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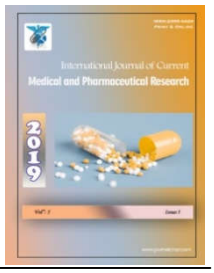

# STUDY OF *Jatropha curcas* AS ANTIFUNGAL AGENT

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**ABSTRACT**

*Jatropha curcas* (Linn) or physic nut is a perennial poisonous shrub grows up to 5m high.It belongs to the family Euphobiaceace. The plant originated from Central America but was spread to other tropical and subtropical countries Africa in Sudan it has found.
The oil from the seed is used as biodiesel. The sap from the stem is used to stop bleeding from wound and the plant is also used as fence from animals.
In the present study,leaves *jatrophacruces* we recollected from Khartoum university faculty of agriculture.
Leaves were washed with distilled water to remove dirt and soil, then dried, and coarsely powdered.
Hundred Grams of the coarsely powdered plant material were exhaustively extracted for four hours with petroleum ether in a Soxhlet apparatus. Petroleum ether ◌̊ extracted was evaporated with a Rota-vapor under reduced pressure. The extract plant material was air-derided, repacked in Soxhelt and was extracted with methanol for six hours. The methanol extracted by the same method but was filtered and evaporated under reduced pressure using Rota vapor. Extract was dissolved in dimethyl sulphoroxide (DMSO) to prepared three concentrations (12.5%, 25%, and 50%).
Aqueous Extract was prepared by adding 50 ml of distilled water to 5 grams of a samples of the coarsely powdered plants materials in conical flask with occasional shaking in water bath (60 C) for 5mints and was then filtered . Three concentrations (12.5%, 25% and 50%). Were made.
The study started by testing the action of extract (petroleum ether ,methanol and Distilled water )of *jatropha cruces* Leaves on known pathogen fungi (*Aspergillus flavus* and *Candida albicans)*.Different Concentrations of were put in well set of media (Sabouraud dextrose agar) after sterilization and Inculcated fungi and control (Ketoconazole and Nystattin) after good growth was obtained the test was Read.
At concentration 50% was found (sensitive) to the petroleum etherextract of leaves of *Jatropha Curcas.L.*Where (25and26) mm of Inhibition zone was recorded.
The concentration of 25% also showed activity to *Candida albicans* similar to *nystatin* (20) mm and moderate activity (sensitive) against *Aspergillus flavus* (17) mm.
Where concentration of 12.5% showed same activity (sensitive) for *Candida albicans,* (16)mm and inactivity against for *Aspergilusflavus.*
Methanolic extract of Leaves at the concentrations of 50% , 25% and 12.5% was found inactivity against *Candida albicans* , but it showed activityagainst *Aspergillus flavus* where itaninhibtary zone of extract (*20*)mm of concentration 50% and concentrations 25% and 12.5% was found inactive against *Aspergillus flavus.* Compared with the control (36) mm. Water extracts of leaves was found inactive against *Aspergillus flavus* and *Candida albicans* at all concentrations used.



## INTRODUCTION

Over the years, plantshave been used as valuable sources of natural products for maintaining animal and human health. Plants theyhave been reported to contain large varieties of chemical substances that possess important preventative and curative therapies (Nascimento *et al*. 2000). About 80% of individuals from developedcountries use traditional medicines which have compounds derived from medicinal plants. Despite the presence of various approaches to drug discovery, plants still remain the main reservoir of natural medicines (Mahomed and Ojewole, 2006). Interest in plants with antimicrobial properties has been revived as a result of antimicrobial resistance. This diseases (Marchese and Shito, 2001). This has

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given scientists to search for newer and alternative microbial compounds from medicinal plants (Aliero and Afolayan, 2006). Besides, the high cost of conventional drugs, particularly in resource limited communities has led to the increased use of plants as an alternative for treatment of infectious diseases. Plant extracts and phytochemicals with antimicrobial properties are of great significance in therapeutic treatments. Their antimicrobial properties are due to compounds synthesized in the secondary metabolism of the plant. The screening of plant extracts and plant products for antimicrobial activity has shown that plants represent a potential source of novel antibiotic protypes (Afolayan, 2003).

Bacteria and fungi are of great Importance for man and animal .*Aspergillus niger* has been reported to cause lung diseases, aspergillos is and otomycosis. Similarly, *Aspergillus flavus*is a human and livestock pathogen associated with aspergillos is of the lungs and sometimes causing corneal, otomycotic and nasoorbital infections. They also produces significant quantity of aflatoxin (Samson *et al.,* 2001).

*Jatropha curcas* (Linn) or physic nut is a perennialpoisonous shrub which grows up to 5m high and belongs to the family Euphobiaceace. (Gadekar, 2006). The plant originated from Central America but was spread to other tropical and subtropical countries and mainly grows in Asia and Africa in Sudan it is found in river Nile State and South kordofan State and Khartoum State.(http.//www.jatropha.wur.nl).

The leaves are usually green to pale green in colour, the flowers are unisexual but occasionally hermaphrodite. The fruits are produced mainly during the rainy season and the seeds mature if the capsule changes from green to yellow (Deghan and Webseter, 1997). The plant has been employed for both medicinal system in Nigeria, the fruits of *J. curcas* and the stem bark of *Cochlospermum planchonii* are combined for the treatment of diabetes mellitus (Igoli *et al*, 2005). Also it is used traditionally for the treatment of pains in the South Eastern part of Nigeria. The use of the aqueous extract of the seed and the nut as a contraceptive have been reported (Gonasekera, *et al*, 1995). The leaf extract also has been shown to have a potent cardiovascular action (Fojas *et al*, 1986). Other uses include; the use of the seeds for making soap, candles, detergents, lubricants and dyes. The bark is used as fish poison.

The oil from the seed is used as biodiesel (Achtem *et al*, 2008). The sap from the stem is used to stop bleeding from wound the plant is also used as fence to protect garden and fields from animals (Gadekar, 2006).

It is a multipurpose plant with several industrial and medicinal applications. *Jatrophacurcas L*. has been considered a potential source of seed oil for the production of biofuel. The plant ethno pharmacological applications are well known, but much of the information is empirical and lacking in scientific validation (Oskoueian. *et. al*.,2011).Terpenoid compounds are the major metabolites found in the Euphorbiaceaefamily. Among the terpenes, diterpenoids have dominated research in Jatropha species with respect to their novel chemical structures and medicinal values [Devappa*et.al*2011]. Recently,(Oskoueian *et al*.,2011)reported that extract of root and latex of *J. curcas* plant which contained phenolics, flavonoid and saponins showed notable antioxidant, anticancer and anti-inflammatory activities. These compounds have been reported to be involved in the biological activities of the plant. Continuous efforts have been carried out to determine the presence of bioactive compounds in various plant materials, in particular, the agro-industrial by-products since they are renewable and abundantly available (Balasundram,*.et.,al*.,2006) Phorbol esters are esters of tetra-cyclic diterpenes which are widely distributed in plant species of the families Euporbiaceae and Thymelaceae. The biological activities such as anti-HIV, anti-malaria,anti-tumor and antimicrobial have been reported by(Goel *et al*.,2007).

*J. curcas* leaves containapigenin and its glycosides, vitexin and isovitexin, stigmasterol, β-sitosterol and gallic acid(Chhabra *et.al*.,1990) while the root and stem contained gallic acid, ellagic acid, quercetin, coumaric acid, benzoic acid and Salic acid (Makkar,*et.al*.,2009).

It has been known that parts of *J. curcas* can be used for wide range of purposes. Extracts from various parts of *J. curcas*, such as seeds, seed oil, and leaves, have shownmolluscicidal, insecticidal, and fungicidal properties (Liu.*et.al*.1997). *J. curcas*extracts were found toinhibit the mycelial growth of Colletotrichummusae thatcauses anthracnose disease in bananas (Thangavelu.et.al.2004). Its leaf extractwas found effective in controlling the fungal pathogen, which causes Azolla disease (Garcia.*et.al*.1990).

***Vernaculars Names***

Common names include: *Jatropha,* physic nut, Barbados nut, purging nut, pig nut, fig nut, and it is sometimes referred to as the biodiesel or diesel tree (Levingston and Zamora. 2006).

***Herbal Medicine***

Herbal medicine sometimes referred to as Herbalism or Botanical Medicine. It is the use of herbs for their therapeutic or medicinal value. Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value Traditional medicine is an important part of African cultures and local medicinal systems vary between different cultural groups and regions (Makhubu 2006) . Herbs are now very popular in developing countries on account of improved knowledge about the safety, efficacy and quality assurance of ethno- medicine. In recent years, secondary plant metabolites (phytochemical) have been extensively investigated as a source of medicinal agents. Thus, it is anticipated that phytochemical with good antibacterial activity will be used for the treatment of bacterial infections. Studies indicate that in some plants there are many substances such as peptides, tannins, alkaloids, essential oils, phenols, and flavonoids among others could serve as sources for antimicrobial production. These substances or compounds have potentially significant therapeutic application against human pathogens including bacteria, fungi and viruses. (Nostro *et al*., 2000).

The success of chemotherapy lies in the continuous search of new drugs to counter the challenges posed by resistant strains of micro organism ( Arora and Keur. 1999),The development of microbial resistance to the available antibiotics has led researchers to investigate the antimicrobial activity of medicinal plants (Bisignano *et al.* 1996). Antibiotic resistance has become a global concern (Westh *et al* 2004) as the clinical efficacy of many existing antibiotics is being threatened by the emergence of multi-drug-resistant pathogens (Bandow *et al,* 2003). Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for

the development of novel drugs because of the great diversity in theirchemical structure. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases .Therefore, researchers are increasingly turning their attention to ethno-medicine, looking for new leads to develop more effective drugs against microbial infections (Benkeblia, 2004);

This has led to the screening of several medicinal plants for potential antimicrobial activity (Colombo and Bosisio, 1996; Iwu *et al*., 1999).

Table 1 Zone of growth Inhibition (mm) Petroleum Ether Extract dis plays antifungal activity against Aspergillus flavusand Candida albicans.

| Organism | Concentration of extraction | | | Positive Control ketoconazole | Negative Control DMSO |
|---|---|---|---|---|---|
| | 50% | 25% | 12.5% | Nystatin | |
| ***Aspergillus flavus*** | 25 | 17 | Zero | 29 | ZERO |
| ***Candida albicans*** | 26 | 20 | 16 | 20 | Zero |

**Table 2** Zone of growth Inhibition (mm) of Methanol Extract dis plays antifungal activity against *Aspergillus flavus*and *Candida albicans*

| Organism | Concentration of extraction | | | Positive Control | Negative Control DMSO |
|---|---|---|---|---|---|
| | 50% | 25% | 12.5% | Ketoconazole and Nystatin | |
| ***Aspergillus flavus*** | 20 | Zero | Zero | 36 | Zero |
| ***Candida albican*** | Zero | Zero | Zero | 20 | Zero |

**Table 3** Zone of growth Inhibition (mm) Aqueous of leaves Extract dis plays antifungal activity against *Aspergillus flavus* and *Candida albicans*

| Organism | Concentration of extraction | | | Positive Control | Negative Control |
|---|---|---|---|---|---|
| | 50% | 25% | 12.5% | Ketoconazole and Nystatin | Distill water |
| ***Aspergillus flavus*** | Zero | Zero | Zero | 22 | Zero |
| ***Candida albican*** | Zero | Zero | Zero | 20 | Zero |

**Table 4** Water extracts of leaves was found

| Organism | Concentration of extraction | | | Positive Control | Negative Control |
|---|---|---|---|---|---|
| | 50% | 25% | 12.5% | Ketoconazole and Nystatin | Distill water |
| ***Aspergillus flavus*** | Zero | Zero | Zero | 22 | Zero |
| ***Candida albican*** | Zero | Zero | Zero | 20 | Zero |

**Table 5** serial dilution for antibiotic

| MIC of | Dilution | Mg per ml |
|---|---|---|
| Ketoconazole | 4 | |
| *Aspergillus flavus* | 5 | 0.31 |
| *Candida albicans* | 4 | 0.62 |

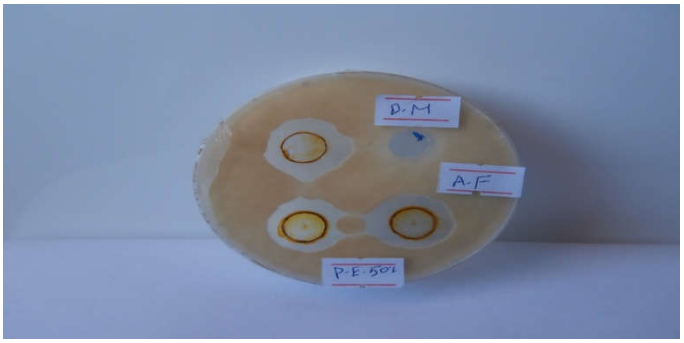


Plate (1)displays zone of growth Inhibition *Aspergillus flavus* using P.E.E. conc. 50% compared with the Control DM.

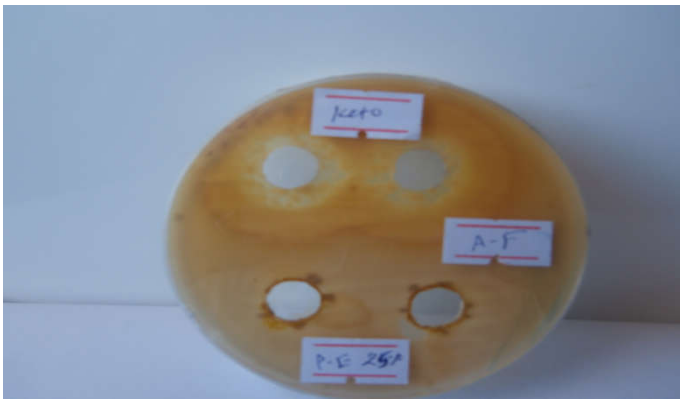


Plate (2) displays zone of growth Inhibition*Aspergillus flavus* using P.E.E. conc. 25% compared with the Control ketocanazole.

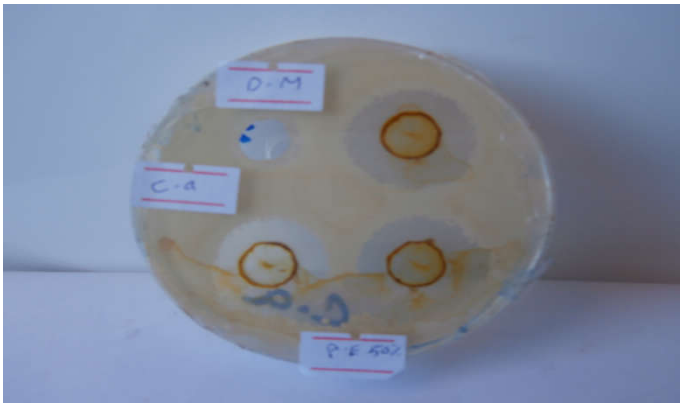


Plate (3) displays zone of growth Inhibition *Candida albican* using P.E.E. conc. 50% compared with the Control DM.

***Chemical Composition***

*Jatropha curcas*has high trypsin inhibitor and lectina activities, which could be inactivated by heat treatment. In addition, high concentration of the antimetabolic, metal-chelating and heat-stable factor, phytic acid, has been reported in *Jatropha curcas*meal (Makkar *et al*., 1998). Apart from these, phorbolesters that are present at high levels in the kernels have been identified as the main toxic agent responsible for toxicity (Makkar *et al*., 1998). After removing the toxic and heat-stable factors through solvent extraction, using 92% methanol, the extracted meal was found to be non-toxic to rats (Makkar and Becker, 1999). The defatted meal has been found to contain a high amount of protein, which ranged between 50% and 62%. Except for lysine, all other essential amino acids in *Jatropha curcas*meal protein have been reported to be in higher concentrations than those of the FAO reference pattern suggested for pre-school children (Makkar, *et al*., 1998). In addition to the more common toxic varieties, a non-toxic variety of *Jatrophacurcas .L.*seeds, that contained negligible amounts of phorbolesters, but similar levels of trypsin inhibitors, lectin activity and phytic acid compared to the toxic variety, has been reported from Papantla region of Veracruz State in Mexico. The non-toxic seed kernels are consumed by local people after roasting. The hydrothermally processed defatted meal of the non-toxic variety did not show any toxicity to rats. However, the growth rates of fish fed diets containing heated *Jatropha* meal were found to be lower than the unheated *Jatropha* meal group. (Makkar and Becker, 1999).Over 90% of the protein in *Jatropha* meal is in the form of true protein. (Makkar *et al*., 1998).

***Uses***

The fact that *Jatropha* oil cannot be used for nutritional purposes without re-detoxification makes its use as energy or fuel source very attractive as biodiesel. In Madagascar, Cape Verde and Benin *Jatropha* oil was used as mineral diesel substitute during the second word.

The wood and fruit of *Jatropha* can be used for numerous purposes including fuel.

The seed of *Jatropha* contains viscous oil, which can be used for manufacture of candle and soap, in cosmetics industry as a diesel/ paraffin substitute or extender. These characteristics along with its versatility make it of vital important to developing countries (Kumar and Sharma, 2008). Economic evaluation at the utilization of *Jatropha* seeds for soap making. Several cases of *Jatropha curcas* nut poisoning in humans after accidental consumption of the seeds have been reported with symptoms of giddiness, vomiting and diarrhea and in the extreme condition even death has been recorded (Makkar and Becker, 1997).

***Other Uses***

***Leaves***

The young leaves may be safely eaten, steamed or stewed. Cooked with goat meat, they are said to advantageously counteract its smell. Pounded leaves are applied near horses' eyes to repel flies in India. HCN (Hydrogen cyanide) is present in the leaves. The extracts of the plants are dangerous to use but water can easily release it over if not too much extract is applied.

***Flowers***

The species is listed as a honey plant. Contains HCN.

***Nuts***

Sometimes roasted and eaten, although they are purgative.
They can be burned like candlenuts when strung on grass. They alsocontain HCN.
They are used as a contraceptive in South Sudan.

***Seeds***

They were used as a contraceptive in South Sudan. The oil has been used for illumination, soap, candles, the adulteration of olive oil, and making Turkey red oil. Turkey red oil, also called sulphonated (or sulfated) castor oil, It the only oil that completely disperses in water. It is made by adding sulfuric acid to pure *Jatropha* oil. It was the first synthetic detergent after ordinary soap, as this allows easy use for making bath oil products. It is also used in formulating lubricants, softeners, and dyeing assistants. The seeds in the zone around Misantla, Veracruz are very appreciated by the population as food once they have been boiled and roasted. It is unclear if this is due to the existence of a non-toxic variety of *Jatropha* in Mexico and Central America, or if the seeds become edible once processed by cooking. It is also similarly reported that *Jatropha* seeds are edible once the embryo has been removed. Again it may be so because of these seeds coming from a local non-toxic variety.

**Roots:** Their ashes are used as a salt substitute. They containHCN and Rotenone.
**Bark:** Used as a fish poison.
**Latex:** Strongly inhibits the watermelon mosaic virus.
**Sap:** It stains linen. Sometimes used for marking.
**Shrub:** Mexicans grow the shrub as a host for the lac insect, which is used in medicine as hepatoprotective and antiobesity drug (Levingston and Zamora. 2006).

***Antimicrobial Activity***

Anti-microbialagent ***is*** a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoan. Antimicrobial drugs either kill microbe (microbiocidal) or prevent it is growth (micro biostatic).

Antibiotics are only those substances that are produced by one microorganism that kill, or prevent the growth, of another microorganism.

The term antibiotic is used to refer to almost any drug that attempts to rid our body of a bacterial infection. Antimicrobials include not just antibiotics, but synthetically formed compounds as well. The discovery of antimicrobials like penicillin and tetracycline paved the way for better health for millions around the world. Before penicillin became a viable medical treatment in the early 1940s, no true cure for gonorrhea, strep throat, or pneumonia. Patients with infected wounds often had to have a wounded limb removed, or face death from infection. Now, most of these infections can be cured easily with a short course of antimicrobials. However, with the development of antimicrobials, microorganisms have adapted and become resistant to previous antimicrobial agents. The old antimicrobial technology was based either on poisons or heavy metals, which may not have killed the microbe completely, allowing the microbe to survive, change, and become resistant to the poisons and/or heavy metals. Antimicrobial nanotechnology is a recent addition to the fight

against disease causing organisms, replacing heavy metals and toxins and may one day be a viable alternative

***Antifungals***

An antifungal drug is a medication used to treat fungal infections such as athlete's foot, ringworm, candidiasis (thrush), serious systemic infections such as Crypto cocci meningitis, and others. Antifungals work by exploiting differences between mammalian and fungal cells to kill off the fungal organism without dangerous effects on the host. Unlike bacteria, both fungi and humans are eukaryotesic .

***General Characteristic of test Organisms***

***Candidaalbicans***

It is the common human pathogen. Unfortunately, it is a common in mouth, vagina and gut. It is often found harmlessly on the skin. Occasionally, the fungus may cause disease in mouth, vagina and bowel, or rarely may be associated with septicaemia, endocarditis , meningitis and lung abscess. The fungus appears on Gram's stain as small oval thin walled yeast and sometimes budding. It is also appears like creamy, medium-size moist dull colonies when cultured on sabouraud dextrose agar. (Klich.,2007).The dimorphic yeast *Candida albicans* is recognized as an increasingly important human pathogen particularly in the host immunocompromised by advanced age, infection or immunosuppressive therapy. *Candidaalbicans It* is often found as a commensal organism in the gastrointestinal tract.(Vera,*et.al*.,2003).*Candida albicans* It continues to be the most common fungal pathogen and a major cause of high morbidity and mortality among immunocompromised patients (Zaoutis *et.al*.,2010). There are reports in the literature of *C. albicans* causingabscess in patients who are immunocompromised, diabeticindividuals, patients with cancer, or those who are on widespectrum antibiotic treatment (Vera *et .al.,*1998).

*Candida* and *Aspergillus species* are the most common agents responsible for invasive fungal infections (IFI) in children. They are associated with a high mortality and morbidity rate as well as high health care costs. Their incidence has dramatically increased within the past two decades (Filioti.*et.al*.2010). In children, invasive *Candida* infection (ICI) is five times more frequent than invasive *Aspergillus* infection (IAI). *Candida spp*. is the third most common agent implicated in healthcare-associated bloodstream infections in children (Richards *et.al*.,1999).(IAI) is more often associated with hematological malignancies andsolid tumors. Strong recommendations concerning prophylactic treatment for IAI have been published (Pappas.*et.al*.,2009). Although *Candida albicans*is still the main *Candida sp*. associated with ICI in children, a strong trend towards the emergence of *Candida* non-*albicans* has been observed. This could be linked to the use of fluconazoleprophylaxis in some patients (Neu.*et al.,*2009).

***Aspergillusflavus***

*Aspergillus flavus* is a fungus. It is a common mold in the environment, and can cause storage problems in stored grains. It can also be a human pathogen, associated with aspergillosis of the lungs and sometimes causing corneal, otomycotic, and nasoorbital infections. Many strains produce significant quantities of aflatoxin.Common clinical syndromes associated with *A. flavus* include chronic granulomatous sinusitis, keratitis, cutaneous aspergillosis, wound infections and osteomyelitis.*A. flavus* grows as a yellow-green mold in culture. Like other Aspergillus species it produces a distinctive conidiophore composed of a long stalk supporting an inflated vesicle. Conidiogenous cells on the vesicle produce the conidia. Many strains of *A. flavus*exhibit a greenish fluorescence under UV light that is correlated with levels of aflatoxin production. (Klich.2007).

*Aspergilli* are ubiquitous in nature and universal in distribution. The diverse Aspergilli group comprises human, animal and plant pathogens, apart from fungi with a plethora of industrial applications. *Aspergillus fumigatus, Aspergillus flavus* and *Aspergillus niger* are known to cause allergic reactions and allergic bronchopulmonary aspergillosis (ABPA) in immuno competent individuals. *A. fumigatus* represents a major cause of morbidity and mortality in the patients of Allergic bronchopulmonary aspergillosis (ABPA) (Dagenais and, Keller 2009). *A. fumigatus, A. flavus and A. niger* are also opportunistic human pathogens in immunocompromised patients such as post transplant cases, HIV etc. where the disease often leads to fatality (Tillie-Leblond and, Tonnel.2009). A number of novel allergens and antigens of diagnostic and therapeutic importance, multifunctional proteins and toxins have been identified and characterized from *Aspergillus* species, particularly from *A. fumigatus* The aflatoxin producing *A. flavus* and ochratoxin producing *A. ochraceus* are plant pathogens infamous for their ability to affect a wide variety of crops ( Bennett.2009).

Aflatoxins are a group of mycotoxins with potent toxicity andcarcinogenicity toward mammals. They are produced by somestrains of *Aspergillus flavus, Aspergillus parasiticus Aspergillus nomius* and *Aspergillus tamarii.* They can befound as contaminants in a wide variety of food and feed commodities (Cottyand Jaime-Garcia.,2007).

***Objectives***

The main Objective of the present work is

To establish well documented baseline information about the antifungal activity of leaves of *Jatropha crucas.L.*

***Specific Objectives***

1. Examination of leaves of *Jatrophcruces.L* using petroleum ether, methanol and water extraction.
2. Evaluation of*in vitro* antifungal activity of *Jatrophcruces.L* against *Candida albicans* and *Aspergillus flavus.*
3. Comparison such as activity of the extracts with the activity of known antifungal like Nystatine and Ketoconazole.

***Rationale***

Decreases the high cost of antifungals drugs, particularly in resource limited communities has led to the increased use of plants as an alternative for treatment of infectious diseases.

## MATERIALS AND METHODS

***Chemicals***

Methanol, Petroleum ether, Peptone water, dimethyl sulphoroxide, (Manufacturered by LOBA CHEMIE. PVT. LTD. India) were used.

***Tested Organism***

***Standard Fungal Organisms***

*Aspergillus flavus(* ATCC 9763)

*Candida albicans*( ATCC 7596)

ATCC: American Type Culture Collection, Rockville, Maryland, USA.

The organisms were obtained from soba Veterinary Research Institute Department of Mycology.

***Culture Media***

1. Peptone waterwas used for Sub Culture *Candidaalbicans*
2. Sabouraud Dextrose Brothwas used for Sub Culture *Asperigellusflavus*
3. Sabouraud dextrose agarwas used for Sensitivity test using agar well diffusion method.

***Antifungal Agent***

Nystatine, Ketoconazole from General Medicine Co. LTD
They were used to compare their activity with *Jatrophacurcas* extraction .

Nystatine (Broad spectrum antifungal used to treat Candidiasis and Ketoconazole was used to treat aspergillosis)

***Plant Material***

The plant *jatropha cruces* was collected from Khartoum university faculty of agriculture.

Leaves were thoroughly washed with distilled water to remove dirt and soil and then dried under shade and coarsely powdered.

***Methods***

***Preparation of Crude Extracts***

Extraction was carried out according to method described by (Harborne, 1984):

***Petroleum Etherextract***

Hundred grams of the coarsely powdered leaves were exhaustively extracted for four hours with petroleum ether (bp-60-80C®) in a Soxhlet apparatus. Petroleum ether ◌extractewas evaporated with a Rota- vapor under reduced pressure. The extractwas air-dried,then repacked in Soxhelt and wasextracted with methanol for six hours. The methanol extractedby the same method.

***Preparation of Different Concentration***

Prepared three concentrations (12.5%, 25%, and 50%).for Petroleum ether and methanol extract
For 50% concentration of bothextract taking 2 gram was dissolvedin 4ml of dimethyl sulphoroxide (DMSO) .from concentrate (50%) taking 1 ml and dissolved in 1ml of dimethyl sulphoroxide (DMSO) to prepared concentrate (25%). From concentrate (50%) taking 1 ml and dissolved in 2ml of dimethyl sulphoroxide (DMSO) to prepared concentrate (12.5%).

The final Solution was kept in refrigerator until used.

***Aqueous Extract***

Aqueous Extract was prepared by adding 50 ml of distilled water to 5 grams of the leaves of the coarsely powdered in a conical flask with occasional shakingin awater bath (60 C) for 5mints . ◌The aqueous extract was then filtered through sterile filter paper .

Three concentrations (12.5%, 25% and 50%). Were made by adding 4ml from aqueous filtered (stock) to 4ml of distilled water to prepared (50%). From concentrate (50%)1 ml was taken and dissolved in 1ml distilled water to prepared (25%). And from concentration (50%) 1ml was taken and dissolved in 2ml of distilled water toprepared (12.5%).

***Preparation of Culture Medium***

Peptone water was prepared by dissolving 0.75 grams of powder in 50 ml of distilled water and distributed in tubes which closed by cotton , autoclave at 121ºC for 15mint.( Cheesbrough,1985).

***Sabouraud Dextrose agar***

Sabouraud dextrose agar with chlorophenicol was used for the maintenance of fungi, was prepared by dissolving 3.9 g of the powder in 100ml distilled water, autoclaved at 121ºC for 15mint and thencooled. Chlonphenicol was added for inhibition of growth of bacteria under flame. The medium was then distributed into sterile Petri dishes, allowed to solidified and kept at 4c® tilluse.( Cheesbrough,1985).

***Preparation of Stock Drug Solution (ketoconazole)***

To prepare stock drug solution (1280mg/liter) 50 ml of inorganic solvent suchas dimethylsulophoxide wasadded to 64 mg of ketoconazole compound and allow to stand for 30 mg. The permit self-sterilization stock solution dispensed in small amount and stored at (-70)c◦ ( Warnock ,1989).

***Preparation of the test Organisms***

***Preparation of Standard Fungal Culture***

The fungal culturewasobtained from Sabouraud dextrose agar slope culture and incubated at 28ºC for 24 hours and then storedtill use (Cheesbrough, 1985).

***In vitro*Testing for antifungal activity *of Jatropha curcas***

Flowing the agar well diffusion method (Moshi *et al*., 2006),aseptically about one colony from each type of fungi culture was inoculated in Peptone water medium and then was incubated at 28ºC overnight, the growth of fungi in Peptone water medium. was immerged sterile swab in Peptone water culture was make striking on surface of Sabouraud dextrose agar "one side of swab used for one plates for each type of fungi and then was incubated on room temperature for half hour. Ware making four wells by core borer (10mm of diameter) in each plates cultured of fungi. By used micropipette about 0.1ml for each concentration of extracts distributed in to two well for each fungal cultured and allowed to diffuse at room temperature for two hour. The plates were then incubated in the upright position at 28ºC for 24 hour. After incubation period the diameters of results growth inhibition zones were read and the average were measured, and mean values were tabulated.

***MIC Determination agar Diffusion Method***

***Test Method***

9ml of dimethylsulophoxide Solution wereadded to each of sterile universal tube numberedfrom (1-6).Was added 1ml of Stock solution was added to tube no. (1) mixwell and was transferred 1ml to tube no.(2).

Repeat this serial dilution though to tube no. (6) Discard 1ml from tube no.(6).Prepared medium ( Sabouraud dextrose agar)

when was medium solidified was placed the plates at37c◦and inoculated with pathogen and was make three wells by core borer(10mm of diameter). Was full each well with 0.1ml of the sterilized suspension of each tube number (1-6) and allowed to diffuse at room temperature for two hour. The plates were then incubated in the upright position at 28ºC for 24 hour. The MIC was then read the lowest drug concentration at which there is no visible fungal growth. (Warnock,1989)

## RESULT

In the Present Study the petroleum ether , methanol and aqueous of leaves of *Jatropha cruces L.* extract were subjected to preliminary screening for their antifangal activity against Aspergillus flavusand Candida albicanscompared with the control (Ketoconazole, Nystatin as positive control and Dimethylesulphoroxid as negative control). Aspergillus flavus and Candidaalbicans.

At concentration 50% was found (sensitive) tothe petroleum etherextract of leaves of Jatropha Curcas.L.Where (25and26)mm of Inhibition zone was recorded.

The concentration of 25% also showed activity toCandida albicanssimilar to nystatin (20) mm and moderate activity (sensitive) against Aspergillus flavus (17)mm. Where concentration of 12.5% showed same activity (sensitive) for Candida albicans, (16)mm and inactivity against for a *spergilusflavus.* Table (1) plat The Methanol extract of leaves at the concentrations of 50% , 25% and 12.5% was found inactive against *Candida albicans*, but it showed same activity against *Aspergillus flavus* where a zone of (*20*)mm at concentration of 50% was recorded compared with the control ketoconazole (36)mm.Table (2) Water extracts of leaves was found inactive against *Aspergillus flavus* and *Candida albicans* at all concentrations used compared with the control Ketoconazole (22)mm and Nystatin (20)mm. Table (Minimums concentration inhibition of *Jatrophacrucas* by petroleum ether extract against *Aspergillus flavus* was achieveddilutions five and against Candida albican was dilation at four and the MIC of control ketoconazole against Aspergillus flavus was reached at dilution four. Jatrophcruscas high active against Aspergillus flavus.

## DISCUSSIONS

Antifungal and antimicrobial activities of extracts from parts of Jatropha species have been reported (Aiyelaagbe*et al*.,2000).moderate antifungal activity against Candida albicans by hexane, chloroform, and methanol extracts from roots of Jatropha podagrica at a concentration of20,000 mg/l. ( Kumar *et al*..2006) reported that 500 mg/l crudeextract from leaves of Jatropha gossypifolia L. completely inhibited eight microorganisms: Bacillus cereus var. mycoides,B. pumilus, B. subtilis, Bordetella bronchiseptica, Staphylococcus epidermidis, Klebsiella pneumoniae, Streptococcus faecalis, and Candida albicans.

It has been known that parts of J. curcas can be used fora wide range of purposes. Extracts from various parts of J.curcas, such as seeds, seed oil, and leaves, have shownmolluscicidal, insecticidal, and fungicidal properties (Solsoloy, 1997) J. curcas extracts were found to be able toinhibit the mycelial growth of Colletotrichum musae that causes anthracnose disease in bananas ( Thangavelu.et.al.,2004) Its leaf extract was effective in controlling the fungal pathogen Sclerotium

This study was demonstrated that the extractby petroleum ether, methanol, aqueous water from J. curcas leaves has fungal activities against important fungal Aspergillus flavus and Candida albicans. The Organisms ware inhibited by the same concentration of Extraction petroleum ether rather than methanol and Distell water; these indicate the presence of active Ingredients in the petroleum ether Extraction than extract in methanol and Distell water.

In the present study, petroleum ether extract of jatrophacurcasleaves showed high activity against Candida albicansthis finding is almost near to finding of ( Aiyelaagbe*et al*.,2000 )but not J. curcas. Where exteract from root of Jatropha podagrica. Showed same activity against Candidaalbicansusing other organic solvent. But( Kumar *et al*..2006) reported complete inhibition of Candida albicansusing of leaves of Jatropha gossypifolia crude extract.

In conclusion the petroleum ether from J. curcas leaves would serve as a natural antifungal against Aspergillus flavus and Candida albicans. Foragricultural applicationsat a low cost and safe practice. However, more work is required in the isolation *and characterization of the active ingredient.*

*Recommendations*

*More studies should be carried to determine the active ingredients of Jatropha curcas Leaves.*
*Further work should be done on other fungi.*
*In vivo sensitivity test should be done on experimental mice.*

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