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In vitro evaluation of various extracts of *Acacia nilotica* (L.) del. against human pathogenic fungi

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Abstract
Acacia nilotica (L.) *Del.* of Mimosaceae is widely distributed in India. It has anti-inflammatory, anti-oxidant, anti-diarrhoeal, anti-hypertensive, insecticidal, anthelmintic, anti-plasmodic, anti-bacterial properties. *Candida albicans* causes candidosis and *Epidermophyton floccosum* causes dermatophytosis. It also invades skin and nails. It is a infectious agent in immunocompromised patient with Behcet's syndrome. Aqueous, ethyl alcohol and ethyl acetate hot extracts of leaves, bark, roots & seeds were investigated for their antifungal activity. The growth inhibition was determined using food poisoning method against human pathogenic fungi. It was observed that ethyl acetate extract showed maximum inhibition than ethyl alcohol extract followed by aqueous extract against the selected human pathogenic fungi.

Keywords: *Acacia nilotica* (L.) Del., antifungal activity

Introduction
Plant based drugs are useful for the treatment of the diverse range of disease and are gaining popularity because of several advantages such as fewer side effect, better patient tolerance, relatively less expensive and acceptance due to a long history of use, especially herbal medicines provide rational means for the treatment of many diseases that are incurable in other system of medicine. According to World Health Organization (WHO) almost 80% people of the developing countries depends on traditional and folk medicines. Search for newer drugs from plants has been on the rise since many of the micro-organisms including fungi are posing serious health related disorders. Probably this may be due to the prolonged and indiscriminate use of antibiotics increase in number of immunocompromised patients. In addition, many of the existing drugs cause various side-effects. Drug development from plant based compounds could be useful in meeting this demand for newer drugs with minimal side-effects. The vast plant biodiversity of our country is presently under explored and many plants could be the source of novel drugs. (S. Madhumathi, *et al*. 2000) [8].

Plants which have been used as medicine over hundreds of years, constitute and obvious choice for study. It is interesting to determine whether their traditional uses are supported by actual pharmacological effects or merely based on folklore. Biologically active compounds from natural sources have always been of great interest to scientists working on infectious diseases. In the recent years there has been a growing interest to evaluate plants possessing antimicrobial activity for various diseases. (Clark & Hufford, 1993) [2]. A number of studies have been reported dealing with antimicrobial screening of extracts of medicinal plants. (Malcom & Sofawora, 1969). The great interest in the use and importance of Indian medicinal plants by the WHO in many developing countries has led to intensified efforts on the documentation of ethno medicinal data of medicinal plants (Dhar *et al*. 1968) [4].

Fungi cause important human disease especially in tropical regions. Despite the existence of potent antifungal agents, resistant or multi-resistant strains are continuously appearing, imposing the need for a permanent search and development of antifungal compounds.

Candida albicans infects skin causing candidosis while *Epidermophyton floccosum* is one of the common causes of dermatophytosis infecting skin (Tinea corporis, tinea cruris, tinea pedis & nail onychomycosis). Candidosis & dermatophytosis are the most common forms of fungal infection in many countries (Odds, 1988, Ribbon 1988) [11, 12]. Treatment demands the use of antifungal agents such as griseoflulvin and Amphotericin B (Koening, 1995). However, the high cost of this kind of treatment, especially in developing countries, the long period of therapy and the possibility of the emergence of resistant strains may hinder the eradication of these diseases (Baker *et al*. 1989, Willocks *et al*. 1991) [1, 14]. The screening the traditional medicinal plants may offer potential resources since there is widespread in rural areas, with much of the population relaying on them (Gadhi, CA. *et al*. 2001) [5].

Acacia nilotica (L.) *Del.* is an important ornamental and medicinal plants of tropical and sub-tropical regions belongs to family Mimosaceae and commonly known as 'Babool'. *Acacia nilotica* (L.) *Del.* is a medium sized, thorny, evergreen tree with a short trunk. The qualitative phytochemical studies of different parts of plant extract showed that, the bark contains terpenoids, tannins, alkaloids, sterols and glycosides. Leaves contain cardiac glycosides and flavanoids, root contains saponins, flavonoids, terpenes, tannins, sterols and seed contain alkaloids, flavonoids, tannins, carbohydrates and sterol.

Materials and Methods

Collection of plant material

The plant parts of *A. nilotica* collected from different regions of Marathwada particularly Nanded District and was immediately identified botanically on the spot in the field by using Flora of Marathwada (Naik, 1998) [10]. The plants parts collected were shredded and dried completely at 50^0C for 72h. The dried material were then ground into fine powder and stored in airtight container at room temp. till extraction.

Cultures

The fungal human pathogen cultures of *Candida albicans* and *Epidermophyton floccosum* were obtained from Department of Microbiology, Government Medical College, Aurangabad and Department of Pathology, Dr. S.C. Govt. Medical College, Nanded. The cultures were maintained on the medium suggested by the respective laboratory and sub-culturing was done fortnightly. The cultures were incubated in an incubator for growth and later were stored in refrigerator.

Extraction of plant material

The plant part powder was added to distilled water/ ethyl alcohol/ ethyl acetate and was allowed to boil for further 4-5 minutes on a water-bath under hood. 10ml of ethanol was used for every gram of powder. The extract was cooled and contents were homogenized thoroughly in a mortar and pestle. The extract was filtered by passing through several layers of muslin cloth. The residual ground powder was re-extracted by boiling in solvent used earlier for 3 minute to ensure the complete removal of contents. The extracts were pooled, centrifuged at 5000 rpm and the volume was adjusted to represent 10 ml/gram of fresh weight of tissue (ml/gfw).

Plant Extract for Antifungal Properties

Antifungal activity of the plant extracts (free from alcohol/ ethyl acetate and converted into aqueous) was evaluated by well-diffusion method expressed by zone of inhibition mm in diameter for *Candida albicans, Epidermophyton floccosum.*

The bioassay was carried out by using 1ml of inoculum ($1X10^6$ colony forming units) prepared from an overnight culture for given test fungi. 1ml of the resultant spore /cell suspension was poured in the petri plate and the plates were poured with respective medium to seed each prepared plate. The medium was allowed to solidify. Using a sterilized cork borer, wells of 5mm diameter were made in the solidified inoculated medium and the plate area uniformly. The wells were filled with 0.5ml of extract. Plates were then incubated aerobically at 37± 2 ºC for 72 h for fungi.

Similarly, wells containing standard concentration of Amphotericin B were used to compare the antifungal property of the plant extract. 1gm of Amphotericin B (Hi-media, Mumbai) was dissolved separately in sterile distilled water and 0.5ml was used to fill the wells.

Results and Discussion

Effect of *A. nilotica* aqueous extracts.

The aqueous extracts of *A. nilotica* exhibited antifungal activity in terms of perfect inhibition of spore germination of test fungi namely *C. albicans* and *E. floccosum* showed that 10% aqueous extracts were inhibitorier to both human pathogenic fungi and with increase in dilution zone of inhibition of fungi was decreased. Seed extract showed maximum inhibition followed by root and bark extract and it was least in leaf extract. The 10% seed extract gave a 16 mm zone of inhibition in case of *E. floccosum* which was more than control i.e. Amphotericin B.

Effect of *A. nilotica* alcoholic extracts.

The alcoholic extract of *A. nilotica* exhibited antifungal activity in terms of perfect inhibition of spore germination of test fungi namely *C. albicans* and *E. floccosum* showed that 10% alcoholic extracts was inhibitorier to both human pathogenic fungi and with increase in dilution zone of inhibition of fungi was decreased. Seed extract showed maximum inhibition followed by root and bark extract and it was least in leaf extract. The 10% seed extract gave a 20mm zone of inhibition in case of *E. floccosum* which was more than control.

Effect of *A. nilotica* ethyl acetate extracts

The ethyl acetate extract of *A. nilotica* exhibited antifungal activity in terms of perfect inhibition of spore germination of test fungi namely *C. albicans* and *E. floccosum* showed that 10% ethyl acetate extracts was inhibitorier to both human pathogenic fungi and with increase in dilution zone of inhibition of fungi was decreased. Seed extract showed maximum inhibition followed by root and bark extract and it was least in leaf extract. The seed extract was more effective in both the test organism compared to control.

It is clear that from the above results the seed extract of the plant showed highest activity in ethyl acetate. However, the activity of leaf was relatively less than the activity of bark of the plant. Similarly, the activity of bark was comparatively less than the root extracts. These results confirm the observations made by various workers with different plant and plant parts. (Cotton 19996, Taylor 1996, Grierson & Afolayan 1999) [3, 13, 6].

Hence from the above results, the seeds of the plant *A. nilotica* can be used for Integrated Paste Management to control growth of human pathogenic fungi and seeds can be utilized to develop antifungal agents.

Table 1: Effect of *A. nilotica* (L.) Del. aqueous extracts on growth of test fungi

Sr. No.	Plant parts	Zone of inhibition (mm)							
		C. albicans				*E. floccosum*			
		10%	5%	2.5%	C	10%	5%	2.5%	C
1	Leaves	6	5	3	14	10	8	5	12
2	Bark	7	6	5	14	12	10	8	12
3	Root	10	8	7	14	14	12	9	12
4	Seeds	12	9	8	14	16	13	11	12

C – Amphotericin

Table 2: Effect of *A. nilotica* (L.) Del. alcoholic extract on growth of test fungi

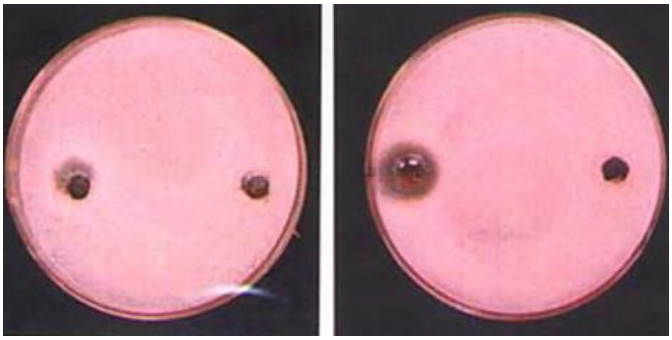
Sr. No.	Plant parts	Zone of inhibition (mm)							
		C. albicans				*E. floccosum*			
		10%	5%	2.5%	C	10%	5%	2.5%	C
1	Leaves	7	6	5	14	14	12	10	12
2	Bark	9	7	5	14	16	14	11	12
3	Root	11	9	7	14	18	16	13	12
4	Seeds	14	12	9	14	20	18	16	12

C-Amphotericin

Table 3: Effect of *A. nilotica* (L.) Del. ethyl acetate extracts on growth of test fungi

Sr. No.	Plant parts	Zone of inhibition (mm)							
		C. albicans				*E. floccosum*			
		10%	5%	2.5%	C	10%	5%	2.5%	C
1	Leaves	17	15	13	14	22	20	18	12
2	Bark	20	18	16	14	26	25	23	12
3	Root	22	19	17	14	28	26	23	12
4	Seeds	26	23	21	14	29	27	27	12

C-Amphotericin



A: *Candida albicans* B: *Epidermophyton floccosum*

Fig 1: Plate showing zone of inhibition *Acacia nilotica* (L.) Del. Extract against.

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