*Available online at* www.ijpab.com

DOI: http://dx.doi.org/10.18782/2582-2845.8399

**ISSN: 2582 – 2845**

*Ind. J. Pure App. Biosci.* (2020) *8*(6), 130-136

Indian Journal of Pure & Applied Biosciences

Peer-Reviewed, Refereed, Open Access Journal

*Research Article*

# In vitro Evaluation of Antifungal Activity of *Balanites aegyptiaca* Del.

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Received: 21.09.2020 | Revised: 25.10.2020 | Accepted: 2.11.2020

**ABSTRACT**

*Repetitive and long-term treatment of plants with chemical fungicides leads to the formation of stable residues which remain in the soil for long periods and adversely affect soil fauna and flora. They may also drain off into ditches and rivers and kill algae and fish. Plants are known to produce a wide array of constitutive and induced antifungal compounds to fight infection. Plants particularly from arid region would be good candidates to explore for novel broad spectrum antifungal compounds to reduce or eliminate our reliance on synthetic chemicals which would be harmful to our environment. In this respect, an attempt was made in this experiment to investigate the antifungal potential of Balanites aegyptiaca Del. an important tree species of Indian arid region. Dried and powdered leaves, root fruit and bark of B. aegyptiaca extracted with ethanol and water using soxhlet extraction apparatus and dried in rotary evaporator and water bath respectively. These extracts were tested against five fungal plant pathogen includes Rhizoctonia solani, R. bataicola, Fusarium solani, Fusarium moniliforme and Alternaria alternata using poison food technique while bevistin was used as standard fungicide. The extract exhibited antifungal activity against five selected fungi. Thus, the current investigation leads to source of new antifungal compound in future.*

***Keywords:*** *Antifungal, Extract, Fungi, Infect, Pathogen.*

## INTRODUCTION

Repetitive and long-term treatment of plants with chemical fungicides leads to the formation of stable residues which remain in the soil for long periods and adversely affect soil fauna and flora. They may also drain off into ditches and rivers and kill algae and fish. Plants are known to produce a wide array of constitutive and induced antifungal compounds to fight infection. Plants particularly from arid region would be good candidates to explore for novel broad spectrum antifungal compounds to reduce or eliminate our reliance on synthetic chemicals which would be harmful to our environment. Plant products have been part of folklores medicine since time of immemorial. Medicinal plants are repository of bioactive compounds containing naturally antifungal properties.

**Cite this article:** Sharma, B., & Verma, N. (2020). In vitro Evaluation of Antifungal Activity of *Balanites aegyptiaca* Del., *Ind. J. Pure App. Biosci. 8*(6), 130-136. doi: http://dx.doi.org/10.18782/2582-2845.8399

A lot of plant extract containing antifungal properties have been reported by many workers (Adekuncle & Ikumpayi 2006; Buwa & Staden 2006, & Parekh, & Chanda 2006). In vitro evaluation of plants for antimicrobial property is the essential primary process for development of eco-friendly antifungal substance of plant origin. In this respect, *B. aegyptiaca* screened in vitro for antifungal potential against five plant pathogenic fungi.

*B. aegyptiaca* belongs to the family Balanitaceae and is found throughout the drier parts of India (Amalraj & Shankanarayan, 1998). It is widely distributed in the Sudano-Sahielian region of Africa (Mohamed et al., 1999), the Middle East and South Asia (Chothani & Vaghasiya, 2011). It is estimated that up to one third of total trees population in central parts of Sudan is from this plant (NCR, 2008) with diverse uses in folk medicine and many other applications (Elfeel & Warrag, 2011). It is indigenous to all dry lands south of the Sahara, extending southward to Malawi in the Rift Valley, and to the Arabian Peninsula (Hall & Waljer 1991; Ndoye et al., 2004; Hammouda et al., 2005; Okia et al., 2011; Chothani & Vaghasiya, 2011 & Al-Thobaiti & Abu Zeid, 2018). Ita drought-tolerant perennial tropical ever green tree belongs to family Zygophyllaceae (Balanitaceae), It is known by various names, e.g. Heiglige in Arabia and desert date in English (Hall & Waljer, 1991), in India it is called Hingota. The name Balanites originally derived from the Greek word which means fruit resemble acorn (Gupta et al., 2012). It is also known by different vernacular names such as Angarvriksha, Balanite, Desert date, Bedeno, Hingot, Soapberry tree, Thorn tree and Egyptian balsam and many more (Rathore et al., 2005). It is a small, branched, spiny shrub or tree up to ten meter height. Stem is pubescent when young, bark dark brown deeply fissured with spiny branches (Koko et al., 2000). Fruit is a long, narrow drupe, 2.5 to 7 cm long, 1.5 to 4 cm in diameter. Young fruits are green turning yellow when mature. *B. aegyptiaca* have many medicinal properties. This plant used as an antihelminth, purgative, vermifuge, and febrifuge and it also used in treatment of skin boils, leucoderma, malaria, wounds, colds, syphilis, liver and spleen disorders, and aches (Mohamed et al., 1999; Chothani & Vaghasiya, 2011; Koko et al., 2000; John et al., 1990; Inngerdingen et al., 2004; Katewa et al., 2004; Khanna et al., 2007; Kubmarawa et al., 2007 & Maregesi et al., 2008).

## MATERIALS AND METHODS

The study was carried out at the Arid Forest Research Institute Jodhpur, Rajasthan. Following steps were included to find out antifungal potential of selected plant species:

### *Collection of Plant Material*

Plant material was collected from some places of Jodhpur District, Rajasthan, India. Plant samples were identified with the help of taxonomic literature, standard flora and herbarium. Collected material was washed thoroughly with running tap water followed by distilled water to remove dirt. After washing and cleaning, material was shade dried at room temperature and finely ground with help of grinder. Powdered material was stored in airtight bottles for further use in preparation of extract.

### *Preparation of Extracts*

Two types of extract aqueous and alcoholic (Ethyl alcohol) were prepared from every collected plant part with the help of Soxhlet apparatus and dried with help of water bath and rotary evaporator respectively. Extract were dissolved in DMSO and solution of different concentrations (10, 20, 30, 40 & 50) were prepared. The effect of extract on selected fungi was tested in vitro by poison food technique [Nane & Thapliyal 1979].

### *Poison Food Technique*

Starter culture of selected fungi was prepared in PDA medium. Plant Extract of different concentration was mixed with cooled molten media in conical flask and poured into petriplates and allowed to solidify at room temperature. A mycelium disk of 5 mm diameter was cut out from periphery of actively growing fungus (4-7 days old culture) with the help of cork borer and aseptically

plated at centre of each petriplate. Three replication of each treatment were maintained, Plate without extract act as negative control and plate with chemical fungicide (.2%) served as positive control. All petriplates were incubated at 25±1˚C for seven days. After incubation the effect of extract was determined by measuring the radial growth of fungi in test plate and compared with control plate. Colony diameter of fungus in each plate was measured in mm. The antifungal activity was assessed in terms of percentage inhibition.

The percentage inhibition was calculated with the help of following formula suggested by Vincet, (1947).

Inhibition Percent = I% = C-T/C X 100

C= Growth of mycelium in control plate (mm)

T=Growth of mycelium in treatment plate (mm) mean of three plates considered as final reading

Mean value and standard error mean were calculated for result of poison food technique.

## RESULT

The antifungal activity of aqueous and alcoholic extract of *Balanites aegyptiaca* plant parts at five different concentration were evaluated in vitro by Poison Food Technique against five plant pathogenic fungi (*Rhizoctonia solani, R. bataticola, Fusarium solani, F. moniliforme & Alternaria alternata*). The result of this study showed that *Balanites aegyptiaca* has ability to inhibit selected fungi in vitro. Both aqueous and ethanolic extract of *Balanites aegyptiaca* leaves fruit, root and bark showed varied result against target fungi. All the ethanolic extracts showed wide range of activity against the targeted fungi as compared to aqueous extract which showed limited antifungal activity. The maximum percentage inhibition (leaves) with aqueous extract (at 50%) for *Rhizoctonia solani,* was 36%, for *Rhizoctonia bataticola* was 29%, for *Fusarium solani* was 37% and *Fusarium moniliforme* was 23% and for *Alternaria alternata* was 27.6%.

The maximum inhibition percentage (leaves) with ethanolic extract (50%) for *Rhizoctonia solani,* was 68.3% for *Rhizoctonia bataticola* was 47%, for *Fusarium solani* was 54% and for *Fusarium moniliforme* was 36% and for *Alternaria alternata* was 51%. It is clearly indicates that the ethanolic extract of *Balanites aegyptiaca* leaves exhibited more antifungal properties against all fungi then aqueous extract. Effect of different concentration (10%, 20%, 30%, 40% and 50%) of ethanol extract on growth of all fungi showed that inhibition of fungus growth increase with concentration of extract. The ethanolic extract of *Balanites aegyptiaca* leaf exhibit maximum inhibition against *Rhizoctonia solani* (68.3%) followed by *Fusarium solani* (54%). All the concentration of ethanolic extract of *Balanites aegyptiaca* leaves was found effective in inhibition of mycelia growth over the untreated control plate. However highest concentration of extract (50%) recorded maximum inhibition. Good antifungal activity was shown by leaf and fruit moderate to mild antifungal activity was shown by root and bark.

The result of antifungal screening of aqueous and ethanolic extract of *Balanites aegyptiaca* are given in tables 1 to 8 and comparative effectiveness is shown with the help of graph 1-4.

**Table 1: Showing inhibition percentage of ethanolic extract of *Balanites aegyptiaca* leaves against selected fungi**

| Fungus species | Concentration of extract/Inhibition Percentage | | | | | |
|---|---|---|---|---|---|---|
| | Control | 10 | 20 | 30 | 40 | 50 |
| *Rhizoctonia solani,* | 0 | 12.6 | 27% | 39% | 52.9% | 68.3% |
| *Rhizoctonia bataticola* | 0 | 7% | 11% | 19.6% | 39% | 47% |
| *Fusarium solani* | 0 | 10% | 21.7% | 37% | 43% | 54% |
| *Fusarium moniliforme* | 0 | 4% | 13% | 17.3% | 28% | 36% |
| *Alternaria alternata* | 0 | 6% | 17% | 28.4% | 37% | 51% |

**Table 2: Showing inhibition percentage of aqueous extract of *Balanites aegyptiaca* leaves against selected fungi**

| Fungus species | Concentration of extract/Inhibition Percentage | | | | | |
|---|---|---|---|---|---|---|
| | Control | 10 | 20 | 30 | 40 | 50 |
| *Rhizoctonia solani,* | 0 | 5% | 12% | 19% | 28% | 36% |
| *Rhizoctonia bataticola* | 0 | 0% | 6% | 14% | 22.3% | 29% |
| *Fusarium solani* | 0 | 3% | 11% | 17% | 25.7% | 37% |
| *Fusarium moniliforme* | 0 | 0% | 0% | 8% | 14.6% | 23% |
| *Alternaria alternata* | 0 | 0% | 8% | 13% | 19.2% | 27.6% |

**Table 3: Showing inhibition percentage of ethanolic extract of *Balanites aegyptiaca* root against selected fungi**

| Fungus species | Concentration of extract/Inhibition Percentage | | | | | |
|---|---|---|---|---|---|---|
| | Control | 10 | 20 | 30 | 40 | 50 |
| *Rhizoctonia solani,* | 0 | 0% | 6% | 11% | 17.5% | 21% |
| *Rhizoctonia bataticola* | 0 | 0% | 3% | 7% | 13% | 18.9% |
| *Fusarium solani* | 0 | 0% | 7% | 12.5% | 19% | 22% |
| *Fusarium moniliforme* | 0 | 0% | 0% | 4% | 9% | 13% |
| *Alternaria alternata* | 0 | 0% | 4% | 9.3% | 14% | 19% |

**Table 4: Showing inhibition percentage of aqoeous extract of *Balanites aegyptiaca* root against selected fungi**

| Fungus species | Concentration of extract/Inhibition Percentage | | | | | |
|---|---|---|---|---|---|---|
| | Control | 10 | 20 | 30 | 40 | 50 |
| *Rhizoctonia solani,* | 0 | 0% | 3% | 7% | 12% | 17.3% |
| *Rhizoctonia bataticola* | 0 | 0% | 0% | 4% | 10.2% | 15.6% |
| *Fusarium solani* | 0 | 0% | 0% | 6% | 12% | 19% |
| *Fusarium moniliforme* | 0 | 0% | 0% | 0% | 3% | 9.2% |
| *Alternaria alternata* | 0 | 0% | 0% | 3% | 11% | 14% |

**Table 5: Showing inhibition percentage of ethanolic extract of *Balanites aegyptiaca fruit* against selected fungi**

| Fungus species | Concentration of extract/Inhibition Percentage | | | | | |
|---|---|---|---|---|---|---|
| | Control | 10 | 20 | 30 | 40 | 50 |
| *Rhizoctonia solani,* | 0 | 29.6% | 43% | 51% | 63.3% | 76% |
| *Rhizoctonia bataticola* | 0 | 4% | 10.7% | 17% | 26% | 39% |
| *Fusarium solani* | 0 | 8% | 19.2% | 31% | 47% | 63% |
| *Fusarium moniliforme* | 0 | 5% | 12% | 21.3% | 34% | 42.8% |
| *Alternaria alternata* | 0 | 10% | 17.5% | 26.7% | 39.5% | 47% |

**Table 6: Showing inhibition percentage of aqueous extract of *Balanites aegyptiaca fruit* against selected fungi**

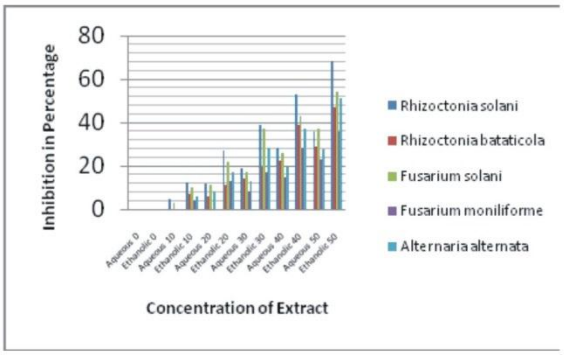
| Fungus species | Concentration of extract/Inhibition Percentage | | | | | |
|---|---|---|---|---|---|---|
| | Control | 10 | 20 | 30 | 40 | 50 |
| *Rhizoctonia solani,* | 0 | 8% | 19.4% | 27% | 39.8% | 48% |
| *Rhizoctonia bataticola* | 0 | 0% | 4% | 11.9% | 18.6% | 26% |
| *Fusarium solani* | 0 | 0% | 10% | 16.6% | 29.3% | 38% |
| *Fusarium moniliforme* | 0 | 0% | 5% | 13% | 19% | 30.5% |
| *Alternaria alternata* | 0 | 3% | 12% | 21.8% | 27.6% | 34% |

**Table 7: Showing inhibition percentage of ethanolic extract of *Balanites aegyptiaca Bark* against selected fungi**

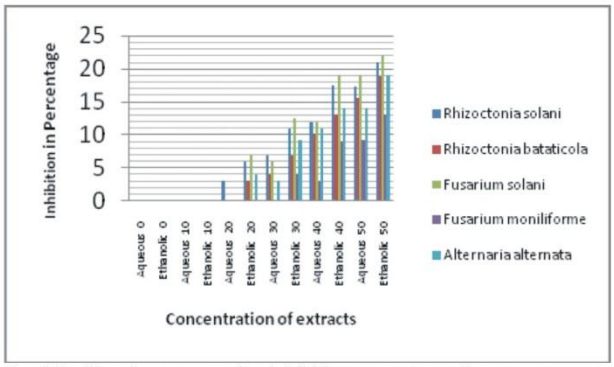
| Fungus species | Concentration of extract/Inhibition Percentage | | | | | |
|---|---|---|---|---|---|---|
| | Control | 10 | 20 | 30 | 40 | 50 |
| *Rhizoctonia solani,* | 0 | 0% | 8% | 17% | 23.6% | 31% |
| *Rhizoctonia bataticola* | 0 | 0% | 4% | 11% | 14% | 22.8% |
| *Fusarium solani* | 0 | 3% | 11.4% | 18% | 25% | 36% |
| *Fusarium moniliforme* | 0 | 0% | 5% | 9.5% | 16% | 27% |
| *Alternaria alternata* | 0 | 6% | 13.8% | 17.3% | 24.7% | 34% |

**Table 8: Showing inhibition percentage of aqueous extract of *Balanites aegyptiaca Bark* against selected fungi**

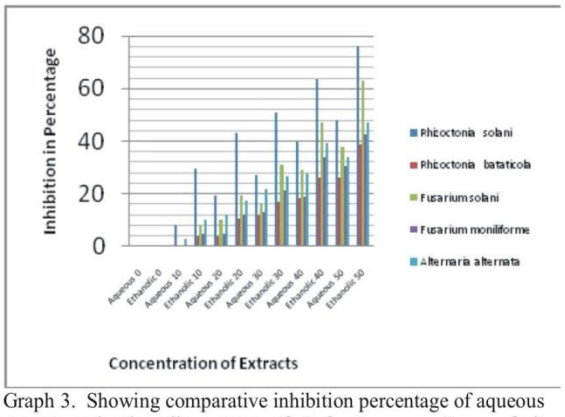
| Fungus species | Concentration of extract/Inhibition Percentage | | | | | |
|---|---|---|---|---|---|---|
| | Control | 10 | 20 | 30 | 40 | 50 |
| *Rhizoctonia solani,* | 0 | 0% | 3% | 8% | 15.3% | 21% |
| *Rhizoctonia bataticola* | 0 | 0% | 0% | 7% | 11.9% | 19% |
| *Fusarium solani* | 0 | 0% | 6% | 14.3% | 19.5% | 28.2% |
| *Fusarium moniliforme* | 0 | 0% | 3% | 7% | 13.1% | 19% |
| *Alternaria alternata* | 0 | 4% | 9% | 12.8% | 21.4% | 27% |



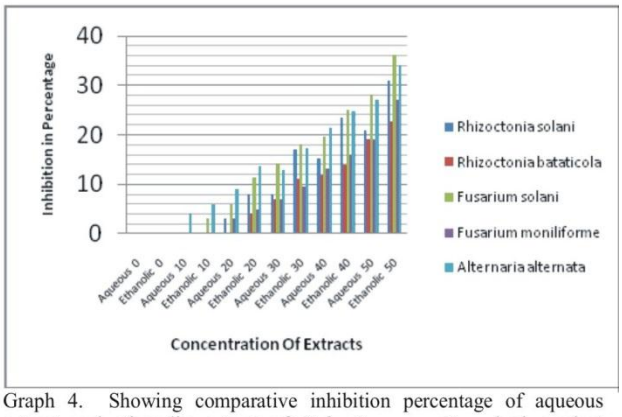

Graph1. Showing comparative inhibition percentage of aqueous extract and ethanolic extract of *Balanites aegyptiaca* leaves against selected fungi




Graph2. Showing comparative inhibition percentage of aqueous extract and ethanolic extract of *Balanites aegyptiaca* root against selected fungi




Graph 3. Showing comparative inhibition percentage of aqueous extract and ethanolic extract of *Balanites aegyptiaca* fruit against selected fungi




Graph 4. Showing comparative inhibition percentage of aqueous extract and ethanolic extract of *Balanites aegyptiaca* bark against selected fungi

## DISCUSSION

The result of this study clearly indicates that that *Balanites aegyptiaca* has ability to inhibit selected fungi in vitro. Both aqueous and ethanolic extract of *Balanites aegyptiaca* showed varied result against target fungi. All the ethanolic extracts showed wide range of activity against the targeted fungi as compared to aqueous extract which showed limited antifungal activity. Effect of different concentration (10%, 20%, 30%, 40% and 50%) of ethanol extract on growth of all four fungi showed that inhibition of fungus growth increase with concentration of extract. Good antifungal activity was shown by leaf and fruit of *Balanites aegyptiaca* while moderate to mild antifungal activity was shown by root and bark.

Methanol extracts extract of the fruit of *Balanites aegyptiaca* were tested on several microbial strains using agar-well diffusion methods (Abdallah et al., 2012). Methanol extracts, particularly at concentration of 100 mg/ml was found to be effective against all bacterial and fungal strains. The alcoholic fruit extract of B. aegyptiaca showed good antifungal (Runyoro et al., 2006 & Maregesi et al., 2008). The microbial activities of ethanol, petroleum ether, and chloroform extract of B. aegyptiaca leaf were also studied by (Kabbashi et al., 2017). These findings are in agreement with our study. Aqueous and

alcoholic extracts of Balanites aegyptiaca Del. were also studied by Khatoon et al., 2014 for antifungal properties against various pathogenic and opportunistic fungi by in vitro agar well diffusion method. All the alcoholic extracts showed wide range of activity. This also supports our research findings. Antifungal potential of B. aegyptiaca also studied by various worker (Al Ashaal, 2010 & Panghal et al., 2011). They also prowed significant antifungal activity of this plant .On the other hand a study done by Abdallah et al., (2012) showed significant antifungal activity of this plant against *Aspergillus* and *Fusarium* species.

## CONCLUSION

The present study leads to conclusion in nutshell that ethanolic extract of *Balanites aegyptiaca* contain significant antifungal potential against selected plant pathogenic fungi. Further investigation is suggested for in vivo effect of this extract.

**Acknowledgments**

The author is greatly indebted to Director General, ICFRE and Director AFRI for providing necessary research facility encouragement and constant inspiration needed during the course of investigation in this project. Special thanks given to supporting associates for their help and cooperation during the project work.

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