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IN-VITRO ANTIMICROBIAL ACTIVITY OF CRUDE EXTRACT OF *FICUS ARNOTTIANA* MIQ. LEAVES (MORACEACE): AN ETHNOMEDICINAL PLANT

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Key words:

Ficus arnottiana, in vitro antibacterial activity, antifungal activity, secondary metabolites.

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ABSTRACT: This study was carried out with an objective to investigate the antibacterial and antifungal potentials of leaves of *Ficus arnottiana* Miq. The aim of the study is to assess the antimicrobial activity and to determine the zone of inhibition of extracts on some bacterial and fungal strains. In the present study, the microbial activity of ethyl acetate, methanol and aqueous extracts of leaves of *Ficus arnottiana* Miq. was evaluated for potential antimicrobial activity against medically important bacterial and fungal strains. The antimicrobial activity was determined in the extracts using agar cup method. The antibacterial and antifungal activities of extracts (50, 100, 200 µl) of *Ficus arnottiana* were tested against two Gram-positive—*Staphylococcus aureus, Bacillus subtilis*; two Gram-negative *Escherichia coli, Pseudomonas aeruginosa* human pathogenic bacteria; and two fungal strains *Fusarium solanii, Candida albicans*. Zone of inhibition of extracts were compared with that of different standards like Doxycycline for antibacterial activity and fluconazole for antifungal activity. The results showed that the remarkable inhibition of the bacterial growth was shown against the tested organisms. The phytochemical analysis of the plants was carried out. The microbial activity of the *Ficus arnottiana* was due to the presence of various secondary metabolites. Hence, these plants can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals research activities.

INTRODUCTION: Antibiotics are one of the most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction. However, over the past few decades, these health benefits are under threat as many commonly used antibiotics have become less effective against certain illnesses not, only because many of them produce toxic reactions, but also due to emergence of drug-resistant bacteria.

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It is essential to investigate newer drugs with lesser resistance. Drugs derived from natural sources play a significant role in the prevention and treatment of human diseases and infections. In many developing countries, traditional medicine is one of the primary healthcare systems. [1, 2] Herbs are widely exploited in the traditional medicine and their curative potentials are well documented. [3] About 61% of the new drugs developed between 1981 and 2002 were based on natural products and they have been very successful, especially in the areas of infectious disease. [4]

Recent trends show that the discovery rate of active novel chemical entities is declining. [5] Natural products of higher plants may give a new source of antimicrobial agents. [6, 7] The effects of plant

extracts on bacteria have been studied by a very large number of researchers in different parts of world. [8] Much work has been done on ethno medicinal plants in India. [9] Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, glycosides, etc., which have been found *in vitro* to have antimicrobial properties.[10, 11] In an effort to expand the spectrum of antibacterial agents from natural resources, *Ficus arnottiana* belonging to Moraceae family has been selected. All parts of *Ficus arnottiana* Miq**.** are used as medicine in indigenous system of medicine; the stem bark is the most potent for medicinal use. The leaves of the plant are used to treat skin diseases. Leaves also have aphrodisiac activity. The roots of the plant are also used as astringent. The bark of the tree was used as astringent, aphrodisiac, demulcent, depurative and emollient. It is also used in inflammation, diarrhoea, purities and diabetes, burning sensation, leprosy, scabies, wound healing and some other skin and vagina diseases. The juice of bark is used as aphrodisiac in Ayurvedic medicinal system and good for lumbago. [12, 13, 14]

In the current investigation carried out, a screening of ethyl acetate, methanol and aqueous extracts of *Ficus arnottiana* leaves against pathogenic bacteria and fungi is done in order to detect new sources of antimicrobial agents.

MATERIALS AND METHODS:

Plant Material: The leaves of *Ficus arnottiana* Miq., belonging to Family Moraceae was collected as Wild plant from the forest of Balawala, Dehra Dun (Uttrakhand, India) in the month of August 2011 was shade dried, powdered and stored. It was authenticated and identified as *Ficus arnottiana* Miq. by Dr. A.S. Sandhu, Garden Supervisor at National Institute of Pharmaceutical and Educational Research (NIPER), Mohali. The sample was submitted to college herbarium with reference number GFA/11/09.

Extraction Procedure: The shade dried, powdered leaves of the plant undergone successive extraction with different solvents as per the polarity. 100g of the coarsely powdered material was exhaustively extracted for 8 hours with hexane (50-70ºC) in soxhlet apparatus. The hexane using rotavapor. The extracted plant material was then air dried and repacked in soxhlet apparatus extract was filtered and evaporated under reduced pressure and exhaustively extracted with chloroform for 8 hours. Then the extract was filtered and evaporated under reduced pressure using rota vapor. The extracted plant material was then air dried and repacked in the soxhlet apparatus and extracted with methanol, ethyl acetate and finally with water and filtered, evaporated using rota vapor.[15]

Preliminary Phytochemical Screening: The hexane, chloroform, methanol, ethyl acetate and aqueous extract extracts were subjected to preliminary phytochemical testing to detect for the presence of different chemical groups of compounds. Air-dried and powdered plant materials were screened for the presence of saponins, tannins, alkaloids, flavonoids, triterpenoids, steroids, glycosides, anthraquinones, coumarin, saponins, fats, carbohydrates, reducing sugars, starch, protein, and amino acids, as described in literatures.[16, 17]

Test Microorganisms and Growth Media: The following microorganisms *Staphylococcus aureus* (MTCC 87), *Escherichia coli* (MTCC 40), *Pseudomonas aeruginosa* (MTCC 424), Bacillus subtilis (MTCC 121) and fungal strains *Candida albicans* (MTCC 183) and *Fusarium solanii* (MTCC 2935) were chosen based on their clinical and pharmacological importance. [18] The bacterial strains obtained from Institute of Microbial Technology, sector 39, Chandigarh. The strains of *E. Coli, Pseudomonas aeruginosa, Bacillus subtilis, Pseudomonas aeruginosa* were maintained on Nutrient broth at 37 °C and *Candida albicans, Fusarium solni, Fusarium oxysporum* maintained on Sabouraud dextrose incubated at 25°C for 4 days. The fungal growth was harvested and washed with sterile normal saline and the suspension was stored in refrigerator till used.

Antimicrobial Activity:

Determination of zone of inhibition method:

In vitro antibacterial and antifungal activities were examined for ethyl acetate, methanol and aqueous extracts. Antibacterial and antifungal activities of plant part extracts against four pathogenic bacteria (two Gram-positive and negative) and two pathogenic fungi were investigated by the agar cup

method.[19-21] Each purified extracts were dissolved in dimethyl sulfoxide, sterilized by filtration using sintered glass filter, and stored at 4°C. For the determination of zone of inhibition, pure Gram-positive, Gram-negative, and fungal strains were taken as a standard antibiotic for comparison of the results. All the extracts were screened for their antibacterial and antifungal activities against the *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis* and the fungi *Candida albicans,* and *Fusarium solanii*. The sets of three dilutions (50, 100, and 200 µl) of *Ficus arnottiana* extract and standard drugs were prepared in double-distilled water using nutrient agar tubes. For evaluation of anti fungal activity, instead of nutrient agar, Sabouraud dextrose agar was used. The inoculated medium was incubated at 25°C for two days for the fungal strains. Fluconazole (5mg) was used as standard drug.

RESULTS AND DISCUSSION:

Preliminary phytochemical screening: It was found that ethyl acetate, methanol and aqueous extracts of *Ficus arnottiana* leaves contained tannins, flavonoids, saponins, triterpenoids, steroids, glycosides, anthraquinones, reducing sugars, carbohydrates, proteins, and amino acids.

Microbial activity:

The antimicrobial activity of the extracts of *Ficus arnottiana* were studied in different concentrations (50, 100, and 200 µl) against four pathogenic bacterial strains, two Gram-positive (*Staphylococcus aureus* MTCC 87, *Bacillus subtilis* MTCC 121) and two Gram-negative (*Escherichia coli* MTCC 40, *Pseudomonas aeruginosa* MTCC 424), and twofungal strains (*Candida albicans* MTCC 183 and Fusarium solanii MTCC 2935). These strains have been selected for the basis of its application purpose of further formulation study.

Antibacterial and antifungal potential of ethyl acetate, methanol and aqueous extracts were assessed in terms of zone of inhibition of bacterial growth. The results of the antibacterial and antifungal activities are presented in **Tables 1, 2, 3, 4** and **5**. Antimicrobial activities of various extracts were determined by Agar Cup Method. Zone of inhibition were measured in mm (including bore diameter 4 mm). FAEAE showed maximum antimicrobial activity against *S. aureus* in 200 µl concentration (**Table 1, Fig. 1**). FAME showed less antimicrobial activity in comparison to FAEAE (**Table 2, Fig. 2**). FAAE showed good antimicrobial result in almost all microbial strains at a concentration of 200 µl (**Table 3, Fig.3**).

FAEAE and FAME do not show antimicrobial activity against *E. coli* and *P. aeruginosa*. FAEAE and FAME showed maximum zone of inhibition against *S. aureus* at a concentration of 200 µl. FAAE showed maximum zone of inhibition against *S. aureus* and *B. subtilis* at a concentration of 200 µl.

1) **Antibacterial activity of Ethyl acetate extract:**

TABLE 1: EFFECT OF ANTIBACTERIAL ACTIVITY OF FAEAE CONTAINING DIFFERENT CONCENTRATION AGAINST DIFFERENT MICROORGANISMS

Dose → / Microorganism ↓	50 µl	100 µl	200 µl	Standard (Doxycycline)
Escherichia coli (MTCC 40)	–	–	–	–
Staphylococcus aureus (MTCC 87)	11	14	19	30
Pseudomonas aeruginosa (MTCC 424)	–	–	–	–
Bacillus subtilis (MTCC 121)	5	7	15	30

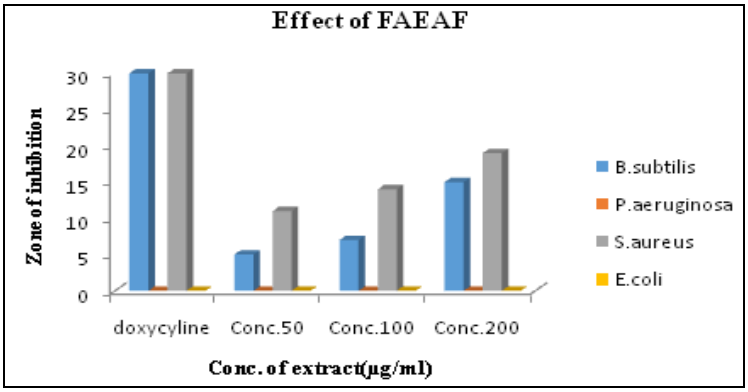

FIG.1: EFFECT OF ANTIBACTERIAL ACTIVITY OF FAEAE CONTAINING DIFFERENT CONCENTRATION AGAINST DIFFERENT MICROORGANISM

2) Antibacterial activity of Methanol Extract:

TABLE 2: EFFECT OF ANTIBACTERIAL ACTIVITY OF FAME CONTAINING DIFFERENT CONCENTRATION AGAINST DIFFERENT MICROORGANISM

Dose → / Microorganism ↓	50 µl	100 µl	200 µl	Standard (Doxycycline)
Escherichia coli (MTCC 40)	0	0	0	0
Staohylococcus aureus (MTCC 87)	14	16	20	33
Pseudomonas aeruginosa (MTCC 424)	0	0	0	0
Bacillus subtilis (MTCC 121)	2	4	7	31

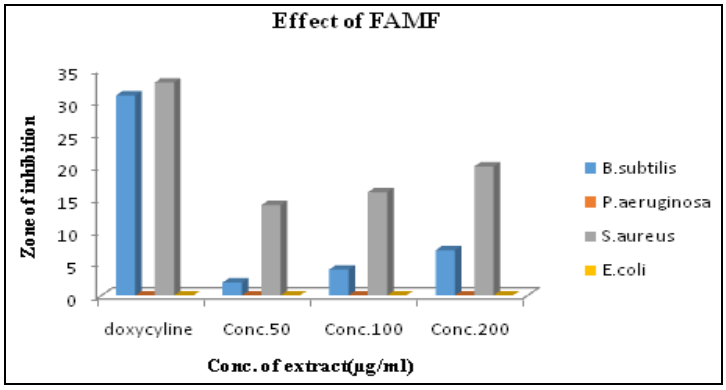

FIG.2: EFFECT OF ANTIBACTERIAL ACTIVITY OF FAME CONTAINING DIFFERENT CONCENTRATION AGAINST DIFFERENT MICROORGANISM

3) Antibacterial activity of Aqueous Extract:

TABLE 3: EFFECT OF ANTIBACTERIAL ACTIVITY OF FAAE CONTAINING DIFFERENT CONCENTRATION AGAINST DIFFERENT MICROORGANISM

Dose → / Microorganism ↓	50 µl	100 µl	200 µl	Standard (Doxycycline)
Escherichia coli (MTCC 40)	0	0	0	0
Staohylococcus aureus (MTCC 87)	12	15	20	30
Pseudomonas aeruginosa (MTCC 424)	9	12	15	18
Bacillus subtilis (MTCC 121)	12	15	20	24

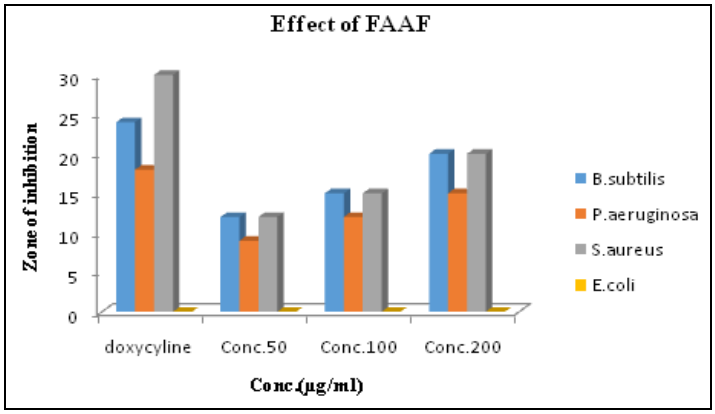

FIG. 3: EFFECT OF ANTIBACTERIAL ACTIVITY OF FAAE CONTAINING DIFFERENT CONCENTRATION AGAINST DIFFERENT MICROORGANISM

FIG. 4: ANTIBACTERIAL ACTIVITY OF *FICUS ARNOTTIANA* MIQ LEAVES OF FAME, FAEAE AND FAAE
(1, 2, 3 = concentration of extract and s = standard drug)

Antifungal Activity by Agar Cup Method:
Antifungal activity of various extracts were determined by Agar Cup Method. Zone of inhibition were measured in mm (including bore diameter 4 mm). FAEAE showed maximum antifungal activity against *S. aureus* in 200 µl concentration. FAAE (**Table 4, Fig. 5**) showed more antifungal activity as compared to the FAME. Also, FAME do not show any antifungal activity against Candida albicans (**Table 5, Fig.6).**

1) Antifungal Activity of Aqueous Extract:

TABLE 4: EFFECT OF ANTIFUNGAL ACTIVITY OF FAAE CONTAINING DIFFERENT CONCENTRATION AGAINST DIFFERENT MICROORGANISM

Strains → / **Test Extract conc. (µg/ml) ↓**	***Candida albican***	***Fusarium solanii***
50	1.9	1.4
100	2.8	2.2
200	4.2	2.5
Fluconazole	3.5	2.3

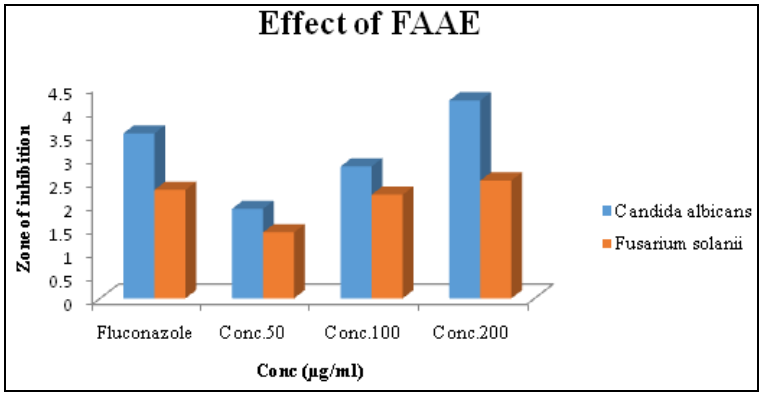

FIG.5: EFFECT OF ANTIFUNGAL ACTIVITY OF FAAE CONTAINING DIFFERENT CONCENTRATION AGAINST DIFFERENT MICROORGANISM

2) Antifungal Activity of Methanol Extract:

TABLE 5: EFFECT OF ANTIFUNGAL ACTIVITY OF FAME CONTAINING DIFFERENT CONCENTRATION AGAINST DIFFERENT MICROORGANISM

Strains → **Test Extract conc. (µg/ml) ↓**	***Candida albican***	***Fusarium solanii***
50	0	1.2
100	0	2
200	0	2.5
Fluconazole	3.2	2.2

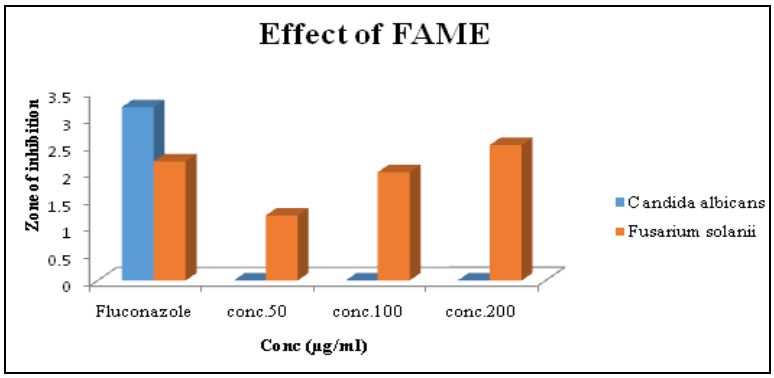

FIG.6: EFFECT OF ANTIFUNGAL ACTIVITY OF FAME CONTAINING DIFFERENT CONCENTRATION AGAINST DIFFERENT MICROORGANISM

Therefore, the antifungal activity of both the extracts showed the following order (**Fig. 7**)

FAAE > FAME

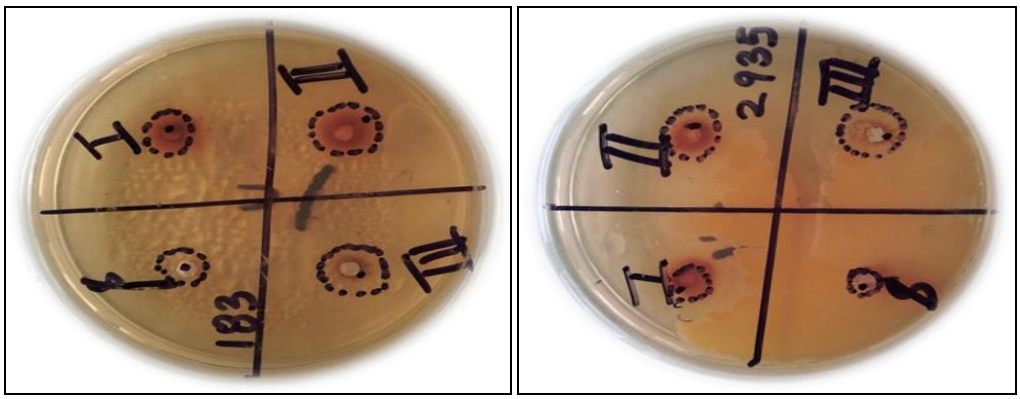

FIG. 7: ANTIFUNGAL ACTIVITY OF *FICUS ARNOTTIANA* MIQ LEAVES OF FAAE AND FAME
(I, II, III = concentration of extract and s = standard drug)

CONCLUSION: The present study justified the claimed uses of *Ficus arnottiana* leaves in the traditional system of medicine to treat various infectious disease caused by the microbes. *In vitro* antimicrobial study was performed by Agar Cup method. The various types of extracts and fraction were investigated for its antibacterial and antifungal activity on four bacterial and two fungal species. They are *Staphylococcus aureus* (Gram positive, MTCC 87); *Bacillus subtilis* (Gram positive 121); *Escherichia coli* (Gram negative, MTCC 40), *Pseudomonas aeruginosa* (Gram negative, MTCC 424); *Candida albican* (Fungus, MTCC 183); *Fusarium solanii* (Fungus, MTCC 2935). *Staphylococcus aureus* play a significant role in invasive skin diseases including superficial and deep follicular lesions. Bacillus subtilis causes endocarditis, tissue necrosis. *Escherichia coli* mostly causes gastroenteritis and urinary tract infection, respiratory system infection and joint infection. Candida albican causes yeast infection. *Fusarium solanii* causes myeloma of the foot. It is a chronic pseudotumorous infection of skin. The antimicrobial activity of various extracts and fraction were compared with the standard drug (Doxycycline- Antibacterial drug; Fluconazole- Antifungal drug).

Antibacterial Activity:

FAEAE showed maximum antimicrobial activity against *S. aureus* in 200 µl concentration. FAME showed less antimicrobial activity in comparison to FAEAE. FAAE showed good antimicrobial result in almost all microbial strains at a concentration of 200µl. FAEAE and FAME do not show antimicrobial activity against *E. coli* and *P. aeruginosa*. FAEAE and FAME showed maximum zone of inhibition against *S. aureus* at a concentration of 200 µl. FAAE showed maximum zone of inhibition against *S. aureus* and *B. subtilis* at a concentration of 200 µl.

Antifungal Activity:

FAEAE showed maximum antifungal activity against *S. aureus* in 200 µl concentration. FAAE showed more antifungal activity as compared to the the FAME. Also, FAME do not show any antifungal activity against *Candida albicans*. The present results will form the basis for the selection of plant species for further investigation in the potential discovery of new natural bioactive compounds. Further studies which aimed at the isolation and structure elucidation of antibacterial active constituents from the plant have been initiated

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REFERENCES:

1. Farnsworth NR. Ethno pharmacology and future drug development: The North American experience. J Ethnopharmacol. 2011; 38:145–52. Pub Med
2. Houghton PJ. The role of plants in traditional medicine and current therapy. J Alter Complement Med.2012; 1:131–43. PubMed
3. Dubey NK, Kumar R, Tripathi P. Global promotion of herbal medicines: India's opportunity. Curr Sci.2009; 86:37–41.
4. Cragg GM, Newman DJ. Biodiversity: A continuing source of novel drug leads. Pure Appl Chem.2009; 77:7–24.
5. Lam KS. New aspects of natural products in drug discovery. Trends Microbiol. 2011; 15:279–89.PubMed
6. Runyoro D, Matee M, Olipa N, Joseph C, Mbwambo H. Screening of Tanzanian medicinal plants for anti-Candida activity. BMC Complement Altern Med. 2011; 6:11. PMC free article Pub Med
7. Shahidi BH. Evaluation of antimicrobial properties of Iranian medicinal plants against Micrococcus luteus, Serratia marcescens, Klebsiella pneumonia and Bordetella bronchoseptica. Asian J Plant Sci. 2012; 3:82–6.
8. Reddy PS, Jamil K, Madhusudhan P. Antibacterial activity of isolates from Piper longum and Taxus baccata. Pharmaceutical Biol. 2013; 39:236–8.
9. Maheshwari JK, Singh KK, Saha S. Economic Botany Information Service, NBRI. Lucknow: 2014. Ethno botany of tribals of Mirzapur District, Uttar Pradesh
10. Dahanukar SA, Kulkarni RA, Rege NN. Pharmacology of medicinal plants and natural products. Indian J Pharmacol. 2011; 32:S81–118.
11. Cowan MM. Plant products as anti-microbialagents. Clinical Microbiol Rev. 2014; 12:564–82.PMC free article [PubMed
12. Chopra R.N., S.L Nayar and I.C. Chopra.2012. Glossary of Indian Medicinal Plants. CSIR, New Dehli, ISBN: 8172361262.
13. Kirtikar K.R., Basu B.D.2012. Indian Medicinal Plant 2, 2333.
14. Farswan M, Mazumder PM, Parcha V. Hypoglycaemic effect of *Ficus arnottiana* Miq bark extract on streptozotocin induced diabetes in rats. Natural product radiance. 2013; vol 8: 478-482.

15. Harbourne JB. Phtochemical methods:a guide to modern technique of Plant Analysis. (2nd edition) Chapman and hall London1 2013; (19): 37-168.
16. Khandelwal KR. 2nd ed. Pune: Nirali Prakashan; 2013. Practical Pharmacognosy; pp. 149–56.
17. Kumar A, Ilavarasan R, Jayachandran, Decaraman M, Aravindhan P. Phytochemicals Investigation on a tropical plant in South India. Pak J Nutr. 2011; 8:83–5.
18. McCracken WA, Cowsan RA. New York: Hemispher Publishing Corporation; 1983. Clinical and Oral Microbiology; p. 512.
19. Alzoreky NS, Nakahara K. Antibacterial activity of extracts from some edible plants commonly consumed in Asia. Int J Food Microbiol. 2012; 80:223–30. PubMed
20. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by standardized single disc method. Am J Clin Pathol. 1966; 36:493–6. PubMed
21. Rios JL Recio MC, Villar A. Screening methods for natural products with antimicrobial activity: A review of the literature. J Ethnopharmacol. 1988; 23:12749. Pub Med

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