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Biochemical and antifungal screening of *Ficus johannis* Sub sp. *afghanistanica*

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Abstract
The present research paper deals with reports on Biochemical and Antifungal screening of the crude extract of leaves of *Ficus johannis Boiss*. Sub sp. *afghanistanica* (Warb.) Browicz. This paper also gives the information on preliminary phytochemical presents in the studied specimen.

Keywords: *Ficus johannis*, preliminary, phytochemicals, antifungal activity

Introduction
Ficus johannis Boiss. Sub sp. *afghanistanica* (Warb.) Browicz, is the plant specimen of family Moraceae. Number of *Ficus* plant species used in traditional medicines to cure diseases as *Ficus amplissima* (Karuppusamy *et al.* 2013) [5], *F. racemosa, F. glomerata, F. glumosa, F. carica, F. religiosa and F. benghalensis* (Haq Nawaz *et al.* 2019) [4] are known from ancient times as herbal medicines to treat various ailments in human beings (Deepa, *et al.* 2018) [3] and *F. heterophylla* (Dhakite *et al*. 2020) [2] also which ensure that these plant specimens shows several biological activities as anticancer, hepatoprotective, hypoglycaemic, antitumor, antioxidant, anthelmintic, analgesic, antimicrobial activity, anti-parasitic, hypolipidemic, anti inflammatory, antibacterial, anti-ulcerogenic, mucoprotective, gastroprotective, antifungal, antiviral, antimalarial, and antiparasitic activities (Abdel-Hameed E-SS *et al.* 2014, Konyalıoglu *et al*. 2005 and Haq Nawaz *et al.* 2019) [1, 6, 4].
The preliminary phytochemical studies of *Ficus johannis* Boiss. Sub sp. *afghanistanica* (Warb.) Browicz, done by Tadavi *et. al.* (2023) [7] but there is no information on the antifungal screening of this specimen and hence this is done and presented in this present communication.

Materials and Methods
Sample Collection: The leaves of *Ficus johannis* Boiss. Sub sp. *afghanistanica* (Warb.) Browicz., were collected in a separate sterile polythene bags from the Tumsar town (Bhandara district Maharashtra state, India) were examined and identified with the help of regional floras and Taxonomic experts.

Preparation of Solvent Extracts: The plant material was stored in desiccator's setup and used for extraction, soxhlet apparatus using various solvents according to their polarity as a methanol, ethanol and chloroform extract.

Phytochemical Evaluation: The extracts were subjected to find out the preliminary phytoconstituents present in it *viz*. carbohydrates, phenolic compounds and tannins mostly.

Antimicrobial Activity Evaluation Techniques: The following conditions used for the screening of antimicrobial activity:
1. There should be intimate contact between the test organisms and substance to be evaluated.
2. Required conditions should be provided for the growth of microorganisms.
3. Conditions should be same through the study.
4. Aseptic / sterile environment should be maintained.

Various methods have been used from time to time by several workers to evaluate the antimicrobial activity. The evaluation can be done by Broth Dilution Method to evaluate the antibacterial activity.

It is one of the non automated *in vitro* bacterial susceptibility tests. This classic method yields a quantitative result for the amount of antimicrobial agents that is needed to inhibit growth of specific microorganisms. It is carried out in tubes with Macro dilution Method in Tubes and Micro dilution format using plastic trays. Following common standard strains were used for screening of antibacterial and antifungal activities: The strains were procured from Institute of Microbial Technology, Chandigarh.

E. coli	*P. aeruginosa*	*S. aureus*	*S. pyogenus*
MTCC 443	MTCC 1688	MTCC 96	MTCC 442

C. albicans	*A. niger*	*A. clavatus*
MTCC 227	MTCC 282	MTCC 1323

Methods Used for Primary and Secondary Screening

Each synthesized drug was diluted obtaining 2000 microgram /ml concentration, as a stock solution.

Primary screen: In primary screening 1000 micro/ml, 500 micro/ml, and 250 micro/ml concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms.

Secondary screen: The drugs found active in primary screening were similarly diluted to obtain 200 micro/ml 100 micro/ml, 50 micro/ml, 25 micro/ml, 12.5 micro/ml, 6.250 micro/ml, and concentrations.

Reading Result

The highest dilution showing at least 99% inhibition zone is taken as MIC. The result of this is much affected by the size of the inoculums. The test mixture should contain 10^8 organism/ ml

Antimicrobial Activity Result

Table 1: Minimal Bactericidal Concentration (µg/mL)

Drug	*E. coli*	*P. aeruginosa*	*S. aureus*	*S. pyogenus*
Species	MTCC 443	MTCC 1688	MTCC 96	MTCC 442
Dried powder of plant sample	100	62.5	125	50
Gentamycin	0.05	1	0.25	0.5
Ampicillin	100	100	250	100
Chloramphenicol	50	50	50	50
Ciprofloxacin	25	25	50	50
Norfloxacin	10	10	10	10

Table 2: Minimal Fungicidal Concentration (µg/mL)

Drug	*C. albicans*	*A. niger*	*A. clavatus*
Species	MTCC 227	MTCC 282	MTCC 1323
Dried powder of plant sample	250	500	500
Nystatin	100	100	100
Greseofulvin	500	100	100

*Bold values indicate significant activities as compared to standards

Results and Discussion

The powder sample of leaves of *Ficus johannis Subsp. afghanistanica* were evaluated for their in-vitro antibacterial activity against *S. aureus* (MTCC 96), *S. pyogenus* (MTCC 442), *E. coli* (MTCC 443), *P. aeruginosa* (MTCC 1688) strains, and *in vitro* antifungal activity against *C. albicans* (MTCC 227), *A. niger* (MTCC 282) and *A. clavatus* (MTCC 1323) strains by using micro broth dilution method. The standard strains used for screening of antibacterial and antifungal activities were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, Mueller-Hinton broth was used as the nutrient medium to grow and dilute the drug suspension for the test bacteria. The minimum inhibitory concentration (MIC) measurement was determined as [(Ac-At)/Ac] × 100

Where Ac is an average of six replicates of light absorption values of the negative controls, and it was an average of six replicates of light absorption values of the tested compound. The MIC (µg/mL) values for and standard antibiotics Gentamycin, Ampicillin, Chloramphenicol, Ciprofloxacin, Norfloxacin, and antifungal agents Nystatin and Griseofulvin are recorded in Table 1 &2.

The results mentioned in Table 1 Minimal Bactericidal Concentration indicate that the equal antibacterial activity against *E. Coli,* strain as compared with the positive controls Ampicillin, and showed lower activity towards Chloramphenicol, Ciprofloxacin, Norfloxacin. The antibacterial activity of *P. aeruginosa* strain showed excellent activity as compared with the standards Ampicillin. The antibacterial activity of *S. aureus* strain showed very good activity as compared with the standards Ampicillin. The antibacterial activity of *S. pyogenus* strain showed excellent activity as compared with the standards Ampicillin and showed equal activity towards the Chloramphenicol & Ciprofloxacin.

The results mentioned in Table 2 Minimal fungal Concentration indicate very good antifungal activity against *C. Albican* strain as compared with the standard drug Griseofulvin and lower activity against *A. Niger* & *A. Clavatus* as compared with the standard drugs Nystatin & Griseofulvin.

Conclusion

The dried powder sample of *Ficus johannis Subsp. afghanistanica* were evaluated for their in-vitro antibacterial activity against *S. aureus* (MTCC 96), *S. pyogenus* (MTCC 442), *E. coli* (MTCC 443), *P. aeruginosa* (MTCC 1688) strains, and in-vitro antifungal activity against *C. albicans* (MTCC 227), *A. niger* (MTCC 282) and *A. clavatus* (MTCC 1323) strains by using micro broth dilution method.

The antibacterial activity of *P. aeruginosa, S. Aureus & S. pyogenus* strain showed excellent activity as compared with the standards Ampicillin. The antifungal activity against *C. Albican* strain shows good as compared with the standard drug Griseofulvin.

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