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ANTIOXIDANT ACTIVITY OF CRUDE EXTRACT FROM ENDOPHYTIC FUNGI OF PHYSALIS ANGULATA L.

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ABSTRACT:

Endophytes, including bacteria and fungi, inhabit host plants and establish mutualistic relationships that are beneficial to both parties. These microorganisms derive nutritional benefits from the host plant, such as access to sugars and other organic compounds, while the plants takes benefits in terms of increased tolerance to biotic and abiotic stresses. Additionally, endophytes assist for the plant growth by various mechanisms, including the production of growth hormones and the enhancement of nutrient uptake. The secondary metabolites induced by endophytic fungi are specifically useful in the treatment of various diseases or ailments, showcasing their potential in pharmaceutical applications. Besides of medicine, these metabolites also have valuable applications in agriculture, where they can contribute to crop protection and yield improvement.

In the present exploration, we focused on isolating crude extracts from the stems of Physalis angulata using a method involving potato dextrose agar media followed by a fermentation process. This process entailed multiple steps, including culturing, sub-culturing, and stable fermentation of the plant endophytes to obtain the desired crude extract. Various solvents, such as chloroform, n-butanol, and ethyl acetate, were employed to facilitate the extraction of bioactive secondary metabolites from the medicinal plant stem. To check the biological activity of the crude extract, we conducted an in vitro assay to determine its free radical scavenging activity. Specifically, we used the ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)) radical cation decolourization assay, which measures the capacity or power of the extract to inhibits the oxidation by assessing its ability to neutralize free radicals. The results indicated that the endophytic crude extract exhibited potent antioxidant activity, highlighting its potential utility in applications requiring antioxidant properties.

Keywords: Endophytic fungi, Isolation, PDA, Physalis angulate, Crude extract, Antioxidant, Bioactive Secondary Metabolites, fermentation.

1. INTRODUCTION

Antioxidants shows a crucial role in reducing the contingency of abiding diseases by neutralizing pernicious molecules called free radicals. Free radicals molecule can destruct cells and which leads to the development of various diseases conditions such as heart disease, cancer, and neurodegenerative disorders. Antioxidants help to prevent or slow down this damage by stabilizing free radicals, thereby reducing the oxidative stress and inflammation that are associated with chronic diseases. Additionally, antioxidants may support overall health by promoting proper immune function and maintaining cellular integrity1. The importance of consuming a diet rich in plant Nutrients products, which are generally found in all organized part of the plant such as fruits and vegetables, cannot be overstated in decreasing the incidence of degenerative diseases like cancer and arteriosclerosis. These secondary plant compounds, also known as phytochemicals, encompass a diverse array of compounds like flavonoids, polyphenols, carotenoids, and glucosinolates, among others. Antioxidants are defined as "substances that prevent or slow damage to cells caused by free radicals, which are harmful molecules that result from normal bodily processes and environmental exposures" (National Institutes of Health [NIH], 2021). Antioxidants are compounds that can inhibit or delay the process of oxidation and protect the damage of the cells caused by the free radicals. These highly reactive free radicals can lead to oxidative stress and contribute to various diseases.

Plants or Herbs are the bloated source of natural antioxidants, providing a wide array of compounds such as tocopherols and polyphenols. These antioxidants are abundant in various plant-based foods including spices, fruits, green plants, cereals, grains, seeds, teas, and edible oils. Tocopherols, commonly known as vitamin E, and polyphenols, found in colourful fruits and vegetables, are well-known for their potent antioxidant properties. They help in neutralize free radicals and decrease oxidative cell damage and thus enhance overall health and potentially reducing the risk of chronic diseases.

In addition to plants, antioxidants can also be obtained from marine organisms such as algae, fishes, and marine bacteria. These marine-derived antioxidants offer a distinct profile of bioactive compounds that may confer unique health benefits. For example, certain algae species contain carotenoids and other antioxidants that protect against oxidative stress and inflammation. Fish and shellfish provide omega-3 fatty acids, which exhibits the antioxidant, anti-inflammatory properties, contributing to cardiovascular health. Marine bacteria induce a wide range of bioactive secondary compounds, including antioxidants, with potential applications in pharmaceuticals and functional foods.

The diverse sources of natural antioxidants from both plant and marine origins offer a broad spectrum of health-promoting compounds. Incorporating these antioxidant-rich foods into the diet can help maintain cellular health, decreases the fortuity of various chronic diseases, and help overall well-being.

2. MATERIAL AND METHODS

Plant information: **Physalis angulate, P. peruviana** belonging to the family Solanaceae.
Synonyms: Cape gooseberry, goldenberry, and Peruvian groundcherry.

Fig.1: GOOSEBERRY PLANT

P. peruviana L., commonly called cape gooseberry in English-speaking countries, is sought after mainly for its juicy, fleshy yellow-orange fruit that grows inside a calyx, which protects it against insects, birds, pathogens, and extreme climatic conditions (Puente et al., 2011). This plant is used in medicine for the treatment of various diseases such as anticancer, antimicrobial, anti-inflammatory, and antipyretic, diuretic and for the treatment of malaria, asthma, arthritis, hepatitis, and dermatitis.

Phytochemical evaluation of this plant have demonstrated that presence of anolides, steroids, alkaloids, glycosides, and flavonoids.

Various types of phytochemicals were present in the plant, such as terpenes and phenolic compounds. Terpenes class, monoterpenes, diterpenes, carotenoids are present. Amid the phenolic compounds, flavonoids.

1. COLLECTION AND AUTHENTICATION OF PLANT:

The fresh roots of Plant Physalis angulata L. were collected from Mahagaon Taluka Barshitakli Dist-Akola Maharashtra India and were authenticated by Dr. A. Benniamin, Scientist F & Head of Botanical Survey of India, Western Regional Centre Pune (M.H) 411001.

2. ISOLATION OF FUNGAL ENDOPHYTES:

The Healthy plant Physalis angulata collected from Taluka Barshitakli and preserved to avoid damage to plant. The root of *Physalis angulata* L. were washed with running tape water to remove the soil and other adhering materials from the root. The root surface of the plant were sterilized with 4% Sodium hypochlorite for a few minutes, 70% ethanol for a few seconds, and Distilled water for 1-2 Minutes. The sterilized roots of the plant were cut into multiple pieces of size 5 cm witrh the help of sterilized razer blade. The surface sterilized pieces (3-4) of root were transferred in Potato-dextrose-agar (PDA) Plates. After completion incubation at a temperature of 25-27℃ for one week, the growth of endophytic fungi was observed and further subjected to a subculturing technique to obtain a pure culture of endophytic fungi. After seven days of incubation, pure culture was sent for identification and PCR sequential analysis to Biokart India Pvt Ltd Bangalore Karnataka India.

3. FERMENTATION OF ISOLATED ENDOPHYTIC FUNGI:

Isolated endophytic fungi of pure culture were transferred in an Erlenmeyer flask containing three-liter potato dextrose broth. The flask was incubated without agitation for 22 days at 25-27℃. fungal culture were filtered with the help of Muslin cloth to detach mycelia from the broth. Filtered broth were homogenized centrifuged at 400 rpm to obtained supernatant.

4. EXTRACTION OF ENDOPHYTIC FUNGAL BROTH:

Supernatant from broths were collected and subjected to liquid-liquid extraction using nonpolar solvents, specifically chloroform and n-butanol. The organic phases were separated and evaporated under reduced pressure to yield a crude extract. Phytochemical screening of the crude extract involved the use of Dragendorff's and Wagner's reagents for alkaloids, the Lead acetate and Alkaline reagent tests for flavonoids, the Liebermann-Burchard reaction for steroids, and Ferric chloride and Gelatine tests for tannins. Additionally, chromatographic technic, Thin Layer Chromatography (TLC) of the crude extract were performed using silica gel plates and a solvent system of chloroform: methanol (70:30:10, v/v/v). TLC technic exhibited the presence of secondary metabolites like alkaloids, flavonoids, steroids, and tannins. The developed TLC plates were visualized under UV light and by spraying with specific reagents, confirming the presence of based on the retention factors (Rf values) compared with known standards.

5. DETERMINATION OF ABTS FREE RADICAL SCAVENGING ACTIVITY OF THE EXTRACT:

- The antioxidant effect of the samples extract were studied using ABTS (2, 2'-azino-bis- 3-ethylbenzthiazoline-6-sulphonic acid) radical cation decolorization assay. ABTS radical cations (ABTS+) were produced by reacting ABTS solution (7mM) [36mg/10ml H2O] with 2.45mM ammonium Persulphate [5.6mg in 10ml of H2O].
- The mixture was allowed to stand in the dark at room temperature for 12-16 hours before use. The absorbance was read at 734nm in a Spectro-photometer.
- On the day of the experiment, it was diluted to 10X and was allowed to react with the different concentrations of plant extract in triplicate.
- The extract was weighed (2mg/2ml of ethanol) and different concentration (100µl, 200µl,300µl, 400µl, and 500µl/ml) was made by diluting it with ethanol.
- Similarly, different concentration of Ascorbic acid was made (10µg, 20µg, 30µg, 40µg, and 50µg/ml).
- 1ml of the ABTS + Ammonium Persulphate solution was then added to one ml of sample/standard/ blank and absorbance was measured in the spectrophotometer at 734 nm.
- The standard curve were plotted from the recorded absorbance and (y=mx+c) was obtained from which the concentration equivalent to ascorbic acid were determined.

3. RESULT AND DISCUSSION

Identification:

Macroscopic study:

The growth of endophytic fungi was observed in incubate Plates Potato Dextrose Agar (PDA) media. after seven days of incubation. The fungal colonies appeared distinct, dense, and well-defined structures, indicating successful isolation from the Physalis angulata roots.

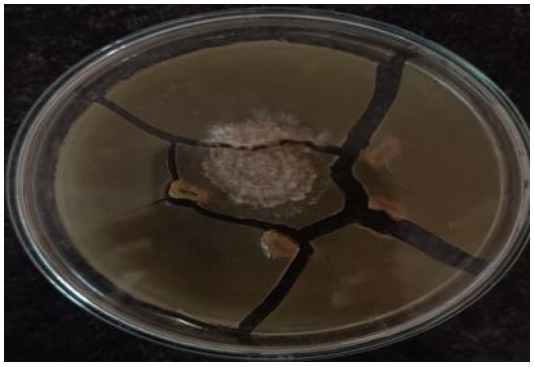

Figure 2: Growth of Endophytic Fungi on PDA Plates

Microscopic study:

Microscopic examination of the endophytic fungi exhibited the presence of well-formed mycelium and spore structures. Staining with iodine-glycerol facilitated the visualization of these structures under the microscope, which confirmed the identity of the fungal isolates.

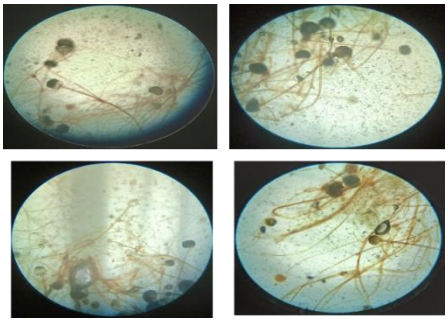

Figure 3: Microscopic Analysis of Endophytic Fungi

Extraction of Endophytic Fungal Broth:

The fermented broth was extracted using nonpolar solvents, chloroform, and n-butanol, followed by separation and drying of the organic phases to obtain a crude extract. Phytochemical analysis intimate the presence of secondary metabolites alkaloids, flavonoids, steroids, and tannins, detected through specific qualitative tests: Dragendorff's and Wagner's reagents for alkaloids, Lead acetate and Alkaline reagent tests for flavonoids, the Liebermann-Burchard reaction for steroids, and Ferric chloride and Gelatine tests for tannins. Thin Layer Chromatography (TLC) further confirmed these findings, utilizing silica gel plates and a solvent system of chloroform: methanol (70:30:10, v/v/v). The developed TLC plates, visualized under UV light and by reagent spraying, showed spots corresponding to these compounds based on their retention factors (Rf values) compared to known standards.

Antioxidant Activity - ABTS Free Radical Scavenging Assay:

The antioxidant capacity of the extract was evaluated using the ABTS radical cation decolorisation assay. The results showed that the extract intimate significant antioxidant activity, which increased with the concentration of the extract.

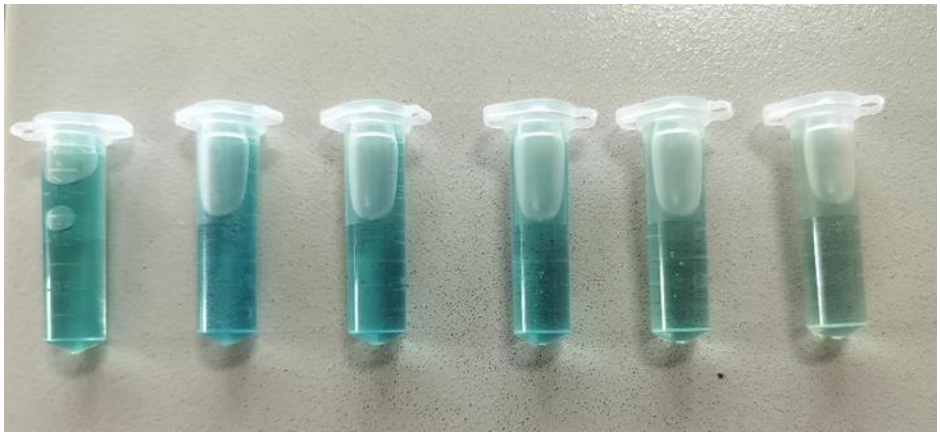

Fig. 4: Different dilution of sample for determination of ABTS Radical Scavenging Activity

Representative image:

(From left to right) -ABTS; S 100µl; S 200µl; S 300 µl; S 400µl and S 500µl/ml)

0.9491	1	2	3	Mean	SD	% Inhibition	Concentration equivalent to ascorbic acid
Sample 100ug/ml	0.8870	0.8056	0.8754	0.8560	0.0440	9.81	1.54
Sample 200ug/ml	0.6892	0.6428	0.6550	0.6623	0.0241	30.21	12.18
Sample 300ug/ml	0.5594	0.5248	0.5449	0.5430	0.0174	42.78	18.74
Sample 400ug/ml	0.4346	0.4318	0.4233	0.4299	0.0059	54.70	24.96
Sample 500ug/ml	0.3253	0.3018	0.3148	0.3140	0.0118	66.92	31.33

Table No. 1: % Inhibition of different dilutions of the sample

Ascorbic AcidConcentration	**Absorbance**	**% Inhibition**
10	0.705	40.25
20	0.523	55.68
30	0.354	70.00
40	0.114	90.34
50	0.002	99.83

Table 2: Absorbance of Ascorbic acid from different concentrations

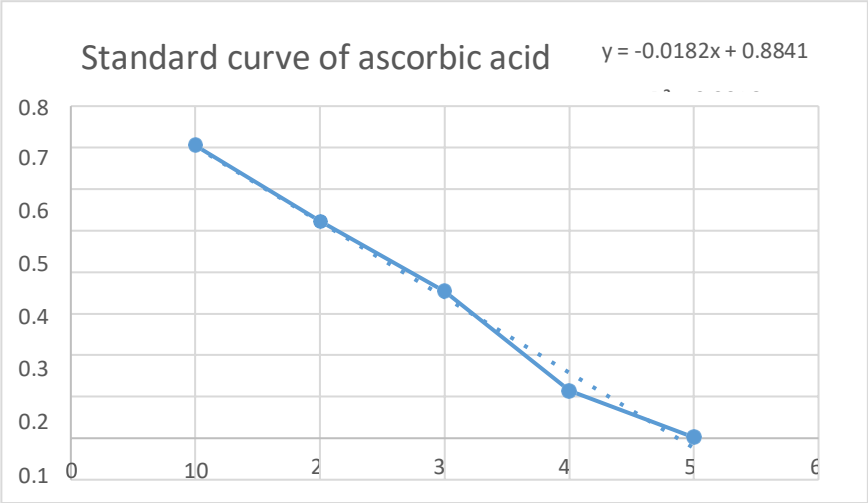

Figure 5: Standard Curve of Ascorbic Acid for ABTS Assay

The standard curve for ascorbic acid were used to actuate the antioxidant equivalent of the extract. The linear equation derived from the standard curve ($y = -0.0182x + 0.8841$, $R^2 = 0.9916$) was utilized to calculate the concentration equivalents.

4. CONCLUSION

The results of this study highlight the potential of endophytic fungi isolated from Physalis angulata as a source of bioactive compounds with significant antioxidant properties. The macroscopic and microscopic identification confirmed the successful isolation, sub-culturing and collection of endophytic fungi, which was essential for subsequent extraction and analysis. The extraction process yielded a crude extract rich in phytochemicals, including alkaloids, flavonoids, steroids, and tannins. These compounds are known for their therapeutic properties, particularly in terms of their antioxidant activity. The presence of these phytochemicals was confirmed through specific qualitative tests and TLC analysis, aligning with the profiles observed in other studies on endophytic fungi.

The ABTS free radical scavenging assay authenticate that the crude extract possessed potential antioxidant activity, with higher up concentrations exhibiting greater inhibition of the ABTS radical. This suggests a dose-dependent relationship, indicating that increasing the concentration of the extract enhances its ability to neutralize free radicals. The observed antioxidant activity is comparable to that of ascorbic acid, a standard antioxidant, further emphasizing the efficacy of the endophytic fungi extract.

These findings offer that the endophytic fungi from Physalis angulata have the potential to be developed into natural antioxidants, which could be valuable in pharmaceutical and food industries. The bioactive secondary metabolites were present in the extract can be explored for their therapeutic applications, particularly in the management of oxidative stress-related diseases.

Future studies could focus on the isolation and Morphological, Molecular characterization of endophytic fungi in bioactive compounds from the crude extract to understand their specific contributions to the overall antioxidant activity. Additionally, investigating the in vivo effects of these extracts could provide further insights into their potential health benefits and safety profiles.

This study underscores the significant antioxidant potential of endophytic fungi isolated from Physalis angulata. The results pave the way for further exploration into the use of these fungi

and their metabolites as natural antioxidants in various applications, contributing to the development of novel therapeutic agents and functional foods.

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