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In vitro Anti-Diabetic Potential of Loranthacean Hemi-Parasite *Helicantes elasticus* (Desv.) Danser Collected from Six Different Hosts

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ABSTRACT

In vitro anti-diabetic potential of methanolic extract of *Helicanthes elasticus* (Desv.) Danser growing on six different hosts and thereby comparing the host's influence on this parasitic plant for this efficacy was studied and statistically analysed. The results showed that Hemiparasite collected from *Hevea brasiliensis*, *Citrus maxima*, *Anacardium occidentale* and *Saraca asoca* were found highly effective in this regard. Highest inhibition on α-amylase was shown by *H.elasticus* collected from *Hevea brasiliensis* where as inhibition of α-glucosidase was found to be highest in hemi-parasite obtained from *Citrus maxima*. The host plants selected under study was also found good in reducing the blood glucose level. The study clearly reveals the fact that, anti-diabetic potential of *H.elasticus* have significantly influenced by the host trees.

Keywords: *Helicantheselasticus*; Loranthaceaa; Anti-diabetic; Methanolic extract; Hemi-parasite

1. INTRODUCTION

Plants and plant products have been used for various purposes since the pre-historic period. Various phytochemicals present in plants impart a wide array of positive health effects and hence why ancient people use plants not only as food but also for alternative medical treatment. The plant-derived drugs serve as a prototype to develop more effective and less toxic medicines[1]. Various ailments from simple wounds to highly dangerous diseases like cancer, hepatitis, etc. are effectively remedied by using various plants and their products fromthe ancient period onwards. Interest in medicinal plants as a re-emerging health aid has been increased by rising costs of prescription drugs and also for bio-prospecting of new plant-derived drugs[2]. India being a land of rich biodiversity provides a treasure for such natural medicinal sources and many people in various parts of the country still depend on such natural resources as a final solution for their health problems. For time immemorial various plants in the Indian subcontinent acted as powerful and keen sources against several contagious diseases and were named "OTTAMOOLI" (means 'effect even in single dose') in Ayurveda by the people of Kerala.

Diabetes mellitus is a chronic disorder of carbohydrate metabolism by increased blood glucose levels. Several reasons were predicted to be the cause of diabetes from genetic to modern dietary habits. According to the WHO, the global prevalence of diabetes is expected to increase from 4 per cent in 1995 to 5.4 per cent by 2025[3], with developing countries being the main victims[4]. The latest IDF (International Diabetes Federation) diabetes atlas provides the fact that 537 million adults all over the world are living with diabetes at present and it will rise to 783 million by 2045. Commonly practiced treatment of diabetes includes diet control and physical exercises, oral anti-diabetic drugs, and finally periodic insulin administration.Oral hypoglycemic agents are highly effective but their use was restricted due to various side effects like liver disorders, flatulence, abdominal pain, renal tumors, and hepatic injury[5]. Due to these adverse after-effects associated with synthetic drugs, anti-diabetic plants were explored as they are safer, cheap, and more effective[6]. In recent years, Chinese herbs have been attracting attention as a cause of hypoglycemia, and it is estimated that more than thousands of plants are used as folk medicine for diabetes[7]. Several species of herbal drugs have been described in scientific literature and prescribed due to their good effectiveness, fewer side effects in clinical trials, and relatively low costs[8].

Helicanthes elasticus (Desv.) Danser, a member of Loranthaceae is a widely occurring hemiparasitic shrub regarded as an under utilised medicinally significant plant growing in India[9]. Cytotoxic, anti-tumor,and immunomodulatory activity of this plant were studied[10] which reveals its anti-cancerous potential. Anti-asthmatic and anti-anaphylactic activity were attributed to this plant due to the presence of polyphenols[11]. The leaves are used for removing stones from the urinary bladder and kidney and they also possess anti-abortion property[12].

In this study, methanolic extracts of *Helicanthes elasticus* (Desv.) Danser growing on six different host plants and the respective hosts were examined for their efficacy in reducing blood glucose levels through *in vitro* α-amylase inhibition and α-glucosidase inhibition assay.

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2. METHODOLOGY

2.1. Preparation of the Plant Extract

About 20 gm of the powdered samples of both parasite and host was extracted separately in 200 ml methanol for 10 hrs in the soxhlet apparatus. It was then filtered, the extract concentrated on a rotary evaporator and the semi-dried extract stored in an airtight bottle

2.2. *Invitro*α-Amylase Inhibition Assay

1000 µL of the starch solution was mixed with 1000 µL of the α-amylase enzyme (purchased from HiMedia) in 5 test tubes. 10, 20, 50, and 100 µg/mL of extract were added to 4 test tubes (Test samples) and keeping one without extract as control. All the test tubes were incubated for 3 min. After incubation 500 µL of 96 mM DNS reagent (0.438 g in 20 mL distilled water) was added to all the test tubes and again incubated for 15 min. Then solutions in each test tube were made up to 6 ml with distilled water. OD of these samples was measured at 540 nm. Then a set of another 4 test tubes were prepared with 4 different concentrations of the sample (10, 20, 50, and 100 µg/mL). All test tubes were made up to 6 mL with distilled water and labeled as extract control. Test tubes were subjected to incubation of 15 min. Blank was prepared with 1000 µL starch and 500 µL DNS reagent. These samples were also made up to 6ml with distilled water. Optical densities of the samples were measured at 540 nm and the assay was repeated in triplicates.

$$Inhibition = \frac{AC_{540} - AT_{540}}{AC_{540}} \times 100$$

AC= Absorbance of control solution, AT= Final absorbance of the test sample

2.3 *In vitro*α-Glucosidase Inhibition Assay[14]

The extract was pre-incubated with the enzyme prior to the addition of the substrate, pnitrophenyl-αD glucopyranoside (PNPG). Glucosidase activity was measured by using spectrophotometry to determine the color developed by the release of nitrophenol resulting from the hydrolysis of the substrate PNPG by glucosidase.

The α-glucosidase inhibitory activity was performed with a set of five test tubes. To all the test tubes 600 µL of potassium phosphate buffer was added. Then 10, 20, 50, and 100 µg/mL of extract was taken in 4 corresponding test tubes. The samples were vortexed and incubated at 37 °C for 15 min. After incubation 25 µL of 5 mM PNPG (0.015 g in 10 ml distilled water) was added and again incubated at 37 °C for 15 min. Finally, the reaction was terminated by adding NaOH. For blank, the reagents were added in the reverse order. The control however doesn't have any sample or test solution. The absorbance of all the samples was measured at 405 nm using a visible spectrophotometer and the procedure was repeated in triplicates. The per cent inhibition of enzyme activity by the test sample was calculated as,

$$Inhibition = \frac{AC_{540} - AT_{540}}{AC_{540}} \times 100$$

AC= Absorbance of control solution, AT= Absorbance of the test sample

2.4 Statistical analysis

All results are expressed as mean ± standard deviation in tables and graphs. The statistical significance of the results from parasites grown on different hosts was analysed by a two-way ANOVA followed by a post-Tukey HSD. Pearson correlation method was used to determine the relationship between activity between the parasite lineage and each host.

3. RESULTS

3.1 *In vitro*α-Amylase Inhibition Assay

α-amylase inhibition assay of *Helicanthes elasticus*(Desv.) Danser samples collected from six different hosts aregiven in

Table 1. Alpha amylase inhibition assay of *H.elasticus* collected from six different hosts

Concentration (µg/mL)	HEN	HEH	HEC	HES	HEA	HEM
10	13.96±1.21	9.55±0.51	20.67±0.68	13.43±0.83	7.45±1.08	4.51±1.28
20	18.47±0.82	23.19±0.57	34.84±1.26	17.63±0.33	21.09±1.3	11.65±1.04
50	31.06±0.23	65.9±0.76	49.95±0.37	32.63±0.58	46.38±0.88	34.84±0.87
100	40.5±0.85	74.92±1.03	56.77±0.61	43.86±0.89	56.35±0.71	45.33±0.68

HEN: *H.elasticus*(Desv.) Danser obtained from *Nerium oleander* L (**NO**).,**HEH**: *H.elasticus*(Desv.) Danser obtained from *Hevea brasiliensis*(Willd.exA.Juss.)Mull. Arg. (**HB**), **HEC**: *H.elasticus*(Desv.) Danser obtained from *Citrus maxima*(Burm.)Merr.(**CM**), **HES**: *H.elasticus*(Desv.) Danser obtained from *Saraca asoca*(Roxb.) Willd.(**SA**), **HEA**: *H.elasticus*(Desv.) Danser obtained from *Anacardium occidentale* L(**AO**)., **HEM**: *H.elasticus*(Desv.) Danser obtained from *Murraya koenigii*(L.) Spring.(**MK**)

Table 2. Alpha amylase inhibition assay of six host plants under study

Concentration (µg/mL)	NO	HB	CM	SA	AO	MK
10	18.85±1.8	34.42±0.63	12.62±1.06	3.27±0.56	15.11±1.49	4.67±0.99
20	21.96±0.78	37.69±0.69	20.4±2.3	7.79±1.06	19±1.06	24.92±0.86
50	24.77±1.93	45.17±1.22	34.27±0.85	13.89±1.61	26.01±0.98	44.08±1.68
100	27.57±1.63	55.3±0.52	48.29±0.79	18.85±1.21	33.49±1.06	51.4±2.01

Table 3. Two-way ANOVA for Alpha Amylase inhibition assay conducted in six accessions of *H.elasticus*

Source	Type III Sum of Squares	df	Mean Square	F	Sig
Corrected Model	26382.579[a]	23	1147.069	1.556	0.0001
Intercept	75064.563	1	75064.563	1.018	0.0001
Plant	3935.825	5	787.165	1.068	0.0001
Concentration	19877.493	3	6625.831	8.987	0.0001
Plant * Concentration	2569.26	15	171.284	232.316	0.0001
Error	35.39	48	737		
Total	101482.532	72			
Corrected Total	26417.968	71			

Dependent Variable- Percentage of inhibition. Superscript a: R Squared = 0.999 (Adjusted R Squared = 0.998)

Table 4. Pearson correlation effect between *H.elasticus* and respective hosts in Alpha amylase inhibition assay

Parasite	HEN	HEH	HEC	HES	HEA	HEM
Pearson correlation (r)	0.981*	0.94	0.969*	0.988*	0.978*	0.966*
Sig.(2 tiled)(P)	0.019	0.06	0.031	0.012	0.022	0.034
N	4	4	4	4	4	4
Host	NO	HB	CM	SA	AO	MK

*Correlation significant at the 0.05 level (2 tiled)

Table 5. Alpha glucosidase inhibition assay of *H.elasticus* collected from six different hosts

Concentration (µg/mL)	HEN	HEH	HEC	HES	HEA	HEM
10	10.89±1.63	10.09±0.92	17.5±0.73	16.07±2.01	17.93±0.58	8.23±0.77
20	18.33±1.58	14.74±0.85	28.15±0.55	29.22±1.64	35.99±0.36	14.74±1.07
50	33.86±2.09	25.5±1.07	57.77±1.05	46.48±1.87	56.04±0.89	24.7±0.94
100	51.13±1.6	36.12±1.51	67.07±1.96	57.24±0.85	61.89±1.64	35.46±1.64

Table 6. Alpha glucosidase inhibition assay of six host plants under study

Concentration (µg/mL)	NO	HB	CM	SI	AO	MK
10	5.04±1.77	16.13±0.78	13.41±0.68	15.12±1.04	17.44±1.51	18.15±0.86
20	7.16±0.53	32.36±1.98	25.3±1.25	27.22±2.8	25.3±0.85	34.27±1.58
50	17.24±1.06	40.42±2.1	38.41±1.04	41.33±1.98	46.77±0.88	56.05±1.35
100	25.2±2.3	48.39±2.06	47.78±1.6	49.5±1.05	59.88±0.54	69.15±1.06

Table 7. Two-way ANOVA for Alpha glucosidase inhibition assay

Source	Type III sum of squares	df	Mean square	F	Sig
Corrected model	23032.9989[a]	23	1001.345	558.128	0.0001
Intercept	75105.252	1	75105.252	4.186	0.0001
Plant	6068.272	5	1213.654	676.404	0.0001
Concentration	15682.439	3	5227.48	2.913	0.0001
Plant * concentration	1282.286	15	85.486	47.644	0.0001
Error	86.125	48	1.794		
Total	98224.375	72			
Corrected total	23119.123	71			

Dependent Variable- Percentage of inhibition. Superscript a: R Squared = 0.996 (Adjusted R Squared = 0.994)

Table 1. The maximum inhibition percentage of 74.92±1.03 was observed in HEH at 100 µg/mL. Among the host samples,a comparatively low percent of inhibition was shown by SA and its value was less than 20 per cent even at 100 µg/mL (Table 2). Inhibition percent were found high in HB at concentration 100 µg/mL followed by MK and CM respectively. Two-way ANOVA conducted proved the statistically significant difference in the interaction effect of plant and concentration on the percentage of inhibition of α amylase with F (48, 15) =232.316, p = 0.0001 at 0.05 significance level (Table 3). Post hoc test with Tukey HSD proved that all groups except HEN and HES (p=0.133) showed statistically significant differences. A Pearson product mean correlation was run to determine the relationship between the inhibition mean value of α-amylase assay between *H.elasticus* and its respective hosts and found there was a very strong positive correlation between host and parasite in the activity except for the samples HEH and its hosts (Table 4).

3.2 *In vitro* α-Glucosidase Inhibition

α-glucosidase inhibition studies of methanolic extract of *Helicanthes elasticus* samples and its six different hosts showed slightly different results from that of α-amylase inhibition assay and progressive nature of inhibition was prevalent in all the samples of *Helicanthes elasticus*. At 10 µg/L HEA had maximum inhibition compared to other samples (Table 5). Among the host plants, MK had maximum inhibition on α-glucosidase followed by AO (Table 6). Interaction effect of plant and concentration is significantly different in α-glucosidaseinhibition assay as proved by Two-way ANOVA (F (48, 15) =47.644, p=0.0001 (Table 7) except for HEM and HEH (p= 0.655), HEC and HEA (p = 0.989) as given by post hoc tests. Except for HEC and its host CM, all the samples of parasite and respective hosts showed a very strong correlation in α-glucosidase inhibition assay (Table 8)

Table 8. Pearson correlation effect between *H.elasticus* and respective hosts in Alpha glucosidase inhibition assay

Parasite	HEN	HEH	HEC	HES	HEA	HEM
Pearson correlation(r)	0.988*	0.984*	0.873	0.999**	0.98*	0.981*
Sig.(2 tiled)(P)	0.002	0.016	0.127	0.001	0.02	0.019
N	4	4	4	4	4	4
Host	NO	HB	CM	SA	AO	MK

*Correlation significant at the 0.05 level (2 tiled)
**Correlation significant at the 0.01 level (2 tiled)

4. DISCUSSION

Helicanthes elasticus (Desv.) Danser obtained from six different hosts has an effective role in the preparation of anti-diabetic formulation as they inhibit alpha-amylase and alpha-glucosidase. This is because of the effective lowering of postprandial hyperglycemia offered by alpha-amylase and alpha-glucosidase inhibition[15]. From the results, it was evident that six samples of *Helicanthes elasticus* responded to these two inhibition assays in different ways at different concentrations. Considering the inhibition effect at 100 µg/mL it was clear that HEN, HEC, HES and HEA were showed more than 50 per cent inhibition on alpha-glucosidase, whereas the action of HEH was observed more on alpha-amylase. HEM was found least effective in both assays. Moreover, such variations were prevalent at all concentrations.It was previously reported that methanolic extract of *Helicanthes elasticus* collected from *Mangifera indica* have good α-amylase inhibition activity and proved to be effective in managing hyperlipidemia and glycogen content which are altered during diabetes mellitus[2].

It was reported that inhibition of α-amylase by medicinal plants is attributed to several possible factors such as fibre concentration and the presence of inhibitors on fibers[16]. Encapsulation of starch and enzyme by fiber reduces the accessibility of starch to the enzyme, resulting in reduced enzyme activity[17]. Inhibition of α-amylase could be attributed to various phytoconstituents as suggested by several researchers such as cardiac glycosides[18], flavonoids,[19] and various phenolic compounds[20]. The inhibition of α-glucosidase could be due to the presence of saponins[21] and alkaloids[22].

The same parasitic plant collected from six different hostshasvarious secondary metabolites in its methanolic extract showed a varied inhibition strategy towards these two enzymes. This emphasises the fact that the impact of hosts in which the parasite grows had a significant influence on the qualitative and quantitative occurrence of varied phytochemicals within it. The difference in anti-diabetic activities of parasitic plants could have been due tohost-related factors which are reflected in their phytochemical profile and such plants have different phytochemistry depending upon the host so also their activities[23]. It was reported that the medicinal properties of *Dendrophthoe falcate*, a member of Loranthaceae vary in effects respective to different hosts to which it establishes a relation[24].

Anti-diabetic effects of hosts taken in the present study were previously reported by various researchers. The hypoglycemic effect of *Murraya koenigii* leaves was studied[25] and *Nerium oleander* was also found to have anti-diabetic properties[26]. The dried flower and bark powder of *Saracaasoca* were used for treating diabetes in Kerala[27] and its hypoglycemic effect was also proved[28]. Stem bark and fruits were reported to be anti-diabetic in the case of *Citrus maxima*[29]. Similar work has not yet been conducted in *Hevea brasiliensis* but it is reported to contain bioactive compounds like alkaloids and flavonoids[30].

Same plant *H.elasticus* collected from different hosts showed a statistically significant difference in both α- amylase and α- glucosidase inhibition assay as shown by 2-way ANOVA, emphasizing the fact that the difference in anti-diabetic activity might be influenced by the hosts in which they grow. ANOVA table showed that the effect of the plant,as well as the interaction effect of both plant and concentration, had a significant influence on inhibition. Moreover, Pearson correlation studies proved that except HEH and host HB (in α-amylase), HEC,and its host CM (in α-glucosidase), the other parasitic samples have a linear relationship with their corresponding hosts in this activity. This also proved the fact that parasites and hosts have influenced each other to contribute to this activity. The intercellular interactions that occurred between the parasite and hosts during infection affect both partners either positively or negatively. From the results, it could be said that some hosts had a significant influence on the anti-diabetic potential of *H.elasticus* growing on it and these effects may be negative or positive. Some retards the efficacy while some hosts increase it. Thus, non-relationships between host and parasite in their phytochemical and pharmacological effects are rare. The parasitic performance was found to be weakest and total nitrogen content was found to be highest in *Castillega wightii* while attacking leguminous host *Lupinus arboreus*[31]. Hemi-parasites of Loranthaceae would contribute to decreasing the salt content in parasitised hosts particularly those bearing fruits[32] and due to these reasons, the quality of fruits found declined[33]. This is why herbalists traditionally recommend mistletoe harvested from specifichosts to treat or treat specific health problems[34]. A positive signal either directly or indirectly from the host part is compulsory for every parasite to thrive well in that host, otherwise, the survival of the parasite will be a question. These signals might be mechanical support, synthesised food, water and minerals,and some intracellular phytochemical constituents that transmit in both directions. Phytoconstituents of host and parasite get transported between them as means of existence or survival from the parasite's part or as a means of avoidance from the host's part. These compounds produced as secondary metabolites have pharmacological significance because they were the products of defense mechanisms.

5. CONCLUSION

α-glucosidase and α-amylase inhibition assay conducted to prove the antidiabetic efficacy revealed that *H.elasticus* was therapeutically significant in lowering blood glucose level. The parasitic samples responded differently towards both assays indicating the fact that antidiabetic efficacy was found different in parasitic accessionsand it means host plants have significant role in bioactive properties of this hemi-parasitic plant.

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