Antioxidant properties of Crude extract isolated from endophytic fungi of *Crateva religiosa*

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***Abstract*- The health system is facing an enormous issue due to the exponential rise of antibiotic resistance among various bacteria. This has made it urgent to search outside of the "norm" for antimicrobial medicines that could be helpful in combating the many bacteria that cause disease. The endophytes meaning which not only occupies their shelter inside plants but also produces highly potent products that will become massive applicable in medical fields, particularly when it comes to the formulations of various health boosting drugs which may impacts on the pathogenic organisms.**

This experiments looked at the bioactive compounds made by *Crateva religiosa*-associated with fungal endophyte and the antioxidant effect of their crude extracts against important medicinal pathogens. In order to do this, fresh *Crateva religiosa* leaves, stems, bark, fruits, and flowers were gathered from Old Yamuna Bridge Chaka Block Prayagraj and CMP Degree College. The method outlined by was used to isolate endophytic fungi.[13]. The actively growing pure culture of agar blocks (Three milimetre in diameter) were placed in the 500 millilitre Erlenmeyer flask with 100 gram of Potato Dextrose Agar media to cultivate the fungus.

For three weeks, the temperature was between 25 and 280 degrees Celsius. 500 cc of ethyl acetate was then added to the flask to halt the fermentation. The fermenting mixture is separated using Whatman No. 1 filter paper. The incubation of fungi and bacteria was done on SDA media maintaining at 4 degree Celsius. The antimicrobial action was done by agar well diffusion assays by Kirby-Bauer's method after extraction. The antioxidant was done with DPPH Method of the fungal endophyte extract with a measured quantity of 100 µg/ml. fungal endophyte *Lasiodiplodia theobromae* exhibits antioxidant action of inhibition of 88.51% and 55.66%. It was determined that endophytic fungi are present in *Crateva religiosa* plant sections. These endophytes may be used as new metabolites in medicine.

Keywords: **endophytic fungus, secondary metabolites, antimicrobial, antioxidant, *Crateva religiosa.***

I. OVERVIEW

The health system faces a significant issue due to the exponential rise of antibiotic resistance across various bacteria. Antimicrobial resistance has become a global burden in addition to posing difficulty for public health care framework. It is made it urgent for search beyond the box as antimicrobial medicines which could be helpful in combating the many bacteria that cause disease. Because all of its parts have been utilised for medical purposes, the plant *Crateva religiosa* has earned the moniker "miracle plant" over the years.

This plant is said to have, anti-inflammatory, anti-ulcer, pain relief, immunomodulatory, antioxidant, antibacterial, antifungal, anti-cholesterol and wound healing qualities.[1-2] Additionally, it has been discovered that *C. religiosa* forms relationships with a variety of microorganisms that function as symbionts in the plant's numerous tissues. Endophytes are the broad term for these symbiont-oriented germs, which can be either fungus or bacteria.[3-4] Endophyte is a general term used to describe microorganisms that not only have a niche within the plant tissues but also undertake a part or the whole of their life cycle within this host plant without causing any damage.[5] They enter and inhabit the tissues of the host plant via natural openings such as lenticels, stomata, and by injury carried out by insects, air currents,and rain waters, or by plant associates.[6]

It has also been observed that common type of endophyte linked with plant parts, are endophytic fungi. These endophytic fungi have been linked to a variety of bioactive substances, such as triterpenoids, tannins, alkaloids, steroids, and anthracene sides, which lower sugar levels and even have enormous uses in the food, medical, and agricultural sectors.[7–9] The pharmaceutical industry would greatly benefit from these bioactive substances made by endophytic

fungus, particularly since the development of new medications has a considerable impact on these pathogenic microorganisms. The antioxidant and antibacterial qualities of fungal endophytes separated by medicinally important plants have been investigated by numerous researchers worldwide.[10–12] Therefore, the goal of the current study is to examine the secondary metabolites generated by endophytic fungi linked to *Crateva religiosa* as well as the antioxidant and antibacterial characteristics of liquid extracts of these secondary metabolites against important medicinal microorganisms.

II. MATERIALS AND METHODS

Plant material collection

Leaf, stem, bark, fruit, and flower samples were gathered from several areas in the Prayagraj district, including Mahamana Pt. Madan Mohan Malviy park (Minto Park) and Civil lines. Samples from a healthy plant were collected at various times of the year and used in additional experiments.

Isolation of endophytic fungi

The method outlined by Suryanarayanan was used to isolate endophytic fungi.[13]. The samples taken from the *Crateva* factory were cleaned using running tap water to get rid of dust and debris, and then they were allowed to air dry before being processed further. The plant material was sterilised by immersing it in 70% Ethyl Alcohol for 3 mins and 0.5% (NaOCl) sodium hypochlorite for 1min. The leaf material was properly washed with sterile distilled water after being soaked in 70% ethanol once more. Following the samples' air drying, the leaves (3–4 millimetre in diameter and 1 centimetre in length) was fragmented with a sterile scalpel, and the midribs were cut individually. Five to six sample segments were put on the Potato Dextrose Agar (PDA) medium in each Petri dish. To inhibit any bacterial development, 250 mg of chloramphenicol was added to the media. The plates were sealed, placed in a BOD incubator, and checked every day for endophytic fungus development. In order to get pure cultures for further research, the tip of the hypha protrude out as fungal plated on a fresh PDA media without ant supplement of antibiotics.

The extraction of secondary metabolites and cultivation of endophytic fungi

The hypha of growing as pure form (Three millimetre in diameter) were placed in 500 millilitre Erlenmeyer flask with 100 g of Sabouraud Dextrose Agar (SDA) medium to cultivate the fungus. For three weeks, the temperature was between 25 – 28 degree Celsius. 500 cc of ethyl acetate was then added to the flask to stop the fermentation. The fermenting mixture was separated using Whatman No. 1 filter paper. Ethyl acetate was used as an organic solvent to extract the metabolite from the fungus. The crude metabolite was obtained by drying the resulting product in CaCl2 desiccators after ethyl acetate was evaporated using a rotary evaporator at 50^{o}C. After that, the crude extract was stored for bioassay at 25^{o}C in an Eppendorf tube.

Inoculum preparation

The bacterial and fungal cultures were kept at 4^{o}C on nutritional and Sabouraud dextrose agar, respectively. Fungal endophyte cultures were subcultured in liquid Sabouraud dextrose medium for 2-3 days at 25 degree celsius prior to the test. Next, using regular saline as diluents, the concentration of broth was 0.5 McFarland standards.

Antioxidant Activity

Procedure

2 mg of DPPH i.e. 1, 1-diphenyl-2-picrylhydrazyl were dissolve in fifty millilitres of methanol to provide 0.1 mM of DPPH. The solution was maintained as 100 µg/ml, which was obtained by dissolving fungal endophyte metabolites in methanol. The standard was Gallic acid. A combination of methanol and DPPH solution served as the negative control and blank. Microtiter plate wells were filled with reaction mixtures containing 25 µl of DPPH and 25 µl of sample which have to be test, and it was maintained 200 µl with addition of 150 µl of methanol. For thirty minutes, these plates were kept in darkness for avoidance of unwanted reactions. The absorbance was taken at 517 nm by spectrophotometer. By contrasting the samples with a control group that received methanol treatment, % inhibition shown by sample understood as antioxidant property of sample or DPPH Scavenging effect of sample and calculated it. According to the endophytic fungal extract's DPPH antioxidant assay results follows the dosage of 100 µg/ml, with % Inhibition of 88.51 and 55.66%, respectively shown in figure1 and 2. These numbers are similar to the 64.7% inhibition seen at the same dose for the positive control, Gallic acid (Figure 3).

S. No.	Concentration (µg/ml)	*C. religiosa*
1	12.5	55.66
2	25	55.01
3	50	68.55
4	100	75.45
5	150	87.19
6	200	88.51

Fig. Antioxidant (%) activity of *Crateva religiosa*

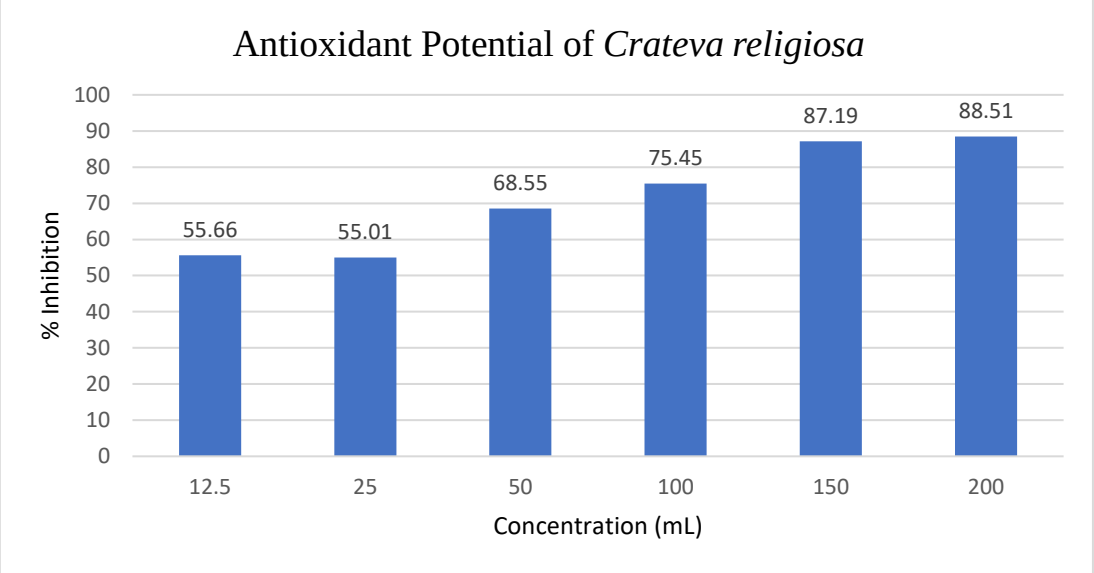

Fig. Antioxidant graph representation of *Crateva religiosa* plant

III. DISCUSSION

In order to investigate the antioxidant and antibacterial properties of bioactive compounds opposed of pathogenic organisms which is medicinal significance, this research reports the separation of fungal endophytes through the plant *Crateva religiosa* in the Prayagraj district. The study's findings indicate that the plant sections of *Crateva religiosa* provide a niche for endophytic fungi, which are significant in medicine. This result is in line with other researchers' findings that endophytic fungus can be discovered in *Crateva religiosa*.[14, 15, and 16]

The balance of antioxidant defence pattern and free radicals production leads to free radical degradation. Cells may be harmed by this oxidative stress.[17-18] Numerous illnesses, including cancer, diabetes, and early ageing, including generation problems, have been connected to oxidative stress conditions.[19] Traditional medicine uses a number of plants that have been proven to contain multiple antioxidant elements with different modes of action.[20]

When compared to the standard (Gallic acid), Liquid culture of fungal endophytes separated from the ethnomedicinal plant was *Crateva religiosa* which shows strong antioxidant action or DPPH action of free radical scavenging. Since these secondary metabolites have been demonstrated to have the capacity to donate protons and may also act as inhibitors of free radical, potentially fulfilling the job of chief antioxidant, they may be a dependable and innovative means of addressing oxidative stress conditions.[21]

IV. CONCLUSION

The endophytic fungi which resides in the sections of *Crateva religiosa*. When compared to information found in the literature, these fungal endophytes of *Crateva religiosa* accounts for a huge origin sink of bioactive compounds since they create secondary metabolites with antibacterial and antioxidant properties. These endophytes may be used as new metabolites in medicine.

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