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Screening of antimicrobial activity of endophytic fungus *Cheatomium globosum* isolated from *Dalbergia sisso*.

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Abstract
The endophytic fungi *Cheatomium globosum* was isolated on potato dextrose agar (PDA) from stems of *Dalbergia sisso*. The *Cheatomium globosum* screened for its antimicrobial activity. Antimicrobial activity in ethanolic extract of *Cheatomium* was tested by using agar well diffusion method. In antibacterial activity *Cheatomium globosum* showed highest zone of inhibition *i.e.* 18 mm against *Staphylococcus aureus* and *Bacillus subtillis*. Similarly, in antifungal activity the maximum zone of inhibition was 40 mm obtained against *Fusarium solani* and *Aspergillus niger*. *Cheatomium globosum* have potential activity against plant pathogenic fungi and bacteria isolated from *Dalbergia sisso*.

Keywords: *Dalbergia sisso*, *Cheatomium globosum*, Antimicrobial, Endophytes, etc

Introduction
Endophytes are microbes; they may be fungi, bacteria and actinomycets. They colonize inside the tissue of plants without causing any disturbance in plant life cycle (Fatma *et. al*., 2010) [1] endophytes completed their whole life cycle within their host plants. Endophytes protect the plants from various diseases and it also helpful to provide nutrition.
Most endophytes have ability to synthesize bioactive compounds which involved in the host endophyte relationship (Kalyanasundaram *et.al.,* 2015) and it serves as potential source of novel natural products for exploitation in medicine as well as in agriculture (Irailton *et.al*., 2015) [3].
Maximum endophytic fungi were reported to produce bioactive metabolites which having a antimicrobial as well as antiviral substances to increase growth and competitiveness of the host plants (Carroll, 1988) [4].
Until the work done on endophytic strains are in small scale, which means there is an opportunity to find new strain and targeting natural product from endophytic microorganisms which useful in different fields such as agriculture, industrial and pharmaceutical. Plant Endophytes synthesizes compounds which have antimicrobial activities but it was reported by very few researchers (Kalyanasundarum *et. al.,* 2015) [1]. In present investigation the endophytic fungi i.e. *Cheatemium globosum* was used against pathogenic fungi and bacteria which causes the crop losses.

Materials and methods
1. Collection of plant sample
Healthy and fresh plant parts like leaves and stem of *Dalbergia sisso*, were collected and from different location of Aurangabad.

2. Sterilization
Collected plant samples were washed in running tap water for 30 minute to remove dust. After washing leaves and stem were cut into pieces under aseptic condition. Samples were sterilized by 0.1% $Hgcl_2$ for 2 minute followed 70% ethanol for 2 minute and then sample were rinsed in sterile distilled water.

3. Isolation
3-4 sterilized segments were placed on PDA (potato dextrose agar) containing petri plates and incubated at 27±1 for 3-5 days. Three replicas were kept for each sample. After incubation fungi are isolated and maintained on PDA slants.

4. Identification
Identification of the fungal isolates was done by slide preparation and examined under light microscope. The identification was confirmed by using standard manuals. (Barnett, 1972) [8].

5. Extraction with solvent
This fungus inoculated on PDB (Potato Dextrose Broth) containing flask and kept in shaking incubator for 20 days at 30°. After 20 days incubation, the fungi were filter through whatman filter paper and collected residue for extraction. The fungal mat was oven dried and extracted with ethanol solvent. The extract was evaporated in water bath and residue was dissolved in 5 ml DMSO in bottles for antimicrobial activity.

6. Antibacterial activity
Antibacterial activity was studied by using agar well diffusion method in aerobic condition. ethanolic extract of endophytes was tested against bacterial pathogens *i.e Staphylococcus aureus*, *Bacillus subtillis*, *Streptococcus sp*, *Pseudomonas aeruginosa* and *E.coli*. after 24 hours incubated at 32°c ± 1 and the zone of inhibition was recorded.

7. Antifungal activity
The Antifungal activity of mycelial extract of ethanol against pathogenic fungi like *Fusarium solani*, *Aspergillus niger*, *Phythium myriotylum*, *Rhizoctonia solani*, *Sclerocium rolfsi* was checked by agar well diffusion method and the zone of inhibition was recorded in mm at 27°c ± 1 after 48 hrs.

Results and Discussion
Dalbergia sisso was collected from Aurangabad region and total 27 endophytic fungi were isolated. Endophytes were identified by using standard manuals (Barnette, 1972) [8]. In

present study, the *Cheatomium* was selected for screening of antimicrobial activity against plant pathogenic fungi and bacteria. Antibacterial and antifungal activity of *Cheatomium* was tested by using well assay. Five bacterial and five fungal pathogens were tested and zone of inhibition was calculated. In antibacterial activity, *Cheatomium* showed maximum inhibition zone against *Staphylococcus aureus* and *Bacillus subtillis* which was 18 mm followed by *Streptococcus sp, Pseudomonas aeruginosa* and *E. coli* were 17mm, 16mm, 15mm respectively (Table no.1, Fig no.1 a-e). Same endophyte reported by Ramesha and Srinivas (2014) [5] for antibacterial activity but they did not find zone of inhibition against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *E.coli*. Rani *et.al.,* (2017) [6] observed that the ethyl acetate extract of *Cheatomium arcuatum* showed 15 mm zone of inhibition against *Staphylococcus aureus* and minimum zone of inhibition against *E.coli* as well as *Pseudomonas aeruginosa*.

Similarly, *C.globosum* was shown significant antifungal activity against *Fusarium solani Aspergillus niger*, *Phythium myriotylum*,*Rhizoctonia solani* and *Macrophomina phaseolina*. The maximum zone of inhibition was recorded against *Fusarium solani* and *Aspergillus niger* i.e. 40 mm and followed by *Phythium myriotylum*, *Macrophomina phaseolina*, *Rhizoctonia solani* 35 mm, 32 mm and 26 mm respectively (Table no. 2, Fig no.2 a-e).

Awad *et.al*. (2014) [7] isolated *C. globosum* from rhizosphere soil of cucumber and revealed potent antibacterial activity in two solvents *i.e.* petroleum ether and ethyl acetate *against Bacillus subtilis, E.coli, Pseudomonas fluorescence* and antifungal activity against *Candida albicans, Fusarium solani, Fusarium oxysporum, Rhizoctonia solani* and *Pythium ultimum*.

Conclusion: The result showed that the ethanolic extract of *Chaetomium globosum* have best antimicrobial properties and it can prefer to use against plant pathogens to control the diseases.

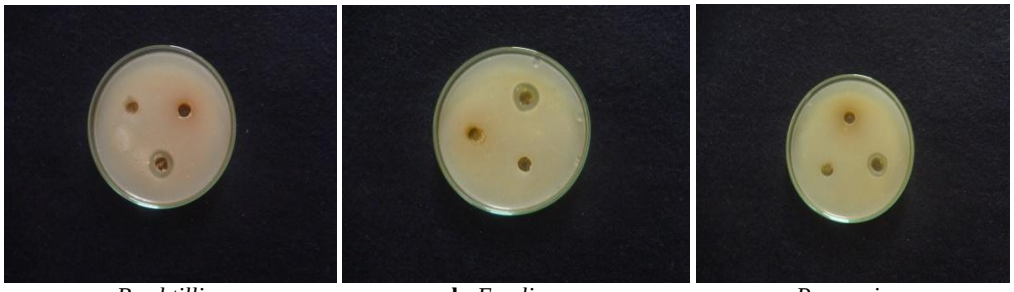
Antibacterial activity

Table 1: Antibacterial activity of *Cheatomium globosum*.

Sr.no	Name of pathogen	Inhibition zone in mm
1	*Pseudomonas aeruginosa*	16 mm
2	*Escherichia coli*	15 mm
3	*Streptococcus sp.*	17 mm
4	*Staphylococcus aureus*	18 mm
5	*Bacillus subtilis*	18 mm

Table 2: Antifungal activity of *Cheatomium globosum.*

Sr.no	Name of pathogen	Inhibition zone in mm
1	*Fusarium solani*	40mm
2	*Phythium myriotylum*	35mm
3	*Rhizoctonia solani*	26mm
4	*Macrophomina phaseolina*	32mm
5	*Aspergillus niger*	40mm



a. *B.subtillis* **b.** *E.coli* **c**. *P.aeruginosa*

d. *St.aureus* **e.** *Streptococcus sp*

Fig 1: Antibacterial activity of *Cheatomium globosum.*

a. *A.niger* **b.** *F.solani* **c.** *M. phaseolina*

d. *Phyhium sp* **e.** *R.solani*

Fig 2: Antifungal activity of Cheatomium globosum.

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