

Full Length Research Paper

Antifungal activity of aerial parts as well as *in vitro* raised calli of the medicinal plant, *Balanites aegyptiaca* Del.

Razia Khatoon^{1,2}, Noor Jahan^{1,2*}, Siraj Ahmad³ and Anwar Shahzad⁴

¹Department of Microbiology, Era's Lucknow Medical College and Hospital, Lucknow-226003, India.

²Department of Microbiology, Jawaharlal Nehru Medical College and Hospital, Aligarh Muslim University, Aligarh-202002, India.

³Department of Community Medicine, Teerthanker Mahaveer Medical College and Research Centre, Teerthanker Mahaveer University, Moradabad- 244001, India.

⁴Plant Biotechnology Laboratory, Department of Botany, Aligarh Muslim University, Aligarh-202002, India.

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The antifungal potential of an endangered medicinal plant, *Balanites aegyptiaca* Del. and its *in vitro* raised calli was evaluated by agar well diffusion method. The alcoholic extract of fruits of parent plant as well as its callus showed good antifungal activity against various pathogenic and opportunistic fungi. Minimum inhibitory concentrations (MIC) of the extracts were determined by broth microdilution method. The MIC of alcoholic fruit extract of parent plant against tested fungi ranged from 3.05 to 24.0 µg/ml, whereas, the MIC of extract of *in vitro* raised callus ranged from 1.53 to 12.0 µg/ml. The present study shows that extracts of *B. aegyptiaca* contain good antifungal activity which could be used in the treatment of fungal infections showing resistance to currently used antifungal agents. As its calli also gave good results, *in vitro* cultivation of the explants may be used to obtain novel antifungal compounds even at places where it does not grow naturally and thus can be used in the treatment of various opportunistic and life threatening fungal infections especially in immunocompromised patients.

Key words: *Balanites aegyptiaca*, *in vitro* raised callus, antifungal activity, agar well diffusion, minimum inhibitory concentration.

INTRODUCTION

Fungi cause opportunistic infections in patients who are immunocompromised, either by an underlying disease process or immunosuppressive agents. Candidiasis has become a major public health problem as an opportunistic infection of HIV/AIDS and considered as a leading fungal infection in immune-suppressed population (Al Ashaal et al., 2010). Patients with cancer often develop serious fungal infections, especially during periods of

cancer treatment. Antibiotic usage for the prevention and treatment of infections in these high-risk patients leads to selection pressures resulting in the emergence and spread of resistant organisms (Panghal et al., 2011).

Nosocomial fungal infections have gained more importance especially in association with or as a consequence of the extraordinary progress in the management of seriously ill patients during the past few decades. However,

*Corresponding author. E-mail: dnoorj@rediffmail.com.

despite this increase, there has been comparatively little progress in elucidating treatment of such nosocomial and opportunistic fungal infections. The treatment of these infections in the immunocompromised patients is a challenging task. Although many antifungal agents have been developed so far but only a few are clinically effective and safe to use. Also, resistance to antifungal agents has been reported in AIDS patients who suffer from recurrent azole-resistant oropharyngeal or oesophageal candidiasis (Chander, 2002).

This situation has forced the researchers to search for new antimicrobial substance from various sources including medicinal plants (Scazzocchio et al., 2001; Erdogru, 2002; Bandow et al., 2003; Parekh and Chanda, 2008). Antimicrobials of plant origin have proved effective in the treatment of several infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Samy and Ignacimuthu, 2000).

But unfortunately, many plant species, possessing medicinally important compounds are disappearing at an alarming rate due to the destruction of their natural habitats (Vanila et al., 2008). Through *in vitro* cultivation, it would be possible to preserve and conserve these important endangered plant species and also *in vitro* cultivation of explants may be used to obtain phytotherapeutic compounds, especially, at places where the parent plants cannot be grown because of the adverse atmospheric conditions (Shahid et al., 2009b).

Balanites aegyptiaca Del. (Zygophyllaceae or Simaroubaceae), known as 'desert date' in English, 'Hingoli' in Hindi and 'Angavriksha' in Sanskrit (Iwu, 1993; Gaur et al., 2008), is a savanna tree that grows in Sahel-Savanna regions and drier parts of middle-belt zones of Nigeria, Ghana, Ivory Coast and India. In India, it is particularly found in Rajasthan, Gujarat, Madhya Pradesh and Deccan. The tree produces date-like fruits between March and October (Chothani and Vaghasiya, 2011).

Various parts of the *Balanites* tree have been used for folk medicines in many regions of Africa and Asia (Hall and Walker, 1991; Mohamed et al., 2000, 2002). Various literatures have revealed antifeedant, antidiabetic, molluscicide, anthelmintic and contraceptive activities in various *Balanites* extracts (Liu and Nakanishi, 1982; Kamel et al., 1991; Ibrahim, 1992; Rao et al., 1997). The bark, unripe fruits, and leaves of this plant are reported to have anthelmintic, antifertility, purgative and antidiysenteric properties (Chopra et al., 1956a, b; Kirtikar and Basu, 1996). The root has been indicated for the treatment of malaria, herpes zoster and venereal diseases (Irvine, 1961; Ayensu, 1978). It is traditionally employed in treatment of jaundice, yellow fever, syphilis, diarrhea, epilepsy, cough, wound healing and even for snake bites (Inngerdingen et al., 2004; Maregesi et al., 2008; Chothani and Vaghasiya, 2011; Abdallah et al., 2012).

Although, *B. aegyptiaca* is a versatile medicinal plant its use is restricted in few localities of Indian sub continents

and parts of Africa. The present study was carried out to evaluate the antifungal potential of medicinal plant *B. aegyptiaca* and its *in vitro* raised callus by testing their activity against an exhaustive range of fungal isolates, including both standard as well as clinical strains.

MATERIALS AND METHODS

Collection of plant materials

The fruit pulp of 15 years old plant of *B. aegyptiaca* was obtained from Tissue Culture Laboratory, Department of Botany, Gujarat University, Ahmedabad (Gujarat) and its *in vitro* cultivation was done in Botanical Garden, Department of Botany, Aligarh Muslim University, Aligarh.

Sterilization of the collected explants

The young explant was washed under running tap water for 30 min to remove any adherent particles and then washed thoroughly in sterile double-distilled water (DDW). Then these explants were kept in 1% (w/v) Bavistin (Carbendazim Powder, BASF India Limited) for 25 min, followed by thorough washing with 5% (v/v) Teepol (Qualigens Fine Chemicals, India), for 15 min by continuous shaking method (Shahid et al., 2007, 2009b).

The treated explant was washed in sterilized DDW 3-4 times under an aseptic condition, to remove the chemical inhibitors. It was then treated with 70% (v/v) ethanol for 30 - 40 s followed by a rapid washing with sterile DDW and then surface sterilized by emersion in a freshly prepared aqueous solution of 0.1% (w/v) HgCl₂ (Qualigens Fine Chemicals, India) (Bhojwani and Razdan, 1996), for 4 min under laminar flow. Finally, the explants were washed 5-6 times with sterile DDW with intervals of 5 min to remove all traces of sterilants (mercury ions).

Inoculation of sterilized explants on MS medium

Murashige and Skoog's (MS) medium (Murashige and Skoog, 1962) containing 2.5 μM of 2,4-dichlorophenoxy acetic acid was prepared in culture tubes (25 × 150 mm, Borosil). The pH of the medium was adjusted at 5.8 ± 0.2. 1% agar (HiMedia Lab. Ltd., India) was added to the medium and it was autoclaved at 15 lb pressure per square inch, 121°C temperature for 15 min. The sterilized explant was then inoculated aseptically and incubated at 25 ± 2°C with relative humidity of 55 ± 5% and exposed for 16 h photocycle of 2,500 Lux intensity (Shahid et al., 2009b). 5 weeks old callus was used for evaluation of the antimicrobial effect.

Preparation of plant extracts

The alcoholic extracts of the plant were tested for antifungal activity. The extracts were prepared according to the method of Singh and Singh (2000) with some modifications (Shahid et al., 2007, 2009a, b). To prepare alcoholic extracts, fresh fruit (15 g) from parent plant was surface sterilized in 70% ethyl alcohol for 1 min and then washed 3 times with sterilized double distilled water (DDW). The calli of the explants were aseptically removed from the culture tubes and all the plant materials, including calli, were grounded with a sterilized pestle and mortar in 150 ml of 95% ethanol and centrifuged at 5000 rpm for 15 min. The resultant supernatant was filtered and taken as the alcoholic extracts which were immediately used for experimentation.

Fungi tested

The clinical fungal strains included in our study were *Candida albicans*, *Candida krusei*, *Candida parapsilosis*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Penicillium* spp. and *Fusarium* spp. isolated in the Department of Microbiology, Jawaharlal Nehru Medical College and Hospital, Aligarh Muslim University, Aligarh, India. The fungal control strains tested were *C. parapsilosis* (ATCC 22019), *C. krusei* (ATCC 6258) and *A. fumigatus* (ATCC 204305), obtained from New Drug Discovery Research, Ranbaxy, Gurgaon, India. The fungi were grown at 25°C and maintained on Sabouraud's Dextrose agar (SDA) slants.

Antifungal susceptibility testing

Antifungal testing was performed on SDA plates using agar well diffusion method (Vanden-Berghe and Vlietinck, 1991; Akinpelu, 2001), with some modifications (Shahid et al., 2007). An inoculum size of 2×10^6 yeast cells or fungal spores was used for inoculating the susceptibility plates. These plates were lawn cultured with fungal suspensions with the help of sterile swabs. Wells of 5 mm diameter were made in each plate using a sterile borer. Plant extracts in a volume of 20 μ l were poured in the wells using a micropipette. 20 μ l of 95% ethanol was used to serve as negative control, whereas, antifungal agent voriconazole (500 μ g/20 μ l) was used as positive control. The plates were kept upright for 5-10 min until the solution diffused into the medium. SDA plates were then incubated aerobically at 25°C in a biological oxygen demand (BOD) incubator for 2-5 days and the zone of inhibition was measured and recorded. All experiments were performed in triplicate.

Determination of minimum inhibitory concentrations (MIC)

MIC was determined by broth micro-dilution method. It was performed according to Clinical and Laboratory Standards Institute (CLSI), formerly NCCLS (1997) for yeasts and NCCLS (2002) for filamentous fungi, with minor modifications (Shahid et al., 2007). Doubling dilutions of the extract was prepared using RPMI-1640 (HiMedia, India) broth supplemented with 0.3 g/L L-glutamine (HiMedia, India), 0.165 mol/L of 3-[N-morpholino] propanesulfonic acid (MOPS) buffer (HiMedia, India) and 0.01% of dimethyl sulphoxide (DMSO) (Qualigens Fine Chemicals, India). Extracts were dissolved in DMSO, and further diluted 1:50 in RPMI-1640 medium, and each resulting solution was used for a doubling dilution series. Microtitre plates were prepared containing 100 μ l of undiluted extracts in the first well, followed by doubling dilutions of extracts from second well. The standardized inoculum of each fungal species was added to the respective dilution wells including the first well. The final concentrations of the extracts ranged from 25×10^3 to 48×10^{-3} μ g/ml. For each test there was a sterility control well containing alcoholic extract in RPMI-1640 broth plus DMSO and a growth control well containing fungal suspension without alcoholic extract. The microtitre plates were incubated at 35°C for 48 h with their upper surface covered by sterile sealers. The lowest concentration that did not show any visible growth was considered the MIC of that extract for the tested fungal species. All the MIC experimentations were performed in duplicate.

Statistical analysis

All the experiments of antimicrobial susceptibility testing were performed in triplicate. The results were expressed as the mean \pm standard error (SE). Data were analyzed statistically by one way analysis of variance (ANOVA) followed by Tukey's multiple analysis test using SPSS Software, Chicago, III, version 10. P values were

calculated by one-sample T-test and $P < 0.05$ was taken as statistically significant.

RESULTS AND DISCUSSION

Antifungal activity of alcoholic extracts of parent plant as well as its *in vitro* raised callus against the tested fungal species is shown in Table 1. Negative control (ethanol) showed the zone of inhibition in the range of 0.00 to 8.67 \pm 0.33 mm. Positive controls (voriconazole) showed the zone of inhibition in the range of 9.33 \pm 0.33 to 13.00 \pm 0.58 mm. The extracts derived from both parent plants as well as callus gave good antifungal activity (Table 1). The alcoholic fruit extract of parent plant showed significant antifungal activity ($P < 0.05$) against *C. albicans* ($P = 0.003$), *C. parapsilosis* ($P=0.012$) and *Penicillium* spp. ($P = 0.017$), with MIC ranging from 3.05 to 24.0 μ g/ml (Figure 1).

Various studies have been under taken previously by different researchers to analyze the antifungal potential of *B. aegyptiaca* (Runyoro et al., 2006; Maregesi et al., 2008; Al Ashaal, 2010; Panghal et al., 2011). They showed significant antifungal activity of this plant against *C. albicans*, which supports our present research findings.

Since we tested an exhaustive range of fungal isolates, both standard and clinical strains as compared to previously studies, we were able to detect a wide spectrum of antifungal activity of this plant extract. In the present study, it was also found that alcoholic fruit extract of *B. aegyptiaca* also showed good antifungal activity against *C. parapsilosis* and *Penicillium* spp., which were not tested in earlier studies. Another study done by Abdallah et al. (2012) showed significant antifungal activity of this plant against *A. niger* and *Fusarium* species. These findings are in contrast with our study. This could be due to different concentrations of extracts used in their study as well as variation in active metabolites present in plant extracts derived from different places.

As seen in Table 1, the alcoholic extract of *in vitro* cultivated callus also showed significant antifungal activity against *C. albicans*, *C. parapsilosis* and *Penicillium* spp. which were comparable to the parent plant extract. In addition, it also showed good antifungal activity against *C. krusei*. The MIC of *in vitro* raised alcoholic callus extract ranged from 1.53 to 12.0 μ g/ml (Figure 2). To the best of our knowledge, this is the first study analyzing the antifungal potential of *in vitro* raised calli of this plant; therefore, our findings could not be compared.

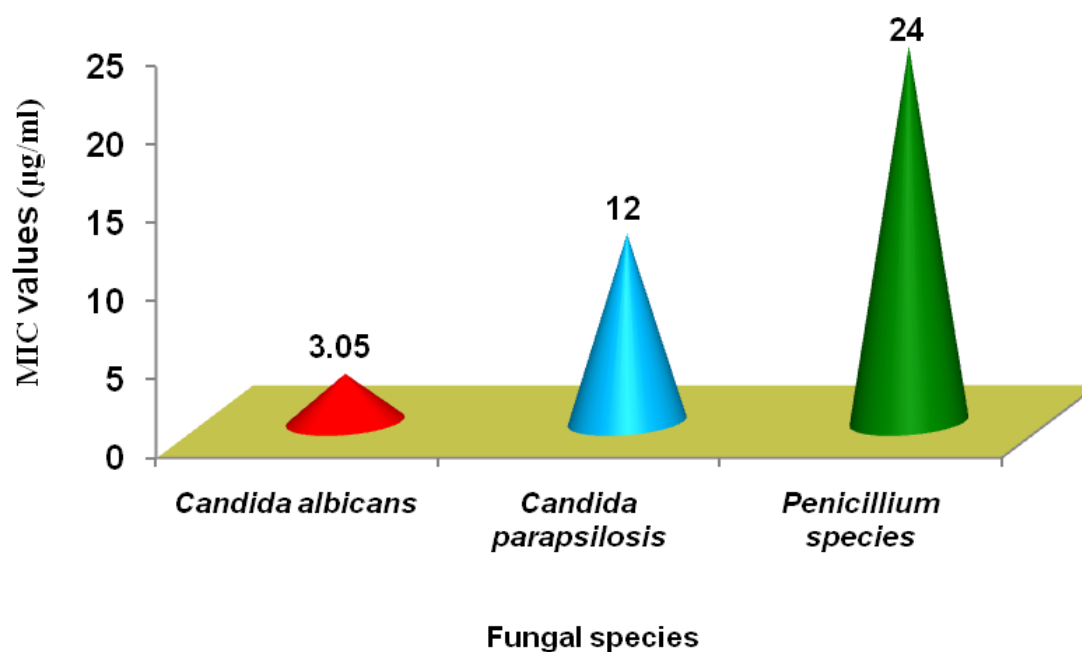
Conclusion

Extracts of *B. aegyptiaca* have remarkable antifungal potential against clinical and standard strains and thus could be used to derive antifungal agents especially against *C. albicans*, *C. parapsilosis* and *Penicillium* species. Hence, it could be used in the treatment of various opportunistic and life threatening fungal infections especially

Table 1. Antifungal activity of alcoholic extracts of parent plant of *B. aegyptiaca* and its *in vitro* raised callus against pathogenic and opportunistic fungi.

Fungi tested	Zone of inhibition (mm) ± SE			
	Alcoholic fruit extract of parent plant ^Δ	Alcoholic extract of <i>in vitro</i> raised callus ^Δ	Ethanol [†] (negative control)	Voriconazole [£] (positive control)
<i>Candida albicans</i>	15.33±0.67 ^a	13.67±0.33 ^a	8.33±0.33 ^b	12.33±0.33 ^b
<i>Candida krusei</i>	0.00±0.00 ^d	12.67±0.33 ^{bc}	7.67±0.33 ^c	11.67±0.33 ^c
<i>Candida parapsilosis</i>	14.67±0.33 ^{ab}	13.00±0.58 ^b	8.67±0.33 ^a	12.67±0.33 ^{ab}
<i>Aspergillus fumigatus</i>	0.00±0.00 ^d	0.00±0.00 ^d	7.67±0.33 ^c	10.33±0.33 ^{de}
<i>Aspergillus flavus</i>	0.00±0.00 ^d	0.00±0.00 ^d	7.33±0.33 ^d	9.67±0.33 ^e
<i>Aspergillus niger</i>	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^e	9.33±0.33 ^f
<i>Penicillium</i> spp.	13.00±0.58 ^c	12.33±0.33 ^c	8.33±0.33 ^b	11.67±0.33 ^c
<i>Fusarium</i> spp.	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^e	9.33±0.33 ^f
<i>C. parapsilosis</i> (ATCC 22019)	14.33±0.33 ^b	13.33±0.33 ^{ab}	8.67±0.33 ^a	13.00±0.58 ^a
<i>C. krusei</i> (ATCC 6258)	0.00±0.00 ^d	13.00±0.58 ^b	8.33±0.33 ^b	12.33±0.33 ^b
<i>A. fumigatus</i> (ATCC 204305)	0.00±0.00 ^d	0.00±0.00 ^d	7.67±0.33 ^c	10.67±0.33 ^d

† = 20 µl of 95% ethanol was used as negative control. Δ = concentration of extracts used in the test, that is, 2 mg/20 µl. £ = concentration of voriconazole used in test, that is, 500 µg/20 µl. Diameter of zone of inhibition is a mean of triplicates ± SE (mm). Differences were assessed statistically using one way ANOVA followed by Tukey's test. P<0.05 was considered as significant. The mean represented by same letter is not significantly different within the column.

**Figure 1.** MIC determination of alcoholic fruit extract of *B. aegyptiaca* against tested pathogenic and opportunistic fungi.

in diabetics, cancer patients and immunocompromised patients. As the extracts from *in vitro* raised callus showed significant and even better antifungal potential as compared to parent plant, *in vitro* cultivation of this plant may be used to obtain phytotherapeutic compounds, even at

places where it does not grow naturally because of adverse atmospheric conditions. In the future, bioactive compounds responsible for antifungal activity could further be enhanced by nutritional and hormonal manipulations of the cultivation medium, as depicted in our study.

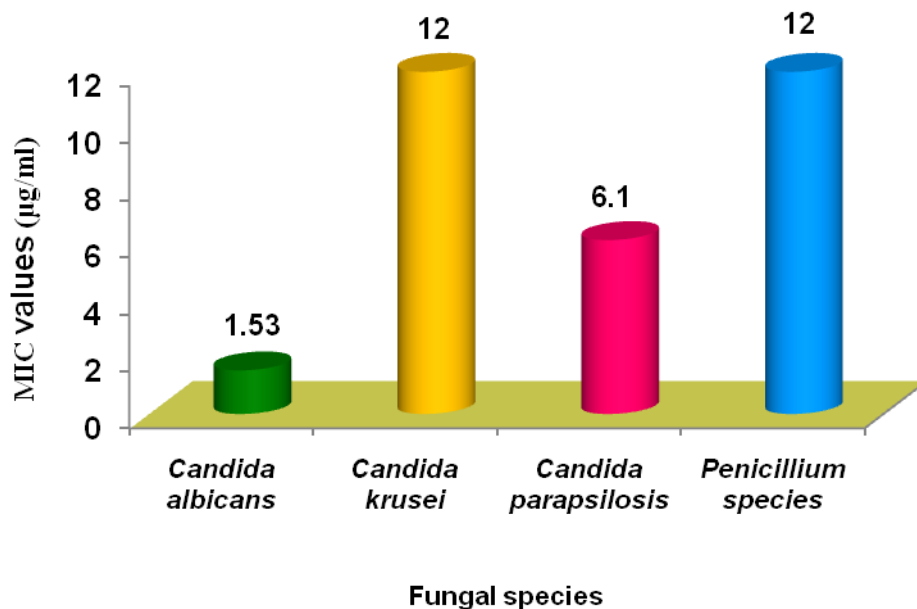


Figure 2. MIC determination of alcoholic extract of *in vitro* raised callus of *B.aegyptiaca* against tested pathogenic and opportunistic fungi.

REFERENCES

- Abdallah EM, Hsouna AB, Al-Khalifa KS (2012). Antimicrobial, antioxidant and phytochemical investigation of *Balanites aegyptiaca* (L.) Del. edible fruit from Sudan. *Afri. J. Biotech.* 11:11535-11542.
- Akinpelu DA (2001). Antimicrobial activity of *Anacardium occidentale* bark. *Fitoterapia.* 72:286-287.
- Al Ashaal HA, Farghaly AA, Abd El Aziz MM, Ali MA (2010). Phytochemical investigation and medicinal evaluation of fixed oil of *Balanites aegyptiaca* fruits (Balantiaceae). *J. Ethnopharmacol.* 127:495-501.
- Ayensu ES (1978). *Medicinal Plants of West Africa*. Reference Publications, Inc. Michigan. p.330.
- Bandow JE, Brotz H, Leichert LIO, Labischinski H, Hecker M (2003). Proteomic approach to understanding antibiotic action. *Antimicrob. Agents. Chemother.* 47:948-955.
- Bhojwani SS, Razdan MK (1996). *Plant tissue culture: theory and practice*. Elsevier. Amsterdam, Netherlands. p.31-32.
- Chander J (2002). *Textbook of Medical Microbiology*. Second edition. Mehta Publishers. New Delhi. p.65.
- Chopra RN, Nayar SL, Chopra IC (1956a). *Glossary of Indian Medicinal Plants, Vol 1*. CSIR Publication. New Delhi. p.32.
- Chopra RN, Nayar SL, Chopra IC (1956b). *Glossary of Indian medicinal plants*. Council of Scientific and Industrial Research. New Delhi. p.168-169.
- Chothani DL, Vaghasiya HU (2011). A review on *Balanites aegyptiaca* Del (desert date): phytochemical constituents, traditional uses, and pharmacological activity. *Pharmacogn. Rev.* 5:55-62.
- Erdoglu OT (2002). Antibacterial activities of some plant extracts used in folk medicine. *Pharm. Biol.* 40:269-273.
- Gaur K, Nema RK, Kori ML, Sharma CS, Singh V (2008). Anti-inflammatory and analgesic activity of *Balanites aegyptiaca* in experimental animal models. *Intern. J. Gr. Pharm.* 2:214-217.
- Hall JB, Walker DH (1991). *Balanites aegyptiaca* Del. A Monograph. School of Agricultural and Forest Science. Banger. University of Wales p.1-12.
- Ibrahim AM (1992). Anthelmintic activity of some Sudanese medicinal plants. *Phytother. Res.* 6:155-157.
- Inngenderingen KCS, Diallo ND, Mounkoro BS (2004). An ethnopharmacological Survey of plants used for wound healing in Dogonland Mali West Africa. *J. Ethnopharmacol.* 92:233-244.
- Irvine FR (1961). *Woody Plants of Ghana*. Oxford University Press, London. p. 878.
- Iwu MM (1993). *Handbook of African Medicinal Plants*. Boca Raton FL. CRC Press. pp.129-130.
- Kamel MS, Ohtani K, Kurokawa T, Assaf MH, el-Shanawany MA, Ali AA, Kasai R, Ishibashi S, Tanaka O (1991). Studies on *Balanites aegyptiaca* fruits: An antidiabetic Egyptian folk medicine. *Chem. Pharm. Bull.* 31:1229-1233.
- Kirtikar KR, Basu BD (1996). *Indian Medicinal Plants*, 3. Allahabad: International Book Distributors. p.1935.
- Liu HW, Nakanishi K (1982). The structure of *Balanites*: Potent molluscicides isolated from *Balanites aegyptiaca*. *Tetrahedron.* 38:513-519.
- Maregesi SM, Pieters L, Ngassapa OD, Apers S, Vingerhoets R, Cos P, Berghe DA, Vlietinck A (2008). Screening of some Tanzanian medicinal plants from Bunda district for antibacterial, antifungal and antiviral activities. *J. Ethnopharmacol.* 119:58-66.
- Mohamed AM, Wolf D, Spiess WE (2000). Recovery and characterization of *Balanites aegyptiaca* Del. Kernel proteins: Effect of defatting, air classification, wet sieving and aqueous ethanol treatment on solubility, digestibility, amino acid composition and sapogenin content. *Nahrung.* 44:7-12.
- Mohamed AM, Wolf W, Spiess WE (2002). Physical, morphological and chemical characteristics, oil recovery and fatty acid composition of *Balanites aegyptiaca* Del. Kernels. *Plant. Foods. Hum. Nutr.* 57:179-189.
- Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15: 473-497.
- National Committee for Clinical Laboratory Standards (NCCLS) (1997). Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard. NCCLS Document M27-A5. NCCLS, Villanova, PA, US.
- National Committee for Clinical Laboratory Standards (NCCLS) (2002). Reference method for broth dilution antifungal susceptibility testing for conidium-forming filamentous fungi; approved standard. NCCLS Document M38-A. Wayne, PA, USA.
- Panghal M, Kaushal V, Yadav JP (2011). *In vitro* antimicrobial activity of ten medicinal plants against clinical isolates of oral cancer cases. *Ann. Clin. Microbiol. Antimicrob.* 10:21-32.
- Parekh J, Chanda S (2008). Antibacterial Activity of Aqueous and Alcoholic Extracts of 34 Indian Medicinal Plants against Some

- Staphylococcus* Species. Turk. J. Biol. 32:63-71.
- Rao MV, Shah KD, Rajani M (1997). Contraceptive efficacy of *Balanites roxburghii* pericarp extract in male mice (*Mus musculus*). Phytother. Res. 11:469-71.
- Runyoro DKB, Matee MIN, Ngassapa OD, Joseph CC, Mbwambo ZH (2006). Screening of Tanzanian medicinal plants for anti-Candida activity. BMC. Complem. Alter. Med. 6:11-20.
- Samy RP, Ignacimuthu S (2000). Antibacterial activity of some folklore medicinal plants used by tribals in western ghats of India. J. Ethnopharmacol. 69:63-71.
- Scazzocchio F, Comets MF, Tomassini L, Palmery M (2001). Antibacterial activity of *Hydrastis Canadensis* extract and its major isolated alkaloids. Planta. Med. 67:561-563.
- Shahid M, Shahzad A, Malik A, Anis M (2007). Antibacterial activity of aerial parts as well as *in vitro* raised calli of the medicinal plant *Saraca asoca* (Roxb.) de Wilde. Can. J. Microbiol. 53:1-7.
- Shahid M, Rahim T, Shahzad A, Tajuddin, Latif A, Fatma T, Rashid M, Raza A, Mustafa S (2009a). Ethnobotanical studies on *Berberis aristata* DC. root extracts. Afri. J. Biotech. 8:556-563.
- Shahid M, Shahzad A, Anis M (2009b). Antibacterial potential of the extracts derived from leaves and *in vitro* raised calli of medicinal plants *Pterocarpus marsupium* Roxb., *Clitoria ternatea* L., and *Sanseveiria cylindrical* Bojer ex Hook. Orient. Pharm. Exp. Med. 9:174-181.
- Singh I, Singh VP (2000). Antifungal properties of aqueous and organic extracts of seed plants against *Aspergillus flavus* and *A. niger*. Phytomorphol. 50:151-157.
- Vanden-Berghe DA, Vlietinck AJ (1991). Screening methods for antibacterial and antiviral agents from higher plants. In Methods in plant biochemistry. Edited by PM Dey, JB Harborne, and Kohostettmann), Academic Press, London. p.47-70.
- Vanila D, Ghanthikumar S, Manickam VS (2008). Ethnomedicinal Uses of Plants in the Plains Area of the Tirunelveli-District, Tamilnadu, India. Ethnobot. Leaflets. 12:1198-1205.