ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 10,Oct 2022

Phytochemical Screening and Determination of Antifungal Activities of *Emblica officinalis* (Amla)

Shobha Nagarajaiah[1] and Keshamma E[2*]

[1]Assistant Professor, Department of Chemistry, Maharani Science College for Women, Maharani Cluster University, Palace Road, Bengaluru, Karnataka, India

[2]Associate Professor, Department of Biochemistry, Maharani Science College for Women, Maharani Cluster University, Palace Road, Bengaluru, Karnataka, India

***Corresponding Author**
Dr. Keshamma E
Associate Professor
Department of Biochemistry,
Maharani Science College for Women, Maharani Cluster University,
Palace Road, Bengalur-560 001,
Karnataka, India

Abstract

Since ancient times, India has been renowned throughout the world for its ayurvedic therapies. Itraconazole and fluconazole have been widely used as preventative measures and therapeutic agents for systemic fungal infections. These antifungal drugs have negative side effects and moreover numerous pathogenic microorganisms are continuously acquiring resistance to them. Therefore, there is resurgence to search for more effective and less toxic novel antifungal agents from plant sources. Hence, in the current study we aimed for phytochemical screening and determination of antifungal activities of leaf extract of *Emblica officinalis* (Amla). Leaves of *E. officinalis* was subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with double distilled water. Results delineated the major phytochemical present in the aqueous

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 10,Oct 2022

(Aq.) extract of leaves of *E. officinalis* were alkaloids, flavonoids, proteins & amino acids, saponins, phenolic compounds, tannins, carbohydrates, reducing sugars. Moreover, quantitative estimation of phytochemicals in Aq. extract of leaves of *E. officinalis* revealed that total polyphenol quantity was found to be highest (24.43 GAE) when compared with total flavonoid quantities (6.83 GAE). Furthermore, Aq. extract of leaves of *E. officinalis* (Amla) possess potential antifungal activity against *Aspergillus niger*, *Aspergillus fumigates*, *Candida tropicalis* and *Penicillium crysogenum.* In conclusion, this study results delineated that leaves of *E. officinalis* (Amla) could be used in herbal medicine for treatment of fungal diseases.

Keywords: *Emblica officinalis*, Amla, Leaves, Aq. Extracts, Antifungal

Introduction

India is well known all over the world for its ayurvedic treatment since ancient time. A wide range of medicinal plant parts like root, stem, flower, fruit, and modified plant organs have been used for extraction of raw drugs.[1,2] The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body.[3] These chemicals are termed as phytochemicals. Phytochemicals are chemicals of plant origin, produced by plants through primary or secondary metabolism. They generally have biological activity in the plant host and play an important role in plant growth. They are naturally present in the plants to defend themselves against various pathogenic microbes by showing the antimicrobial activity by inhibiting or killing microbes. Medicinal plants are richest bio-resource of drugs in traditional system of medicine and it also responsible for different colors, flavors and smell of plant.[4]

Emblica officinalis known as Indian gooseberry or amla, belongs to family Euphorbiaceae, is a medium-sized deciduous tree with gray bark and reddish wood (Figure 1). It is native to tropical southern Asia and possesses very highly characteristic medicinal value. *E. officinalis* is

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 10,Oct 2022

highly nutritious and is one of the richest sources of vitamin-C, amino acids and minerals.[5] It contains several chemical constituents like tannins, alkaloids and phenols.[6] Pharmacological research reports on amla reveals its analgesic,[7] cardio,[8] gastro,[9] nephron,[10] and anticancer properties.[11] *E. officinalis* plant extracts revealed antibacterial / antifungal,[12] antioxidant,[13] and cardioprotective properties.[14]

Figure 1: Showing *E. officinalis* (Amla) plant

Phytochemicals have recently become great interest owing to their versatile application.[15,16] Phytochemicals play an important role in various preparations like food, cosmetics, pharmaceutical, flavours and agrochemical etc. as antimicrobial agents as well as antioxidants. Phytochemical screening is very important in identifying new sources of therapeutically and industrially important compounds like Alkaloids, Flavanoids, Phenolic Compounds, Saponins, Steroids, Tannins, Terpenoids etc.[17] They confer plants with odor (terpenoids), pigmentation (tannins and quinines), and flavor (capsacin).[18] They are a part of plant naturally defense system. These bioactive components are said to be responsible for the antimicrobial effects of plant extracts in-vitro.[19] With this background, present study was conducted with the objectives of phytochemical screening and determination of antifungal activities of leaf extract of *E. officinalis* (Amla).

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 10,Oct 2022

Materials and Methods

Collection of plant material

The leaves of *E. officinalis* (Amla) were collected from Amla plant located around Chikkaballapura, Karnataka, India and washed with tap water for several times and then once with double distilled water. After washing, leaves were shade dried at room temperature and then grounded to fine powder. Powdered sample was stored for further use.

Extraction

Approximately 50 g of dried and coarsely powdered leaves of *E. officinalis* (Amla) were subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with 550 mL of double distilled water. All the extracts were concentrated by distilling the solvent in a rotary flash evaporator and dried at 40ºC. The extracts were preserved in airtight containers and stored at room temperature until further use.

Phytochemical Screening

Phytochemical screening was carried out on the aqueous (Aq.) extracts of leaves of *E. officinalis* (Amla) by using standard procedure to detect phytoconstituents as described by sofora,[20] Trease and Evans[21] and Harborne.[22]

Test for Alkaloids

Approximately 0.2g of Aq. extract of leaves of *E. officinalis* (Amla) was warmed with 2% H_2SO_4 (2.0ml) for two minutes. The reaction mixture was filtered and few drops of Dragendrof's reagent was added to the filtrate. Orange red precipitation showed the presence of alkaloids moiety.

Test for Tannins and Phenolic Compounds

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 10,Oct 2022

The Aq. extract of leaves of *E. officinalis* (Amla) in small quantity was mixed with water and heated on water bath and filtered. To the filtrate, few drops of ferric chloride ($FeCl_3$) was added. A dark green colouration indicate the presence of tannins.

Test for Glycosides

About 0.6g of Aq. extract of leaves of *E. officinalis* (Amla) was hydrolyzed with HCl and neutralized with NaOH solution and few drops of Fehling's solution A and B were added. Formation of red precipitate indicates the presence of glycosides.

Test for Reducing Sugars

The Aq. extract of leaves of *E. officinalis* (Amla) was shaken with distilled water and filtered. Few drops of Fehling's solution A and B were added and boiled for few minutes. Formation of an orange red precipitate confirms the presence of reducing sugar.

Test for Saponins

About 0.2g of Aq. extract of leaves of *E. officinalis* (Amla) was shaken with 5 mL of distilled water and then heated to boil. Frothing (appearance of creamy miss of small bubbles) showed the presence of saponins.

Test for Flavonoids

0.2g of Aq. extract of leaves of *E. officinalis* (Amla) was dissolved in diluted 10%NaOH and few drops of 2M HCl was added. A yellow solution that turns into colorless indicate the presence of flavonoids.

Test for Steroids

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 10,Oct 2022

2 mL of acetic anhydride was added to 0.5g of Aq. extract of leaves of *E. officinalis* (Amla) and then added 2 mL of H_2SO_4. The change of color from violet to blue or green or red showed the presence of steroids.

Test for Terpenoids

0.3g of Aq. extract of leaves of *E. officinalis* (Amla) was mixed with 2 mL of chloroform ($CHCl_3$) and 3 mL of concentrated 6M H_2SO_4 was carefully added to form a layer. Reddish brown coloration at the interface was formed which indicate positive results for the presence of terpenoids.

Test for Proteins and Amino acids

To the 0.3g of Aq. extract of leaves of *E. officinalis* (Amla) few drops of 0.2% ninhydrin solution was added and heated for 5 minutes. Blue colouration indicate the presence of proteins.

Quantitative Estimation of Phytochemicals

Total phenolics

The concentration of total phenolics in the Aq. extract of leaves of *E. officinalis* (Amla) was determined by the Folin-Ciocalteu assay that involves reduction of the reagent by phenolic compounds, with concomitant formation of a blue complex, its intensity at 725nm increases linearly with the concentration of phenolics in the reaction medium.[23] The phenolic content of the extract was determined from calibration curve and were expressed in mg gallic acid equivalent/g of extract powder.

Total flavonoid

Aluminum chloride colorimetric method was used for flavonoids determination in Aq. extract of leaves of *E. officinalis* (Amla).[24] The content was determined from extrapolation of calibration

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 10,Oct 2022

curve which was made by preparing gallic acid solution (0-0.8 mg/ml) in distilled water. The concentration of flavonoid was expressed in terms of mg gallic acid equivalent/g of extract powder.

Evaluation of Antifungal Activity

Fungal Strains

The pathogenic fungal strains *viz. Aspergillus niger*, *Aspergillus fumigates*, *Candida tropicalis*, *Candida albicans*, *Penicillium crysogenum* were isolated from clinical samples of local hospital in and around Chikkaballapura and confirmed by various microscopic evaluation like Gram's staining.[25] Motility, capsule and spore formation was confirmed as per the procedure prescribed by Collins and Lyne.[26] All the fungal pathogens were further confirmed by suitable biochemical tests,[27] and used for antifungal activity studies.

Stock cultures were maintained at 4ºC on the slant of nutrient agar. Active Cultures for experiments were prepared by transferring a loopful of cell organisms from the stock cultures to test tubes of nutrient broth for fungi. Streaking was done on Yeast Extract Peptone Dextrose (YEPD) agar plates and incubated for 24-48 hours at 37ºC in which the assay was performed by disc diffusion method.

Determination of Antifungal Activities

Anti-fungal activity of the Aq. extract of leaves of *E. officinalis* (Amla) was determined by disk diffusion method on Muller Hinton Agar (MHA) medium. Cultured colonies were picked from Yeast Extract Peptone Dextrose (YEPD) agar plates and were added in peptone water and was incubated for around 30 minutes. The MHA medium was poured in the petri plate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moisture with the fungal suspension. The anti-fungal discs were placed on MHA plate with the help of sterile forceps and different concentration of Aq. extract of leaves of *E. officinalis* (Amla) were

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 10,Oct 2022

loaded on discs. Fluconazole was used as positive control and double distilled water was used as negative control. Then all the plates were incubated in upright position at 37ºC for 24-48 hours. The inhibition zones were measured on the underside of the plates.[28]

Results

The major phytochemicals found in Aq. extract of leaves of *E. officinalis* (Amla) were found to be alkaloids, flavonoids, proteins & amino acids, saponins, phenolic compounds/tannins, carbohydrates, reducing sugars. Whereas, phytochemicals *viz.* glycosides, terpenoids and steroids were found to be absent (Table 1).

Table 1: Photochemical screening of Aq. extract of leaves of *E. officinalis* (Amla)

Phytochemical Components	**Aq. Extract of leaves of *E. officinalis* (Amla)**
Alkaloids	+
Flavonoids	+
Glycosides	-
Proteins and Amino acids	+
Reducing sugar	+
Saponins	+
Steroids	-
Phenolic compounds	+
Tannins	+
Terpenoids	-
Carbohydrates	+

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 10,Oct 2022

Quantitative estimation of phytochemicals in Aq. extract of leaves of *E. officinalis* was represented in Table 2. Results revealed that total polyphenols quantity was found to be highest (24.43 GAE) phytochemicals found in Aq. extract of leaves of *E. officinalis* when compared with total flavonoid quantities (6.83 GAE).

Table 2: Quantitative analysis of phytochemicals present in Aq. extract of leaves of *E. officinalis*

Chemical Components	**Aq. extract of leaves of *E. officinalis***
Total phenolics	24.43 GAE
Total flavonoids	6.83 GAE

The highest zone of inhibition (17.5 mm) was observed against *A. fumigates* and lowest zone of inhibition (13.5 mm) was observed against *A. niger* at 100 mg/mL. At 50 mg/mL the highest zone of inhibition (15.5 mm) was observed against *A. fumigates* and lowest zone of inhibition (12.5 mm) was observed against *A. niger*. Whereas the highest zone of inhibition (12.5 mm) was seen against A. fumigates and lowest zone of inhibition (8.0 mm) against *C. tropicalis* at 25 mg/mL. At 12.5 mg/mL the highest zone of inhibition (10.5 mm) was observed against *A. fumigates* and lowest zone of inhibition (5.5 mm) was observed against *C. tropicalis.* The reference standard fluconazole showed highest zone of inhibition (25.5 mm) against *P. crysogenum* and the lowest zone of inhibition (18.5 mm) against at *A. niger*. These findings revealed that the tested Aq. extract of leaves of *E. officinalis* (Amla) possess potential antifungal activity against *A. niger*, *A. fumigates*, *C. tropicalis* and *P. crysogenum* as shown in Table 3.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 10,Oct 2022

Table 3: Antifungal activities of Aq. extract of leaves of *E. officinalis* (Amla)

Fungal strains	Zone of inhibition(mm)					
			Aq. extract of leaves of *E. officinalis* (Amla)			
	Negative Control	**Positive Control**	**12.5 mg/mL**	**25 mg/mL**	**50 mg/mL**	**100 mg/mL**
A. niger	-	18.5	8.5	10.5	12.5	13.5
A. fumigates	-	20.0	10.5	12.5	15.5	17.5
C. tropicalis	-	19.0	5.5	8.0	11.5	15.0
P. crysogenum	-	25.5	8.5	10.0	14.5	16.5

Discussion

The frequency of life-threatening infections caused by pathogenic microorganisms is becoming an alarming factor of morbidity and mortality in immuno-compromised patients in developing countries. Many pathogenic microorganisms are constantly developing resistance to these agents. Since many of the existing antifungal drugs have undesirable side effects or are very toxic, show drug-drug interactions or develop resistance affecting treatment planning. Therefore, it is necessary to search for more effective and less toxic novel antifungal agents that would overcome these shortcomings. The herbal extracts are easily available and cheaper having added advantage of widespread availability, minimal side effects, cost effective and efficiency in long term usage. Considering the side effects and disadvantages of fluconazole, these herbal extracts mainly can be considered as a better alternative anti-fungal agent.[29] Therefore, in the present study was conducted with the purpose of phytochemical analysis and determination of antifungal activities of leaf extract of *E. officinalis* (Amla).

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 10,Oct 2022

Different phytochemicals have various protective and therapeutic effects which are essential to prevent diseases and maintain a state of well-being. Our study results on the qualitative analysis of the Aq. extract of leaves of *E. officinalis* revealed the presence of alkaloids, flavonoids, proteins & amino acids, saponins, phenolic compounds/tannins, carbohydrates, reducing sugars. Moreover, quantitative estimation of phytochemicals in Aq. extract of leaves of *E. officinalis* revealed that that total polyphenol quantity was found to be highest (24.43 GAE) phytochemicals found in Aq. extract of leaves of *E. officinalis* when compared with total flavonoid quantities (6.83 GAE). Furthermore, Aq. extract of leaves of *E. officinalis* (Amla) possess potential antifungal activity against *A. niger*, *A. fumigates*, *C. tropicalis* and *P. crysogenum.*

Fluconazole and itraconazole have been used extensively as prophylactic and treatment for systemic fungal infections.[30,31] Fluconazole resistance has been increasing among patients.[32] Oropharyngeal candidiasis in patients with HIV has shown azole resistant.[33] Phytochemicals derived from plant products serve as a prototype to develop less toxic and more effective medicines in controlling the growth of microorganisms.[34] These compounds have significant therapeutic application against human pathogens including bacteria, fungi or virus. Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new antimicrobial compounds.[35]

The results obtained in the present study are encouraging as this study evidenced for the vide variety of secondary metabolites present in the Aq. extracts of leaves of *E. officinalis*. *E. officinalis* shown considerable antifungal properties. Hence this study supplies as evidence-based study for leaves of *E. officinalis* could be exploited in the management of various human ailments related to fungal infections.

Conclusions

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 10,Oct 2022

In conclusion, this study results revealed the distribution of phytochemicals such as alkaloids, flavonoids, proteins & amino acids, saponins, phenolic compounds/tannins, carbohydrates, reducing sugars in leaves of E. officinalis. Biological activities such as antifungal properties of Aq. extracts of leaves of *E. Officinalis* depicted that leaves of *E. officinalis* could be used in herbal medicine for the treatment of fungal diseases. Further studies are recommended to be carried out to purify these bioactive compounds and could be effectively used in antimicrobial drugs.

References

1. Chandra M. Antimicrobial activity of medicinal plants against human pathogenic bacteria. Int J Biotechnol Bioeng Res. 2013;4(7):653-8.
2. Manikandan A, Rajendran R, Abirami M, Kongarasi K. Antimicrobial activity and phytochemical analysis of *Impatiens balsamina* Seed (Kacittumpai) collected from Coimbatore District, Tamil Nadu, India. Int J Pharm Sci Res. 2016;7(12):5039-43.
3. Sivagnanam S, Chandra AP. Preliminary phytochemical analysis of *Amarathus viridis*. Res J Pharm Biol Chem Sci. 2016;7(1):1-7.
4. Pradeep A, Dinesh M, Govindaraj A, Vinothkumar D, Ramesh Babu NG. Phytochemical analysis of some important medicinal plants. Int J Biol Pharm Res. 2014;5(1):48-50.
5. Srivasuki KP. Nutritional and health care benefits of Amla. J Pharmacogn. 2012;3(2):141-51.
6. Zhang LZ, Zhao WH, Guo YJ, Tu GZ, Lin S, Xin LG. Studies on chemical constituents in fruits of Tibetan medicine *Phyllanthus emblica*. Zhongguo Zhong Yao Za Zhi. 2003;28(10):940-3. PMID 15620182.
7. Perianayagam JB, Sharma SK, Joseph A, Christina AJ. Evaluation of antipyretic and analgesic activity of *Emblica officinalis* Gaertn. J Ethnopharmacol. 2004;95(1):83-5.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 10,Oct 2022

8. Baliga MS, Prabhu AN, Prabhu DA, Shivashankara AR, Abraham A, Palatty PL. Antidiabetic and cardioprotective effects of Amla (*Emblica officinalis* Gaertn) and its phytochemicals: preclinical Observations, Bioactive Food as Dietary Interventions for Diabetes. Biology. 2013;1(4):583-600.
9. Chatterjee A, Chattopadhyay S, Sandip K. Biphasic effect of *Phyllanthus emblica* L. extract on NSAID-induced ulcer: an antioxidative trail weaved with immunomodulatory effect. Evid Based Complement Alternat Med. 2011;1(13):146808.
10. Yokozawa T, Kim HY, Kim HJ, Tanaka T, Sugino H, Okubo T et al. Amla (*Emblica officinalis* Gaertn.) Attenuates age- related renal dysfunction by oxidative Stress. J Agric Food Chem. 2007;55(19):7744-52.
11. Madhuri S. Effect of ProImmu on estrogen induced uterine cytotoxicity in albino rat; 2008 [cited Jul 21, 2023]. Available from: https://www.researchgate.net/publication/343414122_Effect_of_ProImmu_on_estrogen_induced_uterine_cytotoxicity_in_albino_rat.
12. Hossain MM, Mazumder MM, Hossen K, Tanmy SM, Rashid MJ. In vitro studies on antibacterial and antifungal activities of *Emblica officinalis*. Int J Pharm Sci Res. 2012;3(4):1124-7.
13. Golechha M, Bhatia J, Arya DS. Studies on effects of *Emblica officinalis* (amla) on oxidative stress and cholinergic function in scopolamine induced amnesia in mice. J Environ Biol. 2012;33(1):95-100. PMID 23033650.
14. Bhattacharya SK, Bhattacharya A, Sairam K, Ghosal S. Effect of bioactive tannoid principles of *Emblica officinalis* on ischemia reperfusion-induced oxidative stress in rat heart. Phytomedicine. 2002;9(2):171-4.
15. Ruwali P, Negi D. Phytochemical analysis and evaluation of antioxidant activity of *Premna latifolia* Roxb. – A medicinal plant (Family: Lamiaceae), The Pharma. Innov J. 2019;8(5):13-20.
16. Saranraj P, Sivasakthi S, Deepa MS. Phytochemistry of pharmacologically important medicinal plants – a review. Int J Curr Res Chem Pharm Sci. 2016;3(11):56-66.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 10,Oct 2022

17. Akindele AJ, Adeyemi OO. Antiinflammatory activity of the aqueous leaf extract of *Byrosocarpus coccineus*. Fitoterapia. 2007;78(1):25-8.
18. Mallikharjuna LN, Rajanna YN. Sitharam and G.K. Sharanbassapa. 2007, Phytochemical Studies of *Strychnospotatorum* L. f.- A Medicinal Plant, E-Journal of Chemistry;4(4):510-8.
19. Sood A, Kaur P, Gupta R. Phytochemical screening and antimicrobial assay of various seeds extract of *Cucurbitaceae* Family. Int J Appl Biol Pharm Technol. 2012;3(3):401-9.
20. Sofora A. Medicinal plants and Traditional Medicine in Afric. John Wiley Son Ltd. 1993:150-3.
21. Trease GE, Evans WC. Pharmaccology, 11th Edtn. London: Brailliar Tiridel and Macmillian Publishers; 1989.
22. Herborne JB. Phytochemical methods. 3rd ed D.E. and Hall Ltd. London; 1973. p. 135-203.
23. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidative substrates by means of Folin-Ciocalteau reagent, Packer L. Methods Enzymol. 1999; 299:152-78.
24. Ordonez AAL, Gomez JD, Vattuone MA, Lsla MI. Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts. Food Chem. 2006;97(3):452-8.
25. Gram C. Ueber die isolirte Farbung der Schizomyceten in Schnitt-und Trockenpraparaten. Fortschr Med. 1884; 2:185-9.
26. Collins CH, Lyne PM. Microbiological methods. J Microbiol Methods., (3rd. Edition). 1970.
27. Barrow G, Feltham RKA. Manual for the identification of medical bacteria. SteeleCa. 3rd ed. Vol. 1993. Cambridge: Cambridge University Press; 1993. p. 331.
28. Wayne. Method for antifungal disk diffusion susceptibility testing of yeasts; Approved Guideline. 2nd ed. Clinical and Laboratory Standards Institute; 2009.
29. Abu Bakr Siddique M, Ladke KGM, Sunil V, Shirol Pallavi D, Shah Amisha KAV, Ashraf F. Anti -Fungal activity of commercially available extracts of garlic (*Allium sativum*), turmeric (*Curcuma longa*), amla (*Emblica officinalis*): an in vitro study. J Pharm Res Int. 2021;33(45A):437-47.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 10,Oct 2022

30. Meis JFGM, Verweij PE. Current management of fungal infections. Drugs. 2001;61; Suppl 1:13-25.
31. Livermore DM. The need for new antibiotics. Clin Microbiol Infect. 2004;10(4); Suppl 4:1-9.
32. Redding SW, Kirkpatrick WR, Saville S, Coco BJ, White W, Fothergill A, et al. Multiple patterns of resistance to fluconazole in *Candida glabrata* isolates from a patient with oropharyngeal candidiasis receiving head and neck radiation. J Clin Microbiol. 2003;41(2):619-22.
33. Skiest DJ, Vazquez JA, Anstead GM, Graybill JR, Reynes J, Ward D, et al. Posaconazole for the treatment of azole refractory oropharyngeal and esophageal candidiasis in subjects with HIV infection. Clin Infect Dis. 2007;44(4):607-14. doi: 10.1086/511039, PMID 17243069.
34. Ahmad I, Beg AZ. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multiple drug resistant human pathogens. J Ethnopharmacol. 2000;74(2):113-23.
35. Guleria S, Kumar A. Antifungal activity of some Himalayan medicinal plants using direct bioautography. J Cell Mol Biol. 2006; 5:95-8.