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RESEARCH ARTICLE

A comparison of the bioactivity of *Dendrophthoe falcata* on the hosts; *Limonia acidissima* and *Mangifera indica*

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Abstract: *Dendrophthoe falcata* (L.f.) Ettingsh is a hemiparasitic plant, used in Sri Lankan and Indian indigenous medicine to treat various diseases such as asthma, cancerous tumors, diabetes and wounds. Despite the hemiparasite on the host *Limonia acidissima* is used to treat various diseases, any bioactivity study of *Dendrophthoe falcata* grown on the particular host has not been conducted yet. This study aimed to investigate several bioactivities of the hemiparasite grown on *Limonia acidissima* (*La*) and compare with that grown on *Mangifera indica* (*Mi*). Sequential extracts by Soxhlet method from hexane (*La*-HE and *Mi*-HE), ethyl acetate (*La*-EAE and *Mi*-EAE) and methanol (*La*-ME and *Mi*-ME) were investigated for bioactivities. Antioxidant activity was determined using DPPH radical scavenging assay in which *Mi*-ME and *Mi*-EAE showed the highest activity, that is five times more than that of α-tocopherol. Brine shrimp lethality assay was conducted as a preliminary toxicity assay, where *La*-EAE showed the highest activity that is approximately four times that of $K_2Cr_2O_7$. The total polyphenolic content was determined by Folin-Ciocalteu method in which both methanol extracts showed the highest polyphenolic content of which *Mi*-ME was approximately four times more than that of *La*-ME. These results suggest that the bioactivity of the hemiparasite vary considerably with the host. Further, extracts of the hemiparasite on *Limonia acidissima* showing high antioxidant activity coupled with high toxicity justifies its applicability in indigenous medicine.

Keywords: *Dendrophthoe falcata*; *Limonia acidissima*; *Mangifera indica*; bioactivity; host dependence.

INTRODUCTION

Medicinal plants have been extensively used to fight against various diseases for many centuries. These plants have attracted the attention of many researchers with the increasing popularity of the usage of herbal and traditional medicine. *Dendrophthoe falcata* (var. *coccinea*) is one such plant which is a hemiparasite grown on a range of hosts where the genus is spread across tropical regions in Asia, Africa and Australia (Wu *et al*., 2003). *Dendrophthoe falcata* is commonly known as the red honey suckle mistletoe and as "pilla" in Sri Lankan native language. The hemiparasite is used by both Indian and Sri Lankan indigenous medicinal systems to treat various diseases and ailments. It is interesting to note that some studies acknowledge that the applicability of the hemiparasite to treat a particular disease depends on the host it is grown on while some studies do not (Dashora *et al*., 2011a and Chaudhari *et al*., 2014). The hemiparasite on the host *Azadirachta indica* (neem) exhibit antidiabetic action while on the host *Mangifera indica* (mango) is used to treat asthma, menstrual disorders, swelling wounds, ulcers, renal and vesicle calculi (Channabasava *et al*., 2013, Patil *et al*., 2011). The Sri Lankan ethnomedicinal system employs *D. falcata* on the host *Limonia acidissima* (wood apple) to treat cancerous tumors. The immense medicinal potential of *D. falcata* has attracted many researchers and it has been subjected to extensive study which reported to contain many biologically active substances such as quercetin: a flavonoid, β-sitosterol: a steroid, and triterpenes such as β-amyrin and oleanolic acid (Sahu *et al*., 2010, Nair and Krishnakumary 1990). These compounds are shown to possess antifertility, antioxidant, antihyperlipidemic, antimicrobial, and antidiabetic activities (Pattanayak and Mazumder 2009, Dashora *et al*., 2011b, Pattanayak and Sunita 2008, Channabasava *et al*., 2013).

The bioactivity of the hemiparasite *D. falcata* is strongly dependent on the host plant. The specific host hemiparasite relationship governs the type of secondary metabolites produced and hence their therapeutic properties. Reported literature does not consider the variation of observed bioactivity of the hemiparasite, *D. falcata*, with the host where it is grown on. The bioactivities reported for *D. falcata* are highly variable and incomparable, as the effect of the host tree is not considered in these studies and the bioactivities observed for the hemiparasite grown on one host is not seen for another (Chaudhari *et al*. 2014, Patil *et al*., 2011 and Dashora *et al*., 2011b). This study mainly aims to investigate the bioactivity of the hemiparasite on the host *L. acidissima* which is not yet reported despite being used in indigenous medicine in Sri Lanka. In addition, a direct comparison of bioactivity of the hemiparasite *D. falcata* on different hosts has not been reported yet and this study also aims to fill in this gap of knowledge and thereby provide indication for the validity of the claim that the applicability of the hemiparasite to treat a particular disease depends on the host plant. The ethyl acetate and methanol extracts of *D. falcata* on the hosts *L. acidissima* and *M. indica* were evaluated for their antioxidant activity, total polyphenolic

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content and brine shrimp lethality while the hexane extracts were only evaluated for their brine shrimp lethality.

METHODS AND MATERIALS

Plant collection and extraction

The areal parts excluding the leaves of the hemiparasite *D. falcata* on the host *L. acidissima* and *M. indica* were collected from Kurunegala, North Western Province, Sri Lanka in February 2018 and Menikhinna, Central province, Sri Lanka in March 2018 respectively. The collected plant specimens were authenticated as *D. falcata* by the National Herbarium at the Royal Botanical Gardens, Peradeniya. The areal parts of the plant materials were washed and cut into 3 to 4 cm pieces. They were air dried for a period of two weeks and ground to a fine powder. The powdered plant materials were sequentially extracted by Soxhlet method using hexane, ethyl acetate and methanol respectively. Initially, 50 g of powdered *D. falcata* was extracted using 500 mL each from the above-mentioned solvents for 16 hours per solvent. Before changing to the next solvent, the plant material was air dried for 2 hours to remove residual solvent. Extracts obtained from each solvent were concentrated using a rotary evaporator at 30 ^{o}C to obtain the final yield in paste or powder form.

Determination of antioxidant activity

The antioxidant activity was determined by DPPH radical scavenging assay as described by Patil *et al*., (2011), with slight changes. Briefly, a concentration series was prepared in methanol for the ethyl acetate and methanol extracts of *D. falcata* and α-tocopherol as the positive control (Table 1). Then each solution (3.00 mL) was separately mixed with DPPH solution (1 mM, 200 µL) and kept in the dark for 15 minutes. The absorbance was measured at 517 nm using the UV-Vis spectrophotometer (Shimadzu UV-1800). The absorbance of 200 µL of DPPH solution in 3.00 mL of methanol was used as the negative control. All the experiments were carried out in triplicate. The percentage antioxidant activity values were calculated and the IC_{50} values for each extract were determined using probit analysis.

Determination of total polyphenolic content

The total polyphenolic content was determined by Folin-Ciocalteu method as described by Singleton *et al*., (1999), with slight modifications. A volume of 400 µL of gallic acid standard solutions prepared in water (200, 100, 80, 60, 40, 20, 10 ppm) were mixed with Folin-Ciocalteu solution (2.00 mL) and kept for 8 minutes. After that, Na_2CO_3 solution (1.60 mL of 7.5% w/v) was added to each solution and was kept for 1 hour followed by measuring the absorbance using the UV-Vis spectrophotometer at 765 nm. Each experiment was carried out in triplicates to construct the calibration plot. Next, following the same steps, the absorbance values were measured for each extract of *D. falcata* prepared in 70% (v/v) methanol at a final concentration of 6 ppm. The total polyphenolic content was determined by the calibration curve and was expressed as mg of gallic acid equivalent (GAE) per g of dry weight of extract (mg GAE/g).

Determination of brine shrimp lethality

The brine shrimp lethality assay was conducted as a preliminary determination of toxicity studies as described by Pelka *et al*., (2000), which included hatching of brine shrimp nauplii (*Artemia salina*) from artificially prepared sea water and testing the lethality of these nauplii caused by the plant extracts. Brine shrimp eggs (0.010 g) were added to artificial sea water (600.0 mL) and the solution was aerated and illuminated for 24 hours at ambient conditions. The hatched nauplii were transferred to another beaker containing 600.0 mL of artificial sea water and kept for another 24 hours under the same conditions.

A 1000 ppm stock solution for each of the extracts of *D. falcata* was prepared in artificial sea water solution containing 1% (v/v) DMSO (Parra *et al*., 2001). Then, a concentration series of 1000, 500, 250, 125, 50, 5, 1 ppm was prepared for each of the extracts and for $K_2Cr_2O_7$ as the positive control. A 1% (v/v) DMSO solution in artificial sea water was used as the negative control. Each extract solution (4.00 mL) prepared was transferred to a test tube with 10 brine shrimp nauplii (48-hour old) and kept closed at room temperature. After 24 hours the number of surviving nauplii were counted and the LC_{50} was determined by probit analysis.

RESULTS AND DISCUSSION

D. falcata on the hosts *L. acidissima* and *M. indica* exhibited considerable variation of antioxidant activity as depicted by the significantly different IC_{50} values determined by the DPPH radical scavenging assay (Figure 1).

The hemiparasite grown on *M. indica* exhibited a much higher antioxidant activity compared to that on *L. acidissima*. The *Mi*-ME and *Mi*-EAE showed almost five times more activity than the positive control, α-tocopherol, which exhibited an IC_{50} value of 14.29 ppm that lies well

Table 1: Concentration series prepared for each extract and the positive control.

Plant specimen/Control	Extract	Concentration series / ppm
Limonia acidissima (*La*)	Methanol (*La*-ME)	0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 4, 6, 8, 10
	Ethyl acetate (*La*-EAE)	1, 5, 10, 15, 20, 30, 40, 50
Mangifera indica (*Mi*)	Methanol (*Mi*-ME)	1, 2, 3, 4, 5, 6, 7, 8, 9, 10
	Ethyl acetate (*Mi*-EAE)	1, 3, 5, 7, 10, 15, 20, 30, 40
Positive control		5, 10, 15, 20, 25, 30

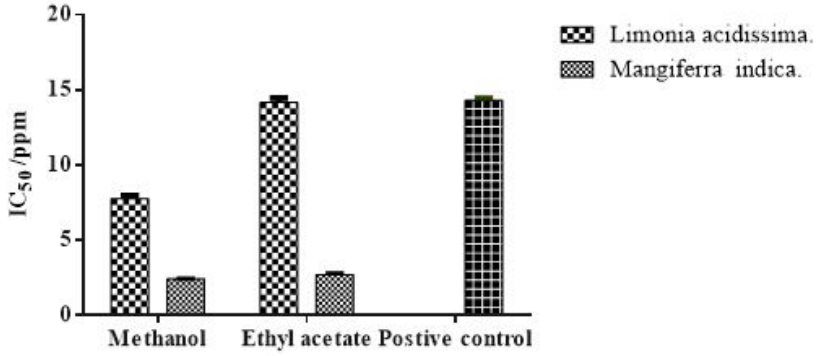

Figure 1: Variation of the IC_{50} values of antioxidant activity of the extracts of *D. falcata*.

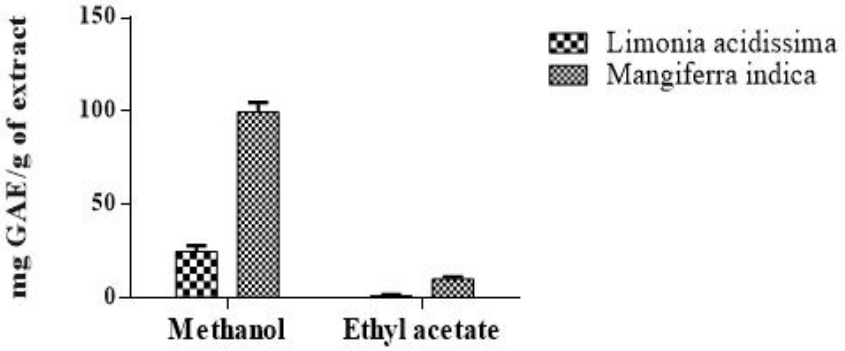

Figure 2: Variation of total polyphenolic content of the extracts of *D. falcata*.

within the reported range of 12 to 45 ppm (Muid *et al*., 2013, Asnaashari *et al*., 2014, Domínguez *et al*., 2005, Mishra *et al*., 2012). Interestingly, even though the extracts of *D. falcata* on *M. indica* showed a much higher antioxidant activity than the positive control, there was a very little variation in the activity between the two extracts. However, the two extracts of *D. falcata* on *L. acidissima* showed considerably more variation in the antioxidant activity in which *La*-ME showed almost two times more activity than the positive control while *La*-EAE and the positive control have similar activity. The hexane extracts of *D. falcata* were not considered for the antioxidant activity assay as phenolic compounds which usually contribute to the antioxidant activity get extracted to polar solvents readily, while non-polar hexane extracts usually show little to no antioxidant activity (Oboh *et al*., 2008).

The antioxidant activity of all extracts except *La*-EAE showed a higher radical scavenging ability than the positive control. The IC_{50} values reported for methanol and aqueous extracts of leaves of *D. falcata* grown on *M. indica* are 77.8 ppm and 144 ppm respectively (Patil *et al*., 2011). Further, another study reports the IC_{50} values for methanol and aqueous extracts for the areal parts of *D. falcata* (host not specified) as 18 ppm and 26 ppm respectively (Dashora *et al*., 2011b). Reported results and the results of the current study suggest that the areal parts of the hemiparasite yield better antioxidant activity than leaves alone. The results of DPPH assay suggest that *D. falcata* on *L. acidissima* is a potent source of antioxidant species which may have great therapeutic potential as indicated by the usage of this plant in indigenous medicinal systems, as free radical damage in biological systems is attributed to many diseases.

The high antioxidant activity observed for *D. falcata* in this investigation suggests that the areal parts of *D. falcata* exhibit potent free radical neutralization ability. This can be attributed by the high polyphenolic content (Chaudhari *et al*., 2014, Patil *et al*., 2011, Dashora *et al*., 2011b). Therefore, the total polyphenolic content of the extracts was determined by the Folin-Ciocalteu method (Figure 2).

The extracts of the hemiparasite on the host *M. indica* showed a substantially higher total polyphenolic content than the extracts of the hemiparasite on the host *L. acidissima*. The *Mi*-ME showed the highest total polyphenolic content of 99.6 mg GAE per g of extract. The *La*-ME interestingly showed almost four times lower total polyphenolic content than *Mi*-ME. The ethyl acetate extracts (*La*-EAE and *Mi*-EAE) also showed a similar variation in the total polyphenolic content where *La*-EAE showed almost seven times lower total polyphenolic content than *Mi*-EAE. Overall, the methanol extracts of the hemiparasite showed a higher total polyphenolic content than the ethyl acetate extracts.

Most polyphenolic compounds act as free radical scavengers, hence samples rich in polyphenolic content often exhibit strong antioxidant activity which is reflected

in this investigation (Fauconneau *et al*., 1997). The extracts of *D. falcata* on *M. indica* which shows the highest total polyphenolic content also showed the highest antioxidant activity compared to the extracts of the hemiparasite on *L. acidissima* which possess lower polyphenolic content. The type and quantity of polyphenolic compounds is greatly influenced by the host-hemiparasite relationship.

In a study it was reported that the total polyphenolic content of a host unspecified *D. falcata* methanol extract was 10.78 mg GAE per g of extract (Pattanayak *et al*., 2012). However, in the current study, 99.6 and 25.1 mg GAE per g of extract were obtained for the *Mi*-ME and *La*-ME respectively. These observed differences in total polyphenolic content in the two investigations can be attributed to probable differences in hosts.

The toxicity of the extracts was evaluated using brine shrimp lethality assay (Figure 3). The *La*-EAE showed the highest toxicity among all the extracts of *D. falcata* with an LC_{50} value of 12.80 ppm which is surprisingly very low even compared to the LC_{50} value of the positive control, $K_2Cr_2O_7$, of 50.40 ppm which lies within the broad range of reported values for 48 hours brine shrimp lethality assay (Naidu *et al*., 2014, Kos *et al*., 2016, Baravalia *et al*.. 2012). According to toxicity indices described by Mungenge *et al*., (2014), all extracts of *D. falcata* on the host *L. acidissima* were highly toxic, exhibiting LC_{50} values less than 100 ppm. On the other hand, *Mi*-HE can be considered as highly toxic, while *Mi*-ME is moderately toxic and *Mi*-EAE is nontoxic. Interestingly, ethyl acetate extracts of the hemiparasites, *La*-EAE and *Mi*-EAE, showed the highest and the lowest toxicity for brine shrimp lethality assay respectively proving the host dependence on the biological activity of the hemiparasite.

The higher toxicity seen in the hemiparasite on the host *L. acidissima* particularly in the ethyl acetate extract (*La*-EAE) correlates well with the Sri Lankan ethnomedicinal usage of the plant on *L. acidissima* to treat cancerous tumors. Anticancer natural products usually possess high toxicity which should selectively exhibit toxicity towards cancer cells. It can be inferred that the extracts of *D. falcata* on *L. acidissima* which show high toxicity would also show higher anticancer activity as literature explains the correlation between the preliminary toxic activity with brine shrimp assay and the anticancer activities (McLaughlin *et al*., 1993). This property coupled with high antioxidant activity could be an added advantage due to their ability to modulate cell signaling pathways and thereby to control cancers by preserving normal cell cycle regulation, inhibiting proliferation and inducing apoptosis, inhibiting tumor invasion and angiogenesis and suppressing inflammation (Valko *et al*., 2007). However, neither reports nor ethnomedicinal records are encountered for the usage of *D. falcata* on the host *M. indica* as an anticancer medication system which is reflected by the relatively lower toxicity values observed for the extracts of the hemiparasite on *M. indica.*

The significant variation of the biological activities of the hemiparasite on the hosts *L. acidissima* and *M. indica* can be attributed to two main factors; the host hemiparasite relationship and the environmental conditions. The host-parasite relationship determines the type of secondary metabolites produced in the hemiparasite as different hosts react in different manners to the hemiparasite which in turn greatly influences the secondary metabolites produced in the hemiparasite on the selected host (Bouwmeester *et al*., 2003). This study illustrates the influence of the host on the type and/or quantity of secondary metabolites produced by *D. falcata* and hence the therapeutic properties of *D. falcata* change with the host species involved. The environmental conditions also play a role in determining the types of secondary metabolites produced (Ramakrishna *et al*., 2011, Pavarini *et al*., 2012, Sampaio *et al*., 2016). *D. falcata* on *L. acidissima* was collected from the North-western province of Sri Lanka which is classified as an intermediate wet dry zone, while *D. falcata* on *M. indica* was collected form the Central province which falls into the wet zone of Sri Lanka. The variation in the environmental conditions in the two zones may have also contributed to the observed difference in the biological activities of the same hemiparasite on the two hosts.

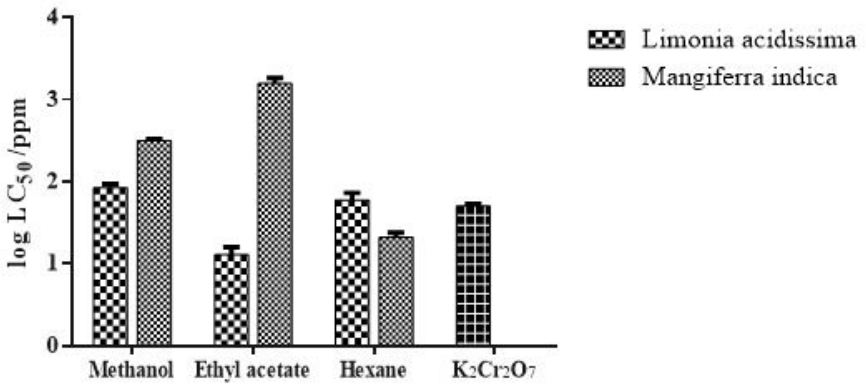

Figure 3: Variation of the LC_{50} values of brine shrimp lethality of the extracts of *D. falcata*.

CONCLUSIONS

The host dependence on bioactivity of *D. falcata* is clearly depicted in the findings of this study. The extracts of *D. falcata* on the hosts *L. acidissima* and *M. indica* exhibited considerable variation in antioxidant activity, total polyphenolic content and brine shrimp lethality. The highest antioxidant activity was shown by the methanol extract of *M. indica* with an IC_{50} value of 2.42 ppm. The highest brine shrimp lethality was shown by the ethyl acetate extract of *L. acidissima* with a LC_{50} value of 12.8 ppm. The extracts of the hemiparasite on *M. indica* showed a much higher total polyphenolic content than the respective extracts of the hemiparasite on the host *L. acidissima,* where *Mi*-ME showed the highest total polyphenolic content which correlates well with the high antioxidant activity observed for this extract as polyphenolic compounds act as free radical scavengers.

The significant variation of bioactivity of *D. falcata* with the host species in this investigation signifies that the metabolism of *D. falcata* is strongly influenced by the host species. This validates the ethnomedicinal claim, that the applicability of *D. falcata* to treat a particular disease depends on the host species.

The ethyl acetate extract (*La*-EAE) of *D. falcata* on *L. acidissima* showed a high antioxidant activity (IC_{50}, 14.16 ppm) coupled with high toxicity on brine shrimp lethality (LC_{50}, 12.8 ppm) which indicates the validity of the usage of the hemiparasite on *L. acidissima* to treat cancerous tumors in Sri Lankan ethnomedicinal system. More experimentation on anti-cancer studies will provide useful insights to confirm its ethnomedicinal applicability.

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STATEMENT OF CONFLICT OF INTEREST

Authors declare no conflict of interest.

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