
A.amara	-	+++	-	-	-	+	++	++	-	-
Loranthus on *A. amara*	-	+	++	-	-	-	++	++	-	++
A. saman	++	+	+	+	-	+	++	++	-	+
Loranthus on *A. saman*	++	+++	+++	+	-	+++	++	++	-	-
A. indica	-	++	++	++	+	++	+	+	-	+
Loranthus on*A. indica*	+	++	-	++	+++	+++	+++	++	-	+++
I.singaporensis	-	-	+++	-	++	-	+	+		-
Loranthus on*I singaporensis*	+	-	+++	-	-	++	++	+++		+
M.hortensis	-	++	+	++	-	-	+	++		-
Loranthus on*M.hortensis*	-	-	++	-	-	++	+++	++		-
L. flos-reginae	-	+++	+++	-	+	-	+	-	-	-
Loranthus on*L. flos-reginae*	-	+++	+	-	+	+	+	+	-	+
M. calabura	-	-	+++	+	-	-	+	-		++
Loranthus on*M. calabura*	-	+++	+++	+	-	++	++	++		+++
P. pterocarpum	+	++	++	-	-	-	-	-	-	-
P. pinnata	-	++	+	+	-	+	+++	++	+	-
Loranthus on *P. pinnata*	+	+++	+++	+	++	++	+++	-	+	+
P. granatum	-	++	-	-	++	+	+	++		-
Loranthus on*P. granatum*	-	++	++	-	-	++	+++	++		-
T. rosea	-	++	+	+	++	++	++	-	-	-
Loranthus on *T. rosea*	+	++	+++	-	++	++	+++	++	-	-
T. argentea	-	++	++	-	-	++	++	++		-
Loranthus on *T. argentea*	-	++	++	-	-	++	++	++		-
T. divaricata	-	-	+++	-	-	+	++	++		-
Loranthus on *T. divaricata*	-	-	-	-	-	++	++	++		-

plant get onto such a big tree when the plants are not within touching distance of each other? Even if the striking distance is maintained between the hosts, how did it jump from one host to other? There is a solid relationship between the *Loranthus* and the birds, as birds are the agents of pollination. In the life cycle of mistletoe, two birds play an important role. One is sunbird, *Cinnyris asiaticus*, for pollination and flower pecker, *Dicaeum erythrorhynchos*, has altered tongues for seed dispersal. Both these birds are common in Kolar and Bengaluru region (www.fes.org.in). The same birds also anchorage on the plants of our college garden, thus spreading the parasite. Birds that disperse the seeds determine how frequently *Loranthus* interacts with its host trees. The fundamental base for the survival of *Loranthus* is determined by the location where *Loranthus* seeds are placed.

The single or primary haustorium develops from the root zone of the embryo of the parasite. The haustorium becomes bulbous and unites with the host. In some species where single haustorium is present, the haustorial strands grow in cambial zone of the host that is internally within the host. These are called cortical strands (www.anbg.gov.au). These strands blowup through the bark and produce shoots with flowers and fruits (Figure 2 N). The epicortical haustoria grow like a vine along the outside of the host branch. These vines send secondary haustoria here and there. In the present study, cortical, epicortical and primary haustoria have been observed (Figure 2 a, b and c).

The frequency of the *Loranthus* is evidently greater and would be an interesting topic to look into the other locations around Kolar district. This gives an insight about the occurrence and host range other than the plants mentioned in the present study. There is an uninterrupted increase in host-*Loranthus* combination which needs to be documented. This gives an idea of dynamic nature of *Loratnhus* species specificity and it is extremely vital to comprehend the relationship among diverse groups of host plants.

Based on the predictions of niche theory, the species that share resources should develop strategies to compete minimum for those resources or otherwise the less competitive species/plant would extirpate. As a general strategy, if both the plants flower at the same time, they may follow pollination niche. To avoid overlap in pollination process, the hypothesis is that the host and parasite plants flower at the same time to attract similar or dissimilar pollinators. Our observations throughout a year followed the finding of Ollerton *et al*., for *Orobanche elatior* and the host *Centaurea scabiosa*, where both the plants maintained the same pollination niche (Ollerton *et al.*, 2007). The pollinators for both host and parasite are not similar, insects help host plants in pollination, whereas, *Loranthus* is effectively pollinated by the birds, despite both have a comparable flowering niche.

Loranthus demands space, time and preference for its host selection. For its preference parasite needs certain chemical signals provided by suitable host to instigate either germination (Bouwmeester *et al*., 2003) or haustorial development (Matvienko *et al*., 2001 and Tomilov *et al*., 2004). Parasite's adaptation to chemical cues is vital after sprouting and in nippy attachment. Following contact on the host, it is evident that parasite displays certain movements either rejecting (allows to grow away) or welcoming(turns round the branch) best before its penetration (Kelly, 1990, 1992). The mechanism and factors that affected the dislodging of *Loranthus* on *Peltophorum* remains indescribable.

Conclusion

The host specificity of *Loranthus* is random. For appropriate establishment of parasite, the identifiable signals, either in chemical form or environmental means, are needed. To minimize the hormone burden on the host, both host and parasite plants entered into flowering nearly at the same time. Varied phytochemicals with different functions have been noted from the leaves of parasite and its hosts. Though parasite havens on the host, it has got its own mechanisms and elements for the synthesis of varied phytochemicals having medicinal value. Further, analysis of these compounds can be beneficial to the individuals as therapeutics.

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