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The Enhanced Antioxidant Capacity of Gall Tissues in *Litchi chinensis* Sonn. as an Adaptive Defence Mechanism Against Mite Infestation

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Abstract: ***Biotic stress, such as herbivory by gall-inducing mites, necessitates a robust defence response in plants. This study investigated the antioxidant activity of normal leaves and leaf galls of Litchi chinensis Sonn. using the 1,1-diphenyl-2-picryl-hydrazil (DPPH) radical scavenging assay. Results were expressed as IC50 values and the Antioxidant Activity Index (AAI). The findings demonstrated a significant increase in antioxidant potency within the gall tissues. The Mature Gall stage exhibited the highest activity, with the lowest IC50 value (198.08±5.94 µg/ml), compared to the Normal Leaf (313.76±9.41 µg/ml). This enhanced activity is strongly correlated with the localised accumulation of secondary metabolites, suggesting that the mite infestation triggers an active metabolic response by the host plant to synthesise protective compounds. This heightened antioxidant capacity serves as a critical defence mechanism to mitigate oxidative stress and may play a role in maintaining the cellular viability of the gall structure, thereby influencing the host-parasite relationship.***

Keywords: *Litchi chinensis*, Plant defence, Galls, Antioxidant assay, DPPH, IC50 value, Oxidative stress

1. Introduction

Reactive Oxygen Species (ROS) are highly reactive molecules that are inevitable byproducts of normal plant metabolism, such as photosynthesis and respiration (Foyer and Noctor, 2013). However, plants also produce elevated levels of ROS as a rapid and critical response to various biotic and abiotic stresses, including pathogen attack, environmental extremes, and herbivory (Suzuki and Mittler, 2006; Mittler, 2017). Uncontrolled accumulation of ROS leads to oxidative stress, causing significant damage to cellular components like lipids, proteins, and DNA, which can ultimately lead to cell death.

To counteract oxidative damage, plants have evolved sophisticated antioxidant defence systems (Mittler, 2017). These systems comprise both enzymatic (e.g., Superoxide Dismutase, Catalase) and non-enzymatic antioxidants (e.g., phenolics, flavonoids, ascorbate, glutathione). These non-enzymatic compounds, often classified as secondary metabolites, are potent radical scavengers and play a crucial role in mitigating stress and enhancing resistance against pests and pathogens (Shapiguzov et al., 2012). The synthesis and localised accumulation of these metabolites are hallmarks of an active plant defence response against localised biotic intrusion.

This principle of localised defence is highly relevant to the system investigated in this study. The focus of this research, *Litchi chinensis* Sonn., is an economically significant subtropical fruit tree (Menzel, 2002) whose production is severely challenged by pests, notably the gall-inducing mite (*Aceria litchii*) (Menzel and Waite, 2005). This mite infestation triggers the host plant to form abnormal, blister-like galls on the leaf. These galls represent a localised, highly modified plant tissue environment in which the host attempts to contain and respond to the physical and chemical cues of the infesting parasite. Understanding the metabolic changes within these gall tissues is key to characterising the plant's adaptive response.

While the morphological and physiological aspects of gall formation have been widely studied, the specific role of the antioxidant system in the host's defence within these unique tissues remains less explored. Given that the formation and maintenance of galls, combined with the presence of feeding mites, constitute a state of continuous biotic stress, it is hypothesised that the gall tissues of *Litchi chinensis* would exhibit an enhanced antioxidant capacity compared to normal, healthy leaf tissues.

This study, therefore, aims to investigate the antioxidant potential of normal and gall tissues of *Litchi chinensis* using the 1,1-diphenyl-2-picryl-hydrazil (DPPH) radical scavenging assay. By quantifying the antioxidant activity index and IC50 values, we sought to determine if the mite infestation actively modulates the plant's metabolic pathways to synthesise protective secondary metabolites, thereby providing an adaptive defence mechanism against the sustained oxidative challenge.

2. Materials and Methods

Antioxidant Activity and IC50 Value Analysis

The antioxidant activity of normal and galled *Litchi chinensis* tissues was assessed using the 1,1-diphenyl-2-picryl-hydrazil (DPPH) radical scavenging assay, performed according to the method described by Brand-Williams et al. (1995).

Plant Extract Preparation

Normal leaves and galls of *Litchi chinensis* infested by the gall-inducer, the mite *Aceria litchii*, were collected, gently washed, and shade-dried. The gall samples were carefully separated into three distinct developmental stages based on morphology and size: young galls, mature galls, and old galls. The dried samples were powdered, and 8 g of crude powder from each stage was extracted using a Soxhlet apparatus with 80% methanol for 24 hours. The extracts were filtered and vacuum-evaporated to complete dryness. The resulting crude extract was resuspended in 10 ml of 80% methanol, and

working dilutions ranging from 20 to 100 µg/ml were prepared for the assay.

Standard and DPPH Solution Preparation

A standard antioxidant solution of 1 mg/ml was prepared in 80% methanol using ascorbic acid. This stock solution was used to prepare working standard dilutions ranging from 20 to 100 µg/ml. A 0.004% (w/v) DPPH solution was prepared by dissolving 0.004 g of DPPH in methanol.

Estimation of Antioxidant Activity

The antioxidant activity of the extracts was estimated by combining 3 ml of the 0.004% methanolic DPPH solution with 1 ml of the test samples, the ascorbic acid standard, or a methanol blank. After a 30-minute incubation period, the absorbance was measured spectrophotometrically at 517 nm. The reduction of the DPPH radical, indicated by a drop in absorbance and a change in colour, was used as a measure of free radical scavenging ability.

The percentage inhibition (%I) was calculated using the following formula:

$$\text{Percent inhibition} = \frac{A\_\{blank\} - A\_\{sample\}}{A\_\{blank\}} \times 100$$

where A_{blank} is the absorbance of the methanol blank, and A_{sample} is the absorbance of the sample or working standard.

The antioxidant activity index (AAI) was calculated to compare the samples against the ascorbic acid standard:

$$\text{Antioxidant Activity Index} = \frac{\text{IC50 of the standard Ascorbic acid (µg/ml)}}{\text{IC50 of the sample (µg/ml)}}$$

The IC50 value, defined as the concentration of the sample required to inhibit 50% of the DPPH radicals, was determined from the linear regression plot of per cent inhibition against the sample concentration. All samples were prepared in triplicate.

3. Results and Observations

The DPPH scavenging activity assay demonstrated that galled leaves of *Litchi chinensis* were significantly more active as antioxidants than normal leaves. A key finding is the inverse relationship between antioxidant activity and the IC50 value: a lower IC50 indicates greater antioxidant potency.

The IC50 value for the normal leaf was 313.76±9.41 µg/ml, which was the highest among all tested samples, confirming its lowest antioxidant activity. In sharp contrast, the mature gall exhibited the lowest IC50 value at 198.08±5.94 µg/ml, thus demonstrating the highest antioxidant activity. The IC50 values for the young and old galls were 233.06±6.99 µg/ml and 303.07±9.09 µg/ml, respectively, both of which were lower than the normal leaf. The standard, ascorbic acid, had an IC50 of 68.09±2.04 µg/ml (Table 1; Figure 1 and 2a).

The antioxidant activity indices further confirmed this pattern. Mature gall showed the maximum antioxidant activity index (0.34±0.01), followed by young gall (0.29±0.01) and old gall (0.22±0.01). The normal leaf showed the minimum antioxidant activity index (0.21±0.01) (Figure 2b).

Table 1: Estimation of antioxidant activity, IC50 value and activity indices of normal leaf and gall tissues of *Litchi chinensis* (NL- Normal leaves, YG- Young gall, MG- Mature gall, OG-Old gall).

S. No.	Conc. (µg/ml)	STD Ascorbic acid	Percentage inhibition by methanolic extracts			
			NL	YG	MG	OG
1	20	32.69	17.54	22.22	26.02	23.39
2	40	43.5	21.05	22.95	28.94	25.43
3	60	51.28	22.95	26.6	30.99	27.63
4	80	53.22	24.56	29.38	34.5	28.65
5	100	57.35	26.6	32.45	36.69	31.14
IC_{50} values		68.09±2.04	313.76±9.41	233.06±6.99	**198.08**±5.94	303.07±9.09
Antioxidant Activity Index (AAI)		1.00±0.03	0.21±0.01	0.29±0.01	**0.34±0.01**	0.22±0.01

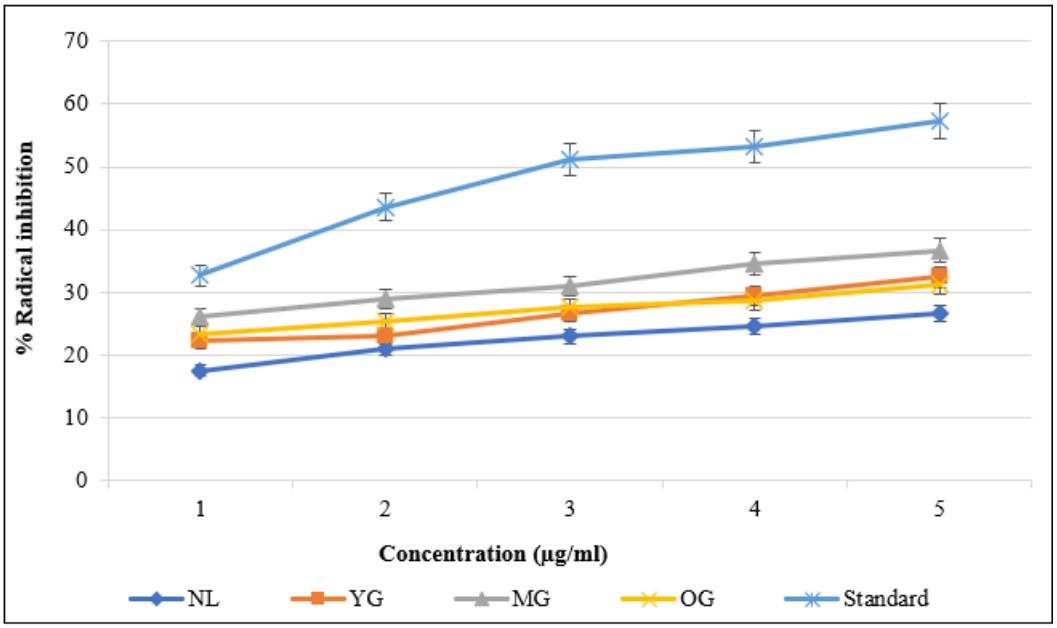
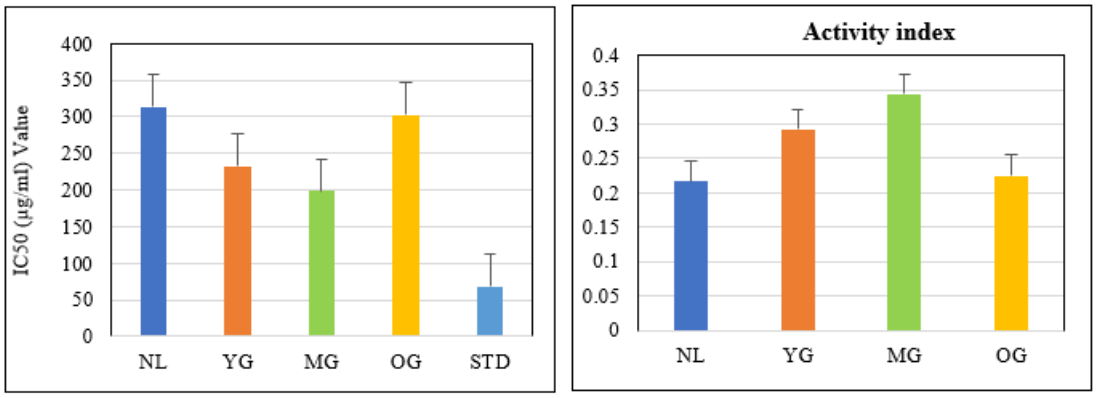


Figure 1: Estimation of percentage free radical inhibition through DPPH assay against ascorbic acid standard. NL- Normal leaves, YG- Young gall, MG- Mature gall, OG-Old gall. The results are represented by the mean value ± standard deviation (n = 3).



(a) **(b)**

Figure 2: (a) IC50 values (µg/ml) (concentration required for 50% inhibition) for DPPH radical scavenging activities of the compounds (NL- Normal leaves, YG- Young gall, MG- Mature gall, OG-Old gall) in comparison with the standard antioxidant Ascorbic acid, (b) Antioxidant Activity Index.

4. Discussion

The results of the DPPH scavenging activity assay strongly indicate that galls on *Litchi chinensis* are significantly more potent antioxidants than normal leaves. The high antioxidant activity in galls aligns directly with the inverse relationship with the IC50 values and supports prior findings that gall tissues have a significantly higher total phenolic content. This consistency confirms the direct link between these two phenomena, which is supported by research demonstrating that enhanced antioxidant activity correlates strongly with greater amounts of total phenolic and flavonoid content in plant extracts (Eshwarappa et al., 2014; Mohamed et al., 2010).

The powerful antioxidant properties of phenolic compounds are primarily attributed to their redox features, which enable them to effectively scavenge free radicals and chelate transition metals (Kessler et al., 2003). The elevated antioxidant activity observed in the litchi galls, especially in the mature stage (198.08 µg/ml), suggests that the mite infestation actively triggers the synthesis of these protective secondary metabolites.

This enhanced antioxidant capacity serves as a key defence mechanism employed by the plant to counteract the oxidative stress induced by parasitic attacks (Chen et al., 2020). The developmental trend observed, highest activity in mature galls and subsequent decline in old galls, is biologically relevant. The mature gall stage represents the phase where the mite is actively developing, placing maximum metabolic demand and stress on the host tissue. The plant responds by maximising antioxidant production to maintain cellular homeostasis and compartmental integrity. Furthermore, the maintenance of high antioxidant levels is proposed to inhibit the senescence of gall tissues (Kot et al., 2018), ensuring the longevity of the gall structure to serve as a continuous nutrient source and defensive sanctuary for the developing mite, thereby influencing the host-parasite relationship. The decline in activity in old galls (303.07 µg/ml) suggests a reduction in metabolite synthesis as the gall senesces after the mite has dispersed. While the crude extract is highly potent, it is still

expectedly less active than the pure standard (Ascorbic Acid IC50 of 68.09 µg/ml).

5. Conclusion

The DPPH radical scavenging assay unequivocally demonstrates that galls induced by mite infestation on *Litchi chinensis* possess significantly higher antioxidant activity than normal leaf tissues. This heightened activity, peaking in the mature gall stage, is strongly correlated with a corresponding increase in secondary metabolites, particularly phenolic compounds. The findings confirm that the host plant actively modulates its metabolic pathways under biotic stress to synthesise a robust chemical defence system. This enhanced antioxidant capacity is interpreted as a critical protective response by the plant, serving to mitigate oxidative stress and potentially extending the longevity of the gall structure, which ultimately influences the ecology and success of the gall-inducing mite. Further research is warranted to isolate and identify the specific phenolic compounds responsible for this high antioxidant potency and to fully elucidate their role in the complex host-parasite interaction.

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