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Atioxidant Activity Of Crude Extracts From Various Parts Of Ethnomedicinal Plant Of Endophytic Fungi Of *Guilandina Bonduc*.

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Abstract

In India, *Guilandina bonduc* is a common plant. Because this plant is frequently utilised in traditional medicine, the biotechnology of endophytic fungi is extremely important because natural resources have decreased of plants. The endophytic fungus that were discovered in *Guilandina bonduc* plant and produced a variety of chemicals were described in this study. The endophytic fungal species were identified by morphological and molecular means using phylogenetic tree analysis. The cultivation method was carried out using Potato Dextrose Broth media. The extraction and evaporation processes were carried out using a rotary evaporator by using ethyl acetate as a solvent. The DPPH method and paper disc diffusion method were used to conduct antioxidant and antibacterial testing. The compound was isolated using chromatographic methods, and its chemical structure was determined by spectroscopic examination. *Lasiodiplodia irregularis* was determined to be an endophytic fungus based on the morphological and molecular study results. 2-Nonynoic acid was the pure chemical that was extracted from this endophytic fungus. This substance was probably going to be used as a starting point for novel antioxidants.

Keywords: *Lasiodiplodia irregularis*, bioactive compounds, endophytic fungi, and antioxidant properties.

Introduction

The *Lasiodiplodia irregularis* plant's secondary metabolites, which include flavonoids, peronemin, isopropanol, betulinic acid, and sitosterol, are effective antioxidants and antibacterials that can boost immunity (Dillasamola et al., 2021; Latief, 2021). However, there are numerous barriers to the production of medicinal plants that lower plant populations, and research is required to identify alternative sources of raw materials for medical applications. Endophytic fungi's biotechnology allows them to live inside plant tissues without endangering their hosts (El Hawary et al., 2020; Mbilu et al., 2018). Endophytic fungi are an attracting target for natural products due to their intriguing range of chemical structures and bioactivity (Tiwari and Bae, 2020; Wen et al., 2022).

Endophytic fungi are known as secondary metabolite stores because research has shown that they produce secondary metabolites with a variety of bioactivities, including antibiotics, antiprotozoals, antivirals, antidiabetics, antiparasitics, anticancerous, antioxidants, and immunomodulatory compounds (Khan et al., 2019; Manganyi and Ateba, 2020). A genus of endophytic fungus with a variety of bioactivities is called

Trichoderma (Morais et al., 2022; Zhang et al., 2021). *Trichoderma* grows significantly more quickly and has a very good ability to adapt to its surroundings. Many secondary metabolites, including isonitrile, diketopiperazine, sesquiterpenes, polyketides, alkyl pyrone, and peptaibol, can be produced by *Trichoderma* (Khanet al., 2020; Wu et al., 2017).

It has been demonstrated that *T. harzianum* and *T. hamatum* create 6-pentyl-α -pyron, which effectively possesses antioxidant and antibacterial qualities against *Acidovorax avenae, Erutimcara favora*, and *Xanthomonas campestris* (Al Rajhi et al., 2022; Baazeem et al., 2021). Alkaloids, tannins, phenolics, triterpenoids, and flavonoids are among the substances found in *Lasiodiplodia irregularis* that are known to have bioactivity, including antipyretic, antibacterial, anticancer, and antioxidant properties (Gu et al., 2022; Karuppiah et al., 2019; Scudelett et al., 2021; Singh et al., 2021). Endophytic fungi's secondary metabolites can serve as useful sources of raw materials for novel medications. The bioactive metabolites have a distinct chemical structure, according to research. Similar metabolites or novel chemicals that differ from their host can be produced by these endophytic fungi (Cruz et al., 2020; El Hawary et al., 2020). This phenomena has the potential to be created from a group of bacteria in order to find novel medications.

Material and Methods

Sample Preparation and Isolation of Endophytic Fungi

Fresh pieces of leaves, roots, bark, stems, fruits, and flowers were utilised. Before the endophytic fungi were isolated, all plant parts were surface sterilised by washing them with water for approximately five minutes. The sample was then submerged in 70% alcohol for approximately three minutes, washed with sterile distilled water for approximately one minute, and finally submerged in 3% NaOCl solution for one minute. Before being inoculated into a petri dish with PDA, the sample was first chopped aseptically into ±3 x 1 cm pieces. The inoculants were then cultured for 3–14 days at a temperature of 25–28^{0}C in a BOD incubator. Fungal endophytes are purified by moving the colonies to fresh petri dishes with media and letting them sit for 48 hours (Setiawan, 2022; Hapida et al., 2021).

Characterization and Identification of Fungal Endophytes

Endophytic fungi were identified using morphological and phenotypic traits. Using a 1000X magnification, microscopic features were observed using the slide culture approach. The resulting macroscopic and microscopic phenotypic traits were then compared with a number of sources (books and journals) for identification requirements (Pitt and Hocking, 2009; Walshet al., 2018; Watanabe, 2002).

Molecular Identification of Fungal Endophytes

The endophytic fungal isolates with the highest potential bioactivity were used for molecular identification. The ITS DNA (rDNA) region was used for the identification. Primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS 4 (5'-TCCTCCGCTTATTGATATGC-3') were employed in the amplification procedure. After that, the sequences were added to BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Additionally, the sequences were aligned using the CLUSTAL W method (in the MEGA11 program), and a phylogenetic tree with a bootstrap value of 1000 was created using the neighbour joining tree method (Tamura et al., 2013).

Cultivation and Extraction

Six blocks of agar with a diameter of six millimetres were used for cultivation. Each endophytic fungal isolate's pure culture was added to 300 millilitres of Potato Dextrose Broth medium. Fifteen glass bottles with a capacity of one litre were used to cultivate the isolate. The cultures were then incubated for thirty days. Filter paper was used to separate the media from the fungal biomass, and ethyl acetate was added to the culture medium in a 1:1 ratio. A rotary evaporator was used to separate the extracts after ten days (Habisukanet al., 2021).

Antioxidant Activity Test

According to Baliyan et al. (2022), the antioxidant activity was measured using the DPPH method, which involved adding 0.2 mL of each extract to a 0.5 mM DPPH solution. For half an hour, the blend solution was incubated in a dark tube. A spectrophotometer was used to measure the absorbance at 517 nm, with gallic acid serving as the standard shown in figure 3. Antioxidant activity was determined using the IC50 value and percentage of inhibition (Abbas et al., 2021).

S. No.	Concentration (µg/ml)	*Guiandina bonduc*
1	12.5	44.42
2	25	71.76
3	50	74.22
4	100	74.47
5	150	85.8
6	200	92.45

Fig1. Antioxidant potential of *Guilandina bonduc* plant

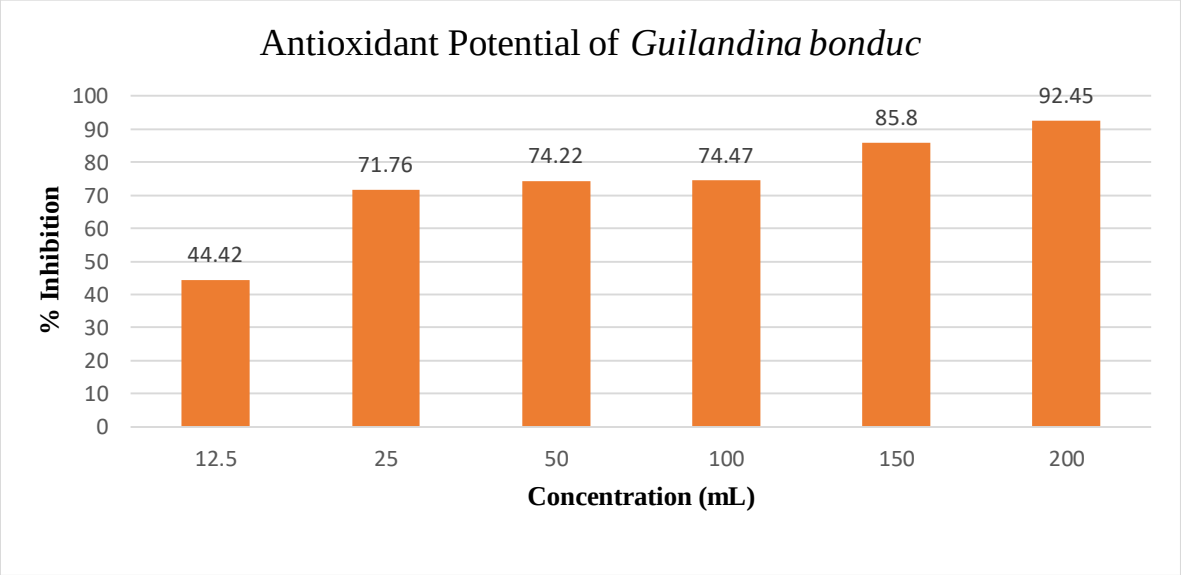

Fig 2. Antioxidant potential of *Guilandina bonduc* plant

	Guilandina bonduc	Standard (Gallic Acid)
12.5	44.42	87.2
25	71.76	90.9
50	74.22	92.2
100	74.47	93.8
150	85.8	94
200	92.45	95.75

Fig 3. Antioxidant activity of *Guilandina bonduc* plant compared with Standard Gallic acid

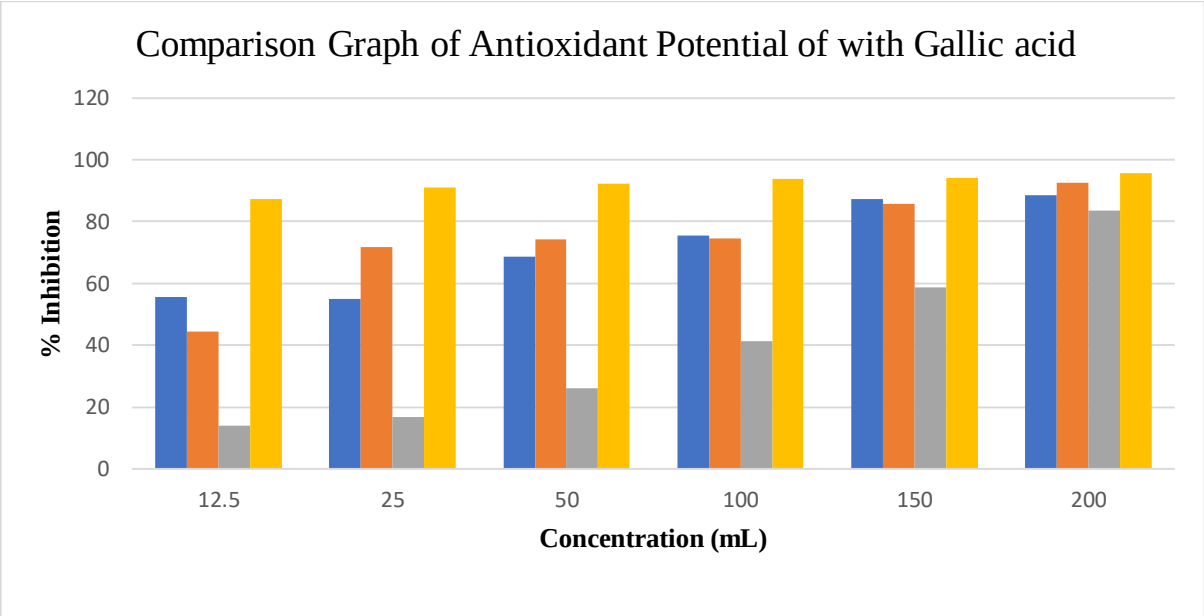

Fig 3. Comparison graph of antioxidant activity with standard Gallic acid

Result and discussion

Isolation and Identification of Fungal Endophytes

Re-isolating distinct pats from *Guilandina bonduc* at the fourth position from the major branch a different site from earlier studies was the continuation of this study. Additionally, isolates were identified both morphologically and molecularly. Microscopic views revealed conidiophores that were globose, short and thick phialides, and hyaline, while macroscopic features revealed colonies that were whitish green, cottony, umbonate, and radiating. As the colony grew older, it turned green. According to molecular identification the isolate was 100% similar to *Lasiodiplodia irregularis*.

Bioactivity of Fungal Endophyte

Ascorbic acid and tetracycline were used as standards to examine the antioxidant activities of *L. irregularis* pure compound and ethyl acetate extract. The ethyl acetate extract of the endophytic fungus *L. irregularis* and its compound's antioxidant property test results are displayed in figure1 and 2. According to the results the compounds produced shows high antioxidant activity (IC50< 100μ g/m. These findings suggest that the chemicals could be used to create novel medicinal materials. According to the literature, the endophytic fungus extract's secondary metabolite composition is comparable to that of the host plant. This suggests that endophytic fungi participate in mutualistic interactions, and replicate secondary metabolites of their host plants.

Conclusion

4-hydroxybenzoic acid was the bioactive substance and isolated from *Guilandina bonduc* plant parts. The antibacterial and antioxidant properties of this chemical were found highly potent. According to studies, this chemical can be modified in a number of ways to be employed as a novel medicinal material.

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