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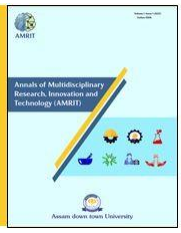

RESEARCH ARTICLE

PLANTS CHEMICAL DEFENSE

Exploring the biochemical defense of *Mikania micrantha* against the parasitic *Cuscuta reflexa*

Smita Deka[1], Uddhav Sarania[1], Priya Borah[1], Nabajit Kalita[1], W James Singha[1] and Hemen Deka[1]*

Ecology and Environmental Remediation Laboratory, Department of Botany, Gauhati University, Guwahati, Assam 781014, India

*Corresponding author: Hemen Deka, *E-mail: hemendeka@gauhati.ac.in*

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Abstract

Parasitic plants exhibit a wide range of morphologies and structurally different haustorial connections, providing a conduit for extracting host resources. Host-parasitism interaction shown by the parasitic plants can impose significant biotic stress on their hosts by extracting nutrients and producing cytotoxic compounds. The present study was conducted to evaluate the biochemical defense mechanisms of the host plant *Mikania micrantha* against the parasitic plant *Cuscuta reflexa* by examining 2,2-diphenyl-1-picryllhyrazyl (DPPH) scavenging activity, hydrogen peroxide (H_2O_2) free radical scavenging activity and reducing power activity of the host plants. Moreover, the influence on the phenolic contents of host plants by *C. reflexa* was also evaluated. The results showed that methanolic extracts from *M. micrantha* infected by *C. reflexa* exhibited significantly higher antioxidant activity, free radical scavenging activity, reduced power capacity, and total phenolic content than control plants without *C. reflexa* infection. The DPPH free radical scavenging activity of *M. micrantha* was notably high at 82.99±1.11% in contrast to 57.11±1.3% in control plants. Similarly, *M. micrantha* showed increased H_2O_2 scavenging activity and reduced power compared to control plants. Furthermore, the total phenolic content was recorded as 44.77±1.35mg/g in infected *M. micrantha*, 40.88±2.05mg/g in *C. reflexa*, and 37.55±1.78mg/g in control plants of *M. micrantha*.

Keywords: *Parasitism, Host-Parasitic interaction, Haustoria, DPPH, H_2O_2, Biochemical defense*

1 Introduction

Parasitic plants are a diverse group of angiosperms that depend partially or fully on host plants for minerals, water, and organic nutrients [1]. They extract resources by attaching to the roots or shoots of their hosts and using specialized structures called haustoria which penetrate the xylem of the host plant and form close connections with its phloem [2]. The close relationship between parasitic plants and their hosts including the direct transfer of compounds from host to parasite suggests that the host species plays a significant role in shaping both the parasites and the animals that feed on them [3]. Additionally, parasitic plants might benefit indirectly by targeting hosts that provide compounds capable of defending the parasite from herbivores [4]. Parasitism often reduces host performance leading to changes in competitive interactions between host and non-host plants [5]. This can trigger a cascade of effects on community structure, diversity, vegetation cycling, and zonation [6]. Moreover, parasitic plants impose significant biotic stress on their hosts by extracting nutrients and water, producing cytotoxic compounds [7].

The *Cuscuta reflexa* is one of the common parasitic plants that parasitize above-ground parts of broad-leaved plants including weeds, field crops, vegetables, and ornamentals throughout the cultivated areas [8]. The *Cuscuta* sp. although green at first often turns orange or purple after parasitizing a host which highlights its reliance on the host for energy instead of photosynthesis [9]. Despite containing chlorophyll, *Cuscuta* sp. is classified as a holoparasite as it cannot reproduce without a host [10]. The

haustoria produced by the *Cuscuta* sp. can penetrate the stem of the host plant by establishing connections with both the xylem and phloem making it a clear example of stem parasitism [11]. Each *Cuscuta* species can parasitize many hosts having a wide physiological tolerance of the metabolism and chemical compositions of host species and this is a unique characteristic of parasitic angiosperms [12]. In agriculture, *C. reflexa* is one of the most important weeds among the members of the genus *Cuscuta*. It causes significant yield loss, especially in Alfalfa (*Medicago sativa*), sugar beet (*Beta vulgaris* L.), and other crop species. The devastating effect is caused by significant growth reduction of the host plants ultimately leading to reduced biomass accumulation and poor seed development of infested host plants.

Mikania micrantha is a fast-growing perennial creeping vine belonging to the family Asteraceae and is native to Central and South America [13]. The effects of the obligate parasite *Cuscuta campestris* on *M. micrantha* for biomass production, physiology, and ecology were reported in recent studies [14]. Different species belonging to the genera *Cuscuta* including *C. campestris*, *C. chinensis*, and *C. australis* can effectively restrain the growth of *M. micrantha* [15, 16]. Moreover, host species are sometimes also affected by the parasitic behavior of *Cuscuta* sp. which involves attaching to the host and extracting water and nutrients via haustoria. This nutrient depletion leads to stunted growth, decreased biomass, and overall vigor in the host plant [17]. The physiological stress caused by *Cuscuta* sp. also negatively impacts the photosynthesis and respiration of the host further hindering growth and reproductive success [10].

Additionally, the *Cuscuta* plant was reported to act as a potential biological control for invasive plants including *Mikania micrantha*, *Ipomoea cairica*, and *Wedelia trilobata* [18]. The presence of *Cuscuta* sp. can alter competitive dynamics in the ecosystem, potentially diminishing the population of host species if it struggles to compete for light and space [19]. Therefore, the parasitism possessed by *Cuscuta* sp. may pose significant challenges to the health and sustainability of host plants like *M. micrantha.*

Parasitic plants are known to exert biotic stress on their host plants impacting their biochemical processes including antioxidant activity. Recent studies indicate that interactions with parasitic plants can stimulate host plants to produce elevated levels of antioxidants as a defense mechanism [20]. This response varies depending on the host species with different host-parasite interactions resulting in distinct levels of total phenolic and flavonoid content which are known to be closely linked to enhanced antioxidant capacities [21]. This variation suggests that host-specific responses to parasitism may play a critical role in shaping the antioxidant profile of the host plant potentially influencing its resilience to biotic stressors. It has been reported that when *Cuscuta* sp. interacts with the host plants it may affect the antioxidant activities of host plants including 2,2-diphenyl-1-picryllhyrazyl (DPPH) scavenging activity, hydrogen peroxide(H2O2) free radical scavenging activities and reducing the power of the host plants and vice versa [22, 23, 24]. Moreover, *Cuscuta* sp. can also influence the phenolic contents of host plants within their habitat [25]. Therefore, the present study was carried out to evaluate the biochemical defense mechanisms of the host plant *Mikania micrantha* against the parasitic plant *Cuscuta reflexa* within the study site.

2 Materials and Methods

2.1 The collection site:

The sampling location of the present study is Gauhati University campus where many parasitic plant species are present. The map indicating the sampling locations is presented in Figure 1. The invasive plant, *Mikania micrantha* where *Cuscuta reflexa* was growing as a parasitic plant was selected for the study. The plants were collected from different areas of Gauhati University. The plant species of *M. micrantha* which was used as a control was not parasitized by *C. reflexa* were also collected for comparative analysis. All the plant species were collected in polybags aseptically and brought into the laboratory for further processing and analysis.

2.2 Preparation of plant extract

Before analysis, the collected plant samples were allowed to shade dry. After that, the dried plant samples were crushed and homogenized into a fine powder. The plant extracts were prepared by mixing dried powdered samples in methanol in 1: 10 (w/v) ratio. The solution was kept in a shaking incubator at 100 rpm and 40o C for 48 hrs. and filtered for further use.

2.3 Analysis of Antioxidant Activities

2.3.1 DPPH free radical scavenging activity

The 2,2-diphenyl-1-picryl hyrazyl (DPPH) activity was measured following Brand-Willium et al. [26] with slight modifications. For the experiment, Butylated hydroxyl toluene (BHT) was selected as standard, and methanol was used as control. The absorbance of the DPPH solution was measured at 517 nm using a UV-VIS spectrophotometer.

2.3.2 H_2O_2 Radical Scavenging Assay

The hydrogen peroxide (H_2O_2) Scavenging ability of the experimental plant samples was determined by the method of Ruch *et al.* [27]. For the assay, plant extracts were dissolved in 0.4 ml of phosphate buffer (pH 7.4) and 0.6 ml of H_2O_2 solution. The mixtures were then incubated for 10 min at 25°C and the absorbance readings were measured at 230 nm.

2.3.3 Reducing Power Assay

The reducing power was assessed using the method of [28]. Briefly, plant extracts were mixed with 2.5ml phosphate buffer (0.2, pH 6.6) and 2.5ml potassium ferricyanide (K3[Fe (Cn)6]). The mixtures were then incubated at 50ºC for 20 minutes.

Afterward, 2.5 ml of 10% Trichloroacetic acid (TCA) was added to each reaction mixture, followed by centrifugation at 3000 rpm for 10 minutes. The resulting supernatant (2.5ml) was then mixed with 2.5ml of deionized water and 0.5ml of ferric chloride (FeCl2) and absorbance was recorded at 700nm.

2.3.4 Total phenolic content

The total phenolic content was estimated by the Folin–Ciocalteu method, as outlined by [29]. Plant extracts were dissolved in ethanol to obtain a test solution of a concentration of 1 mg/ml. One ml of each test solution was further diluted with 9 ml of distilled water, followed by the addition of 200 µl of Folin–Ciocalteu reagent and 600 µl of 2% Na2CO3. The mixture was incubated for 2 hours at 25°C, after which the absorbance was measured at 750 nm using a spectrophotometer. Total phenolic content was expressed as gallic acid equivalents (mg/ml).

3 Results and Discussion

3.1 DPPH Scavenging Activity

The DPPH free radical scavenging activities of the methanolic extracts of the host plant *Mikania micrantha* against the parasitic plant *Cuscata reflexa* have been graphically presented in Figure 2. For a comparative study, the antioxidant activity against DPPH free radicals of the host plant without the attack of its parasitic plants has also been analyzed. The vice versa effect of the host plants on the DPPH scavenging ability of *C. reflexa* has also been presented in Figure 2. The results revealed that the *Mikania micrantha* (host) showed more inhibition percentage than the *M. micrantha* (control) among the different concentrations. The *M. micrantha* (host) showed inhibition percentages of 40.28±1.42%, 49.18±1.7%, 58.54±1.66%, 67.8±1.75%, and 82.99±1.11% for DPPH free radical scavenging activity as against the

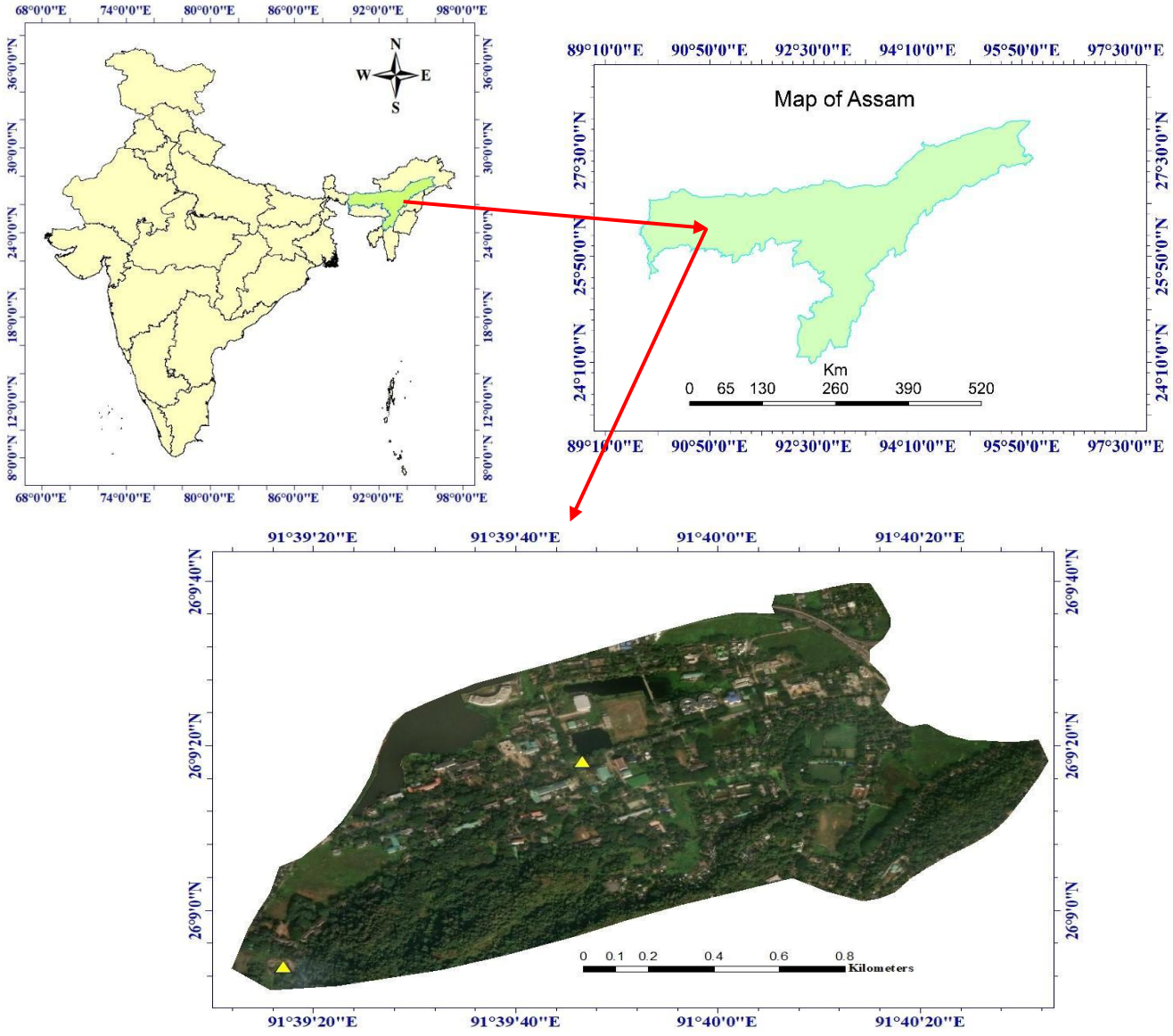

Figure 1: (A) Map of India, (B) Map of Assam, (C) Map of sampling locations

concentrations of 10µg/ml, 20µg/ml, 40µg/ml, 80µg/ml and 160µg/ml respectively. On the other hand, in the case of *Mikania micrantha* (control), the inhibition percentages were recorded as 17.97±1.97%, 24.68±1.4%, 35.29±0.96%, 44.03±0.75%, and 57.11±1.3% as against the concentrations of 10µg/ml, 20µg/ml, 40µg/ml, 80µg/ml and 160µg/ml respectively. The results also disclosed that the IC_{50} value of the standard BHT, *M. micrantha* (host), and *M. micrantha* (control) were recorded as 133.16µg/ml, 469.78µg/ml, and 719.10µg/ml respectively. The results revealed that in the case of the *C. reflexa* parasitizing *M. micrantha*, the inhibition percentage for DPPH free radical scavenging activities were recorded as 35.26±2.06%, 41.96±0.79%, 53.99±0.92%, 68.25±1.8%, and 79.27±1.0% as against the concentrations of 10µg/ml, 20µg/ml, 40µg/ml, 80µg/ml and 160µg/ml respectively.

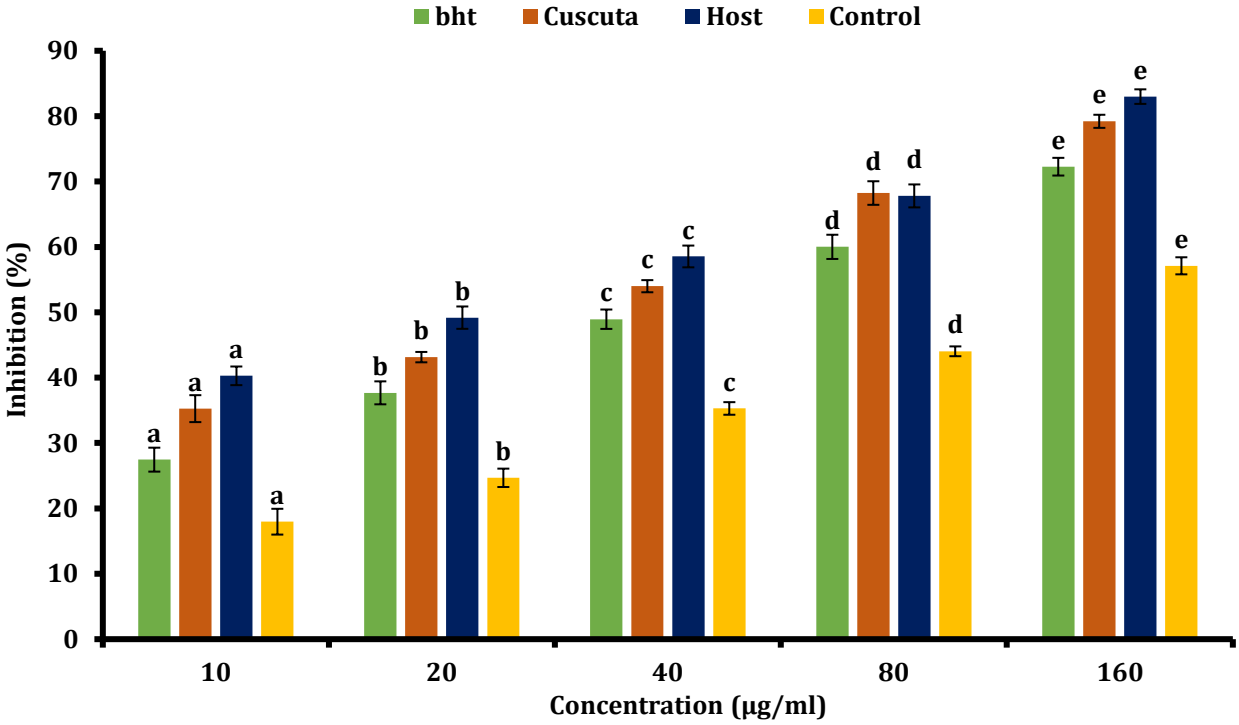

Figure 2: Showing the DPPH scavenging activities of *Mikania micrantha* and *Cuscuta reflexa* under association and in control conditions. Values are Mean ± SD. Different letters above the bars indicate significant differences.

The present study revealed that the DPPH scavenging activity of the host plant samples showed greater inhibition percentages. This can be attributed to the increased antioxidant defense activity of the plants under parasitic attack. The elevated levels of the antioxidant defense system aid the host plants survive under such pressure. This is per the previous findings of Tanruean *et al* [23]. who had reported a higher inhibition percentage in host plants under stressed conditions and displayed potential antioxidant activity. Moreover, it has been stated by the earlier works of Zagorchev *et al.* [1] that plants when attacked by parasites are under constant pressure that often affects their survivability.

3.2 H_2O_2 (Hydrogen Peroxide) Radical Scavenging Activity

The H_2O_2 free radical scavenging activities of the plant extracts of the host plant *M. micrantha* against the parasitic plant *C. reflexa* have been graphically presented in Figure 3. For comparative analysis, the antioxidant activity against DPPH free radicals of the host plant without the attack of its parasitic plants has also been analyzed. The vice versa effect of the host plants on the DPPH scavenging ability of *C. reflexa* has also been presented in Figure 3. The *M. micrantha* (host) showed inhibition percentages of 13.01±0.97%, 17.66±1.7%, 22.65±1.66%, 26.18±1.75%, and 29.95±1.11% for H_2O_2 scavenging activity as against the concentrations of 10µg/ml, 20µg/ml, 40µg/ml, 80µg/ml and 160µg/ml respectively. On the other hand, in the case of *Mikania micrantha* (control), the inhibition percentages were recorded as 5.71±1.11%, 9.12±1.4%, 13.3±0.96%, 17.2±0.75% and 21.04±1.3% against the concentrations of 10µg/ml, 20µg/ml, 40µg/ml, 80µg/ml and 160µg/ml respectively. In the case of the plant species *Cuscuta reflexa*, the inhibition percentage for H_2O_2 scavenging activities were recorded as 8.25±0.89%, 11.95±0.79%, 16.62±0.92%, 20.65±1.8% and 25.18±1.0% when parasitized with *M. micrantha* as against the concentrations of 10µg/ml, 20µg/ml, 40µg/ml, 80µg/ml and 160µg/ml, respectively.

In the case of the H_2O_2 scavenging assay, both the host plant samples showed a higher inhibition percentage than the control plant samples. This implied that the host plants showed better defense against the oxidative stress created due to parasitism. Plants generally exhibit higher capacity to scavenge H_2O_2 when exposed to certain stress conditions. Moreover, higher resistance against parasitism is acquired by plants with increased

accumulation of H_2O_2, ß-Glucanase, and Peroxidases. The antioxidant can interact with reactive oxygen species (ROS) or high levels of free radicals including H_2O_2 radicals to constrain oxidative stress by inhibiting lipid peroxidation and improve resistance power by protecting against potential cell injury caused by paralocations and other environmental stress [30].

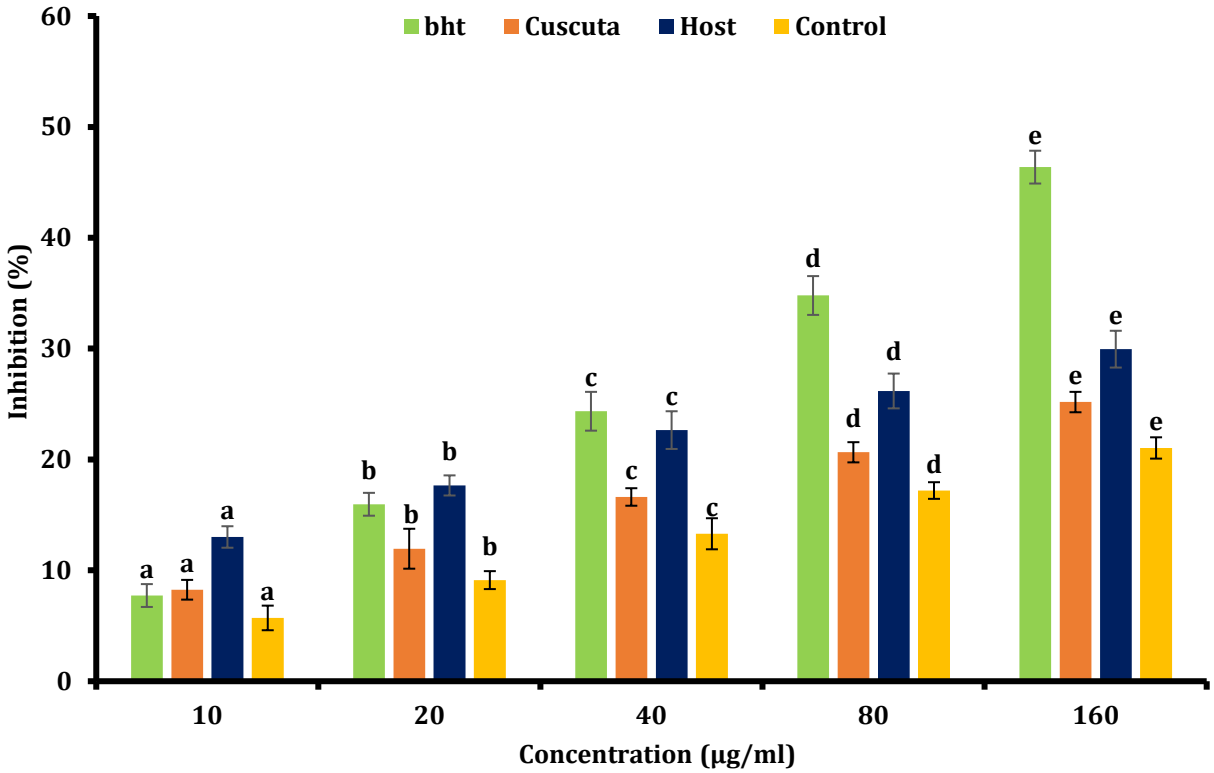

Figure 3: Showing the H_2O_2 scavenging activities of *Mikania micrantha* and *Cuscuta reflexa* under association and control conditions. Values are Mean ± SD. Different letters above the bars indicate significant differences.

3.3 Reducing Power Assay

The results of reducing power activity of different extracts of *Cuscuta reflexa* and their host plant *Mikania micrantha* collected from various locations of Gauhati University have been presented graphically in Figure 4. The results revealed that the plant species *C. reflexa* parasitizing *M. micrantha*, the reducing power activities were recorded as 0.059±0.013nm, 0.196±0.012nm, 0.342±0.013nm, 0.524±0.011nm and 0.741±0.014nm against the concentrations of 10µg/ml, 20µg/ml, 40µg/ml, 80µg/ml and 160µg/ml respectively. The results also revealed that the *M. micrantha* (host) showed more reducing power activity than the *M. micrantha* (control) among the different concentrations. The reducing power activity in the case of *M. micrantha* (host) was recorded as 0.069±0.014nm, 0.222±0.01nm, 0.394±0.01nm, 0.601±0.011nm and 0.796±0.015nm against the concentrations of 10µg/ml, 20µg/ml, 40µg/ml, 80µg/ml and 160µg/ml respectively. On the other hand, *Mikania micrantha* (control) exhibited the reducing power activity of 0.044±0.01nm, 0.156±0.016nm, 0.306±0.014nm, 0.491±0.025nm and 0.696±0.037nm against the concentrations of 10µg/ml, 20µg/ml, 40µg/ml, 80µg/ml and 160µg/ml respectively.

The host plant samples showed maximum reducing power activity than the control plant samples. Soni and Sosa [31] have found a similar trend by evaluating the reducing assay of four important herbs namely *Ocimum sanctum*, *Mentha spicata*, *Trigonella foenum-graecum*, and *Spinacia oleracea* that were grown under stress conditions. The enhanced level of reducing power activity in hosts could also be corroborated by the increased ROS production. It has been suggested that the interactions between reducing power and ROS restrict the oxidative stress in plants by inhibiting lipid peroxidation and enhancing resistant power which ultimately increases the reducing power activity of the host plants [29, 32].

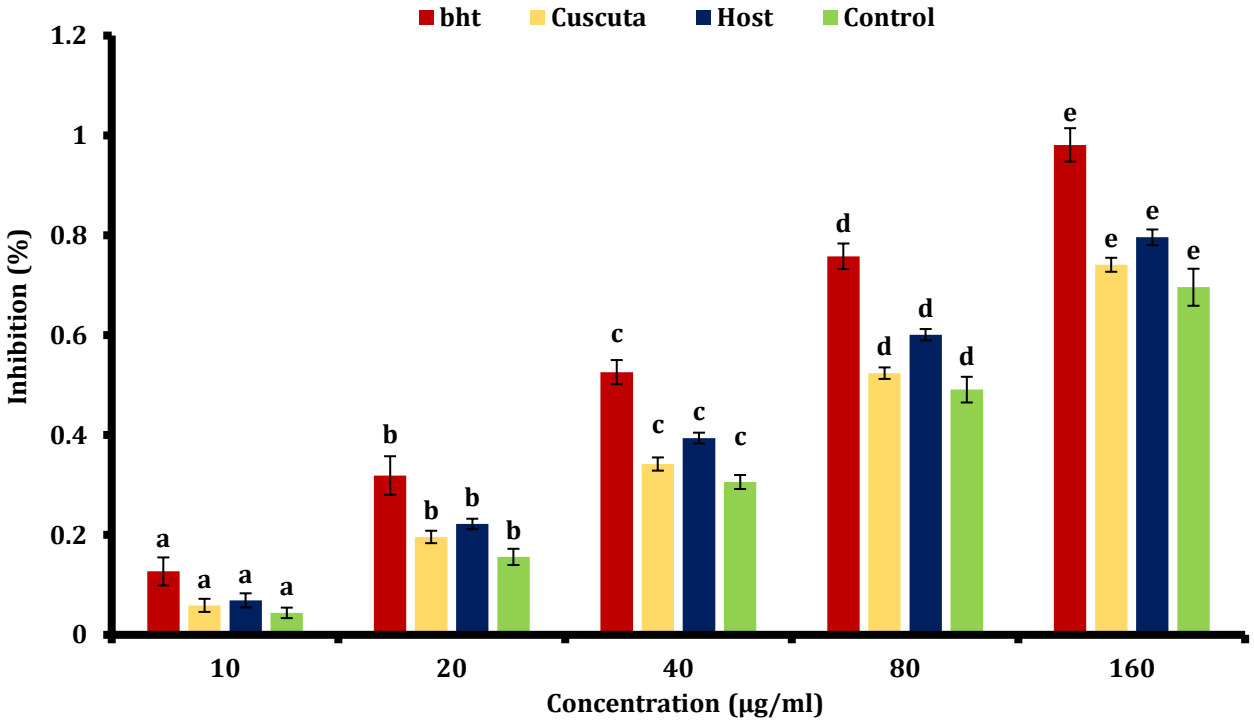

Figure 4: Showing the reducing power scavenging activities of *Mikania micrantha*

3.4 Phenolic content

Polyphenol-containing samples are reduced by the folin-ciocalteu reagent, thereby producing a blue colour complex. From a gallic acid calibration curve, the phenolic contents were estimated and have been presented in Figure 5. The results revealed that the total phenolic content of *C. reflexa*, host plant (*Mikania micrantha*), and control plant (*M. micrantha*) was recorded as 40.88±2.05 mg/g, 44.77±1.35 mg/g and 37.55±1.78 mg/g respectively. The results also indicated that the total phenolic contents for the host plant were more than that of the control plant. The present findings agree with the earlier reports of Amol *et al.* [33] where it has been reported that higher levels of phenolic and flavonoid content were exhibited in *C. grandis* infested with *C. reflexa*. The increasing values of phenolic contents could be responsible for its greater antioxidant activities. Previous research also suggested that the antioxidant activities of plant extracts can be partly attributed to some constituents of phenolic compounds other than fatty acids. Tanruean *et al* [23] reported that the phenolic compounds could exhibit high antioxidant activities especially rutin, catechin, and quercetin, found in the extracts of *C. reflexa* grown on *C. grandis* and *F. racemosa.* However, the unidentified active phenolic compounds in the methanol crude extracts of *C. reflexa* may also play a role in antioxidant activity.

Conclusion

This study focuses on the antioxidant defense mechanisms in *Mikania micrantha* plants parasitized by *Cuscuta reflexa*. For comparison, uninfected *Mikania micrantha* plants served as the control. The antioxidant enzyme activities and phenolic content of both parasitized and control plants were evaluated using the DPPH scavenging assay, H_2O_2 radical scavenging assay, and reducing power assay. The results revealed significant variations between the parasitized and control plants. Methanolic extracts of the parasitized plants demonstrated enhanced antioxidant defense mechanisms compared to the control group. These host plants exhibited higher antioxidant and free radical scavenging activities, greater reducing power, and elevated phenolic content. Overall, the findings suggest that parasitized *Mikania micrantha* plants activate a stronger defense system to cope with oxidative stress induced by parasitism. The enhanced antioxidant activity likely contributes to preventing oxidative damage and bolstering the plant's defense mechanisms under adverse conditions. However, further studies are required to explore the specific antioxidant enzymes involved, molecular mechanisms underlying the defense response, and the long-term impact of parasitism on plant physiology.

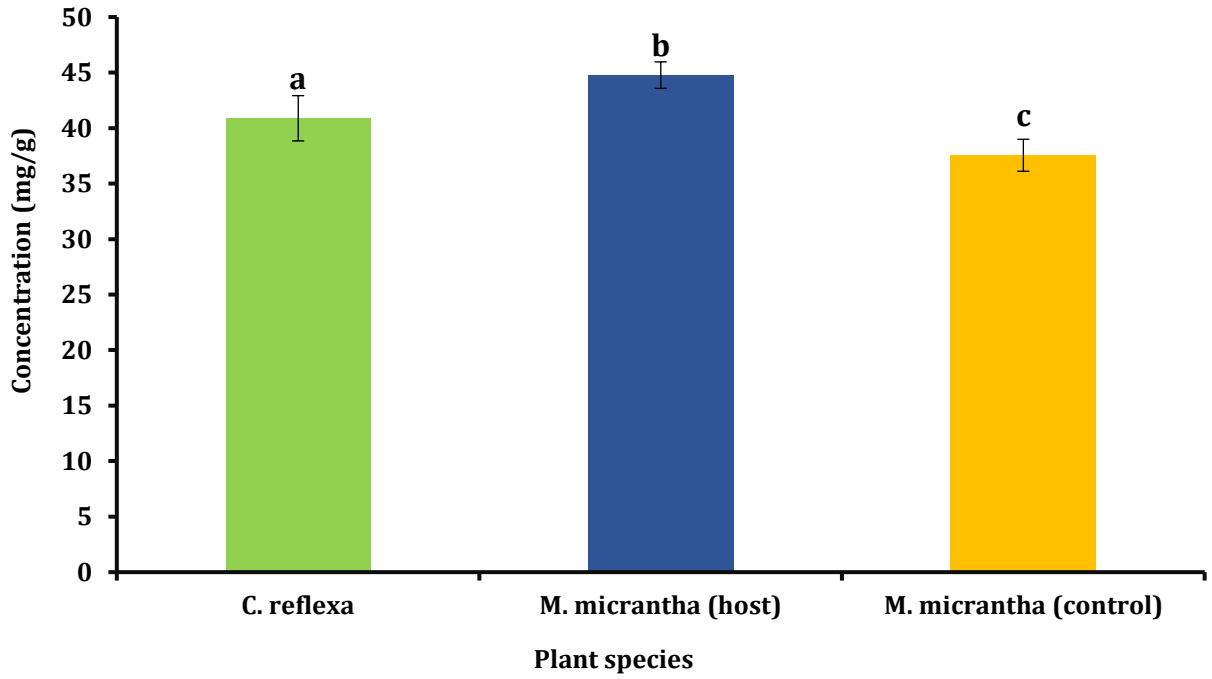

Figure 5: Showing the phenolic content values of *Mikania micrantha* and *Cuscuta reflexa* under association and in control conditions. Values are Mean ± SD. Different letters above the bars indicate significant differences.

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