April 30, 2019

PharmacologyOnLine

Archives • 2019 • vol.1 • 117-135

ANTIFUNGAL PROPERTIES OF METHANOLIC LEAF EXTRACT OF *ANACARDIUM OCCIDENTALE L* (CASHEW) AGAINST FUSARIAL ISOLATES FROM HUMAN AND PLANT ORIGIN

Udoh, Iniekong Philip[1*]; Aladenika, Seto Tunrayo[1]; Eleazar, Clara Idara[2]; Onyebueke, Ebere Adaze[1]; Azubuike, Nkiruka Chinonyelum[1]; Okwuosa, Chukwugozie Nwachukwu[1]; Ibezim, Nnenna Ekpereka[3]; Esimone, Charles Okechukwu[4]

[1]Department of Medical Laboratory Sciences, Faculty of Health Sciences and Technology, College of Medicine, University of Nigeria, Enugu Campus.

[2]Department of Microbiology, Faculty of Biological Sciences, University of Nigeria, Nsukka.

[3]Department of Computer Science and Robotics Education, University of Nigeria, Nsukka.

[4]Depertment of Pharmaceutical Microbiology and Biotechnology, Nnamdi Azikiwe University.

Email address: iniekong.udoh@unn.edu.ng

Abstract

Anacardium occidentale L (Anacardiaceae) widely use in folk medicine for its anti-fungal properties. In the present study, we screened methanolic leaf extract of *Anacardium occidentale* (Cashew) for antifungal activities against fusarial isolates from humans and plants. The methanolic leaf extract was screened against 1265 fusarial isolates from various mycological specimens of both male and female patients seen at different hospitals and 2,140 fusarial isolates from various plants sources from different markets in Enugu State. The fusarial isolates were also screened against various conventional antifungal agents (miconazole, ketoconazole, terbinafine, fluconazole, ciclopirox, voriconazole, and itraconazole) using modified agar disc diffusion method, while susceptibility to methanol leaf extract of the plants (*A. occidentale*) was by modified agar well diffusion method. The antifungal susceptibility test of fusarium isolates to crude methanolic leaf extracts of *Anacardium occidentale* (Cashew) revealed that, the plant leaf extract showed potent antifungal activity against both human and plants fusarial isolates tested. The mean inhibition zone diameter (IZD) of the extract against isolates from both human and plant sources were 25.75±0.46 and 24.23±0.85respectively, while that of vericonazole (most potent) was 15.48±0.69 and 15.24±0.70 for human and plant respectively. This was followed by fluconazole with mean IZD of 13.76±0.78 and 13.99±0.81 for human and plant respectively. However multi-resistance was observed among other antifungal agents tested. There was significant difference between the activity of the extract and that of antifungal agents (P = 0.000). The lowest MIC for *A. occidentale* was 0.0820 µg/ml while that of antifungal agent was < 1µg/ml. The IZD and MICs results further proved that extract has potent antifungal activity. The inhibition zone diameter and the MICs of fusarial isolates to methanol extracts of *A. occidentale* leaf revealed that the extract showed potent antifungal activity against the fusarial isolates tested.

Keywords: *Anacardium occidentale, antifungal, fusarium, susceptibility, Medicinal*

Introduction

Since antiquity medicinal plants are well-known natural sources of remedies for the treatment of various diseases. There is increased patronage of phytomedicinal products all over the world in recent times, hence medicinal herbs are regarded as effective alternatives to the orthodox medicine[1,2].

Potent antifungal and antibacterial chemotherapeutic agents may be obtained from medicinal herbs which represent a rich source. Medicinal plants researches have yielded many valuable drugs and almost on a daily basis new compounds with biological activity from plants are recovered [3]. The use of medicinal plants in the world, and especially in Nigeria contributes significantly to primary health care.

The incidence of fungal infections has increased tremendously as a result of more aggressive treatment for other conditions (for instance use of immunosupresssants, transplantations and the use and abuse of antibiotics), the increased incidence of leukemia, lymphoma and AIDS; greater knowledge of clinical mycology; and the greater accuracy of diagnostic techniques[4-6].

Infections caused by opportunistic fungi are becoming more common as a result of increased used of cytotoxic therapies to treat a variety of disease syndromes. Fusarium, a genus of fungi belonging to the phyllum Ascomycota, class Ascomycetes, order Hypocreales, family Hypocreaceae is most often associated with plant diseases and often causes superficial or subcutaneous infections that become disseminated in heavily immunocompromised individuals, frequently causing death. Fusarium species are therefore increasingly implicated as the causative agents of human mycoses particularly in the immunocompromised patient populations, as well as important plant pathogens [7]. Hence certain groups of Fusarium also exhibit a predilection toward human and plant diseases, a unique characteristic among the fungi [8].

Fusarium infections are an important problem all over the world [9]. Certain fungal species, including *Fusarium* species rarely cause disease but are considered emerging pathogens [9,10]. Since many of the data concerning such infections are gathered from small series or individual cases, it is difficult to evaluate the predisposing factors, natural history, clinical course, treatment and prognosis of these patients. *Fusarium* species are molds that have a worldwide distribution and are found mainly as saprophytic organisms in soil as plant pathogens or as saprophytes on plant debris. *Fusarium* causes serious morbidity and mortality in humans. It has been documented that farmers are mostly infected by fusarial mycoses. There is presently high resistance of fusarial isolates to conventional antifungal agents.

Fusarium species causes serious morbidity and mortality in humans. It has been documented that farmers are mostly infected by fusarial mycoses. There is presently high resistance of fusarial isolates to conventional antifungal agents. The pathogenic relationship between human and plant isolates of *Fusarium* species have not been documented. It has not also been known if human and plant derived isolates of *Fusarium* are equally susceptible to antifungal agents or have similar susceptibility profile. Despite increasing reports of life threatening *Fusarium* infections, little is known about its pathogenesis and management. Treatment of *Fusarium* infections remains difficult due to their resistance to antifungal drugs and as such treatment of disseminated fusariosis is plagued by high failure rates. It is therefore imperative that extensive efforts be made to catalogue the susceptibility/resistance profiles of fusarial isolates from Eastern Nigeria, to currently use antifungal agents; and to uncover new antifungal remedies from alternative (herbal) sources.

Fusarium species are often resistant to antifungal agents, hence development of new antimicrobial compounds for resistant fungi are absolutely

necessary [11-14]. There is therefore need to develop new, cheap and alternative antimicrobial drugs from plant sources for the treatment of infectious diseases to supplement the ever-increasing cost of quality modern drugs. One approach is to screen local medicinal plants for possible antimicrobial properties [15]. Additionally, the search for new and cheaper alternative antimicrobial medicine is imperative whether it is as part of an herbal medicine or as a component of western medicine [16].

The knowledge of medicinal plants and their roles in the treatment of diseases is as old as man. They constitute a predominant mode of managing health problems in developing countries and mostly among the rural populace [17,18]. Herbal medicine is generally believed to be more effective but with fewer side effects compared to synthetic medicines. Many times, this has led to indiscriminate use without appropriate dose resulting in abuse [19]. Various ethnic groups have reported incidences of adverse effects and sometimes life-threatening conditions of these herbal remedies [20,21]. Hence it of vital importance to determine the toxicity profile of medicinal plants even though they have been used for ages to enable scientific documentation on their safety/risk potentials.

Anacardium occidentale L (Anacardiaceae) is believed to possess antifungal properties and is used traditionally for the treatment of several infections by the native of Northeast coast of Brazil [22]. The plant was taken from Brazil to India and East Africa, where it soon became naturalized.

Cashew is a small and evergreen multipurpose tree of the Amazon that grows up to 12-15 m high. It has a thick and tortuous (short), often irregularly shaped trunk with branches so winding that they frequently reach the ground. The leaves are spirally arranged, leathery textured, elliptic to obovate, 4 to 22 cm long and 2 to 15 cm broad, with a smooth margin. The flowers are produced in a panicle or corymb up to 26 cm long, each flower small, pale green at first then turning reddish, with five slender, acute petals 7 to 15 mm long. Cashew trees are often found growing wild on the drier sandy soils in the central plains of Brazil and are cultivated in many parts of the Amazon rainforest and also in Nigeria.

Anacardium occidentale L leaf tea is prepared as a mouthwash and gargles for mouth ulcers, tonsillitis, and throat problems and is used for washing wounds. An infusion and/or maceration of the bark are used to treat diabetes, weakness, muscular debility, urinary disorders, and asthma. The leaves and/or the bark is also used in Brazil for eczema, psoriasis, scrofula, dyspepsia, genital problems, and venereal diseases, as well as for impotence, bronchitis, cough, intestinal colic, leishmaniasis, and syphilis-related skin disorders. North American practitioners use cashew for diabetes, coughs, bronchitis, tonsillitis, intestinal colic, and diarrhea, and as a general tonic[22].

Fungal infections constitute major global challenge. *Fusarium* infections worldwide are an important problem [6]. Certain fungal species, including *Fusarium* species rarely cause disease but are considered emerging pathogens [8,9].

Fusarium species cause a broad spectrum of infections, including superficial infections, such as keratits and onychomycosis, as well as locally invasive and disseminated infections; invasive and disseminated infections occur almost exclusively in severely immunocompromised patients.

Since many of the data concerning such infections are gathered from small series or individual cases, it is difficult to evaluate the predisposing factors, natural history, clinical course, treatment and prognosis of these patients. There is therefore yeaning need to screen and harness medicinal plants for antifungal application. In the present study *Anacardium occidentale L* leaf was screened for antifungal activities and it potential use

for the treatment of various fungal infections was evaluated to authenticate the claims in folk medicine.

Aims of Present Study

To evaluate the Antifungal properties of methanolic Leaf extract of *Anacardium occidentale L* (Cashew) in Enugu state, South Eastern Nigeria

Specific objectives of the present study include:

1. To determine the antifungal properties of methanolic Leaf extract of *Anacardium occidentale L* (Cashew)
2. To determine the *in vitro* antifungal susceptibility profile of the various *Fusarium* species isolated to various conventional antifungal agents
3. Exploiting alternative ways of treating *Fusarium* infection using herbal preparations.

Methods

Collection of samples

One thousand two hundred and sixty-five isolates of *Fusarium* species isolated from different clinical mycological specimens (corneal scrapings, skin and nail clippings) obtain from different hospitals and two thousand one hundred and forty various symptomatic plants samples (tomato seedlings (*Lycopersicum esculentum*), palm fruit (*Elaeis guineensis*), sweet potato (*Ipomoea batatas*), Irish potato (*Solanum tuberosum*), banana (*Musa sapientum*), plantain (*Musa paradisiacal*), pawpaw (*Carica papaya*), avocado pear (*Persea Americana*), water-melon (*Citrullus lanatus*), and pepper (*Capsicum chinense*) within Enugu, Enugu State, Nigeria. Ethical approval for the study was obtained from the Ethical Committee of the Department of Pharmaceutics, University of Nigeria, Nsukka. The isolates were confirmed by standard mycological methods [23].

Fresh Leaves of *Anacardium occidentale* L used for this study were collected from Agulu community, Anambra State, Nigeria. The plant species were identified by a taxonomist in the Department of Botany, University of Nigeria Nsukka. Voucher specimens have been deposited at the Herbarium of the Department of Pharmacognosy, U.N.N.

Preparation of Plant Extract

The leaves sample were washed thoroughly with running tap water and once with sterile water shade- dried for seven days and further dried in an oven regulated at 40°C. The dried leaves were reduced to a coarse powder with an electric grinder and kept in an airtight container prior to solvent extraction [24].

Exactly 750 g portion of the powder was macerated in a total of 2 L of 95 % methanol and was kept over an orbital shaker at 150 rpm for 48 hours until exhaustively extracted at room temperature. The methanol extract was filtered with Whattman No. 1 filter paper, the residue discarded, and the filtrate evaporated yielding solid extract. The extract was also placed under UV rays for 24 hours for sterilization and after which checked for sterility by streaking a sample suspention on sabouraud' dextrose agar plate. The yield, which was 44.2 grams, was stored in sterile screw capped container in a refrigerator (4° C) until needed [25].

Percentage yield of the extract was calculated thus

$$\% \text{ yield} = \frac{\text{Mass of dried extract}}{\text{Mass of powdered leaf extract}} \times 100$$

Phytochemical tests

A preliminary phytochemical analysis of *Anacardium occidentale* L leaves extract was carried out and involved testing for the presence of the following secondary metabolites: tannins, alkaloids, flavonoids, saponins, glycosides, resins, steroids and triterpenes, oils, proteins and carbohydrates using modified methods of Harbourne [26] and Evans [27].

Test Microorganisms

Fusarium species isolated from various mycological specimens of both male and female patients seen at different hospitals and various plants sources from different markets in Enugu State

Maintenance and Standardization of Stock Cultures

Stock cultures of all isolates were stored in Sabouraud dextrose agar slant supplemented with 2% glucose at 4°C. Prior to use, the *Fusarium* isolates were freshly sub-cultured onto Sabouraud dextrose agar and incubated at 28°C for 7-10 days [23].

Susceptibility testing of fusarial isolates to Extract

Susceptibility testing was by modified agar well diffusion technique [25,28,29]. A small portion of the above extract was evaporated to dryness by heating on a water bath and the weight of the extract determined. The weighed methanol extract was dissolved in 2mls dimethyl sulfoxide (DMSO) and the resulting solution diluted to a concentration of 2mg/ml using sterile distilled water.

A sterile Mueller- Hinton Agar supplemented with 2% glucose and 0.5µg of methylene blue per ml (MH- MB) at a depth of 4mm were used. The agar surface was inoculated by using a swab dipped in a isolates cells suspension adjusted to the turbidity of a 0.5 McFarland standard and was spread evenly over the surface. The agar (19.9mls each) was seeded with 0.1ml of standardized inocula (broth cultures of fusarium) and was allowed to set. A total of 5 wells (8mm diameter each) were bored in the agar using a sterile cork -borer. Two drops (about 0.04ml) of 5mg/ml concentration of the extract were carefully placed into each of the wells. Two drops of 2 fold diluted DSMO was put in the centre well as control. The plates were left for 1hour at room temperature for proper diffusion of the extract before incubating at 28°C for 24, 48 and 72hours. Triplicate determinations were made and the average zone of inhibition diameters (IZD) of the extract recorded after the incubation.

Antifungal Susceptibility Testing with standard antifungal agents

Antifungal susceptibility of isolates was established by the modified Agar disc diffusion method of Lass-Flo¨rl *et al.* [30] using single tablet of the following antimicrobial susceptibility test tablets: miconazole 10 µg (MICOZ), ketoconazole 15 µg (KETOC), terbinafine 30 µg (TERBI), fluconazole 25 µg (FLUCZ), ciclopirox 50 µg (CICLO), voriconazole 1 µg (VORI), itraconazole 8 µg (ITRAC), manufactured by Rosco Diagnostica A/S, Taastrupgaardsvej 30, DK-2630 Taastrup Denmark.

Agar disc Diffusion Method

Preparation of Standard Inoculum

The inocula were prepared by overlaying mature slants with sterile saline and gently scraping the surface with a plastic pipette tip. The resultant suspension was drawn up with a sterile Pasteur pipette. The suspensions were then filtered once through sterile gauze to remove hyphae. The inocula were then adjusted with Mueller- Hinton broth supplemented with 2% glucose and 0.5 µg of methylene blue to a 0.5 McFarland standard, providing an inoculum concentration of 1x 10^6 conidia/ml (CFU /ml), which was verified by colony count using improved neubauer counting chamber.

A sterile Mueller- Hinton Agar supplemented with 2 % glucose and 0.5 µg of methylene blue per ml (MH- MB) at a depth of 4mm was used. The agar surface was inoculated by using a swab dipped in a cell suspension adjusted to the turbidity of a 0.5 McFarland standard and was spread evenly over the surface. The antifungal tablets were aseptically placed and gently pressed on the agar. Incubation was at 28°C for 72 hours. Inhibition zone diameters (IZD) of active antifungal drugs were measured and an interpretative chart was used to interpret the

results, in order to determine the agent(s) most suitable for use in antifungal therapy.

MICs of Conventional Antifungal Agents

The MICs of the antifungal agents for *Fusarium* species were calculated from disk zone diameter measurements using standard method of Pfaller, *et al*. [31], where the zone diameter in millimeters from the continuous agar gradient around each disk, which was calibrated with MICs, determined from the standard CLSI M27-A2 broth dilution method by balanced-weight regression analysis. Even though the interpretative breakpoints for *Fusarium* species, have not yet been established, that of Candida species according to Pfaller, *et al*. [31] was used.

MICs of Plant Extract

Plots of logarithmic concentration of the plant extract against the square of inhibition zone diameters (IZD^2) for the fusarial isolates were performed. From the intercept of these graphs on the y- axis, the corresponding MICs of the isolates were obtained.

Statistical Analysis

Data were collected, computed and analyzed statistically using SPSS, version 15.0. Analysis of variance (ANOVA) formulas by Post Hoc Test using Tukey HSD and Games - Howell comparisons were used for multiple value comparisons, while Chi-square tests by cross tabulations were used for Correlations. The correlation tests were also carried out to determine the Pearson coefficient (r) value. Students T-test was used for frequency calculations.

The Probability values were determined by, 0.05 and 0.01 significance level, at 95 % and 99 % confidence limits respectively.

Results

Antifungal Screening Tests of Medicinal Plant Extract Anacardium occidentale (Cashew)

Antifungal susceptibility of selected fusarium isolates to crude methanolic leaves extract of *Anacardium occidentale* (Cashew) revealed that the extract showed potent antifungal activity against the *Fusarium* species tested. The activity of the extract was better than those of the conventional antifungal agents (Fig 1).The percentage yields of the methanol extracts of *Anacardium occidentale* leaves was 5.8%

Phytochemical analysis

This present work revealed that the active compounds include: alkaloids, saponins, terpenoids phenols, steroids, volatile oils, anthraquinones, flavonoids, tannins, cardiac glycosides and reducing sugar.

Susceptibility of Isolates to Antifungal agents Tested

The antifungal susceptibility patterns of all the *Fusarium* species isolated were tested to selected antifungal agents and the sensitivity rates are shown in Figure 2. Voriconazole showed activity against the highest proportion of the fusarial isolates (58.0 % and 64.8 % for humans and plants respectively). Distribution of activity of other antifungal agents against the *Fusarium* isolates from human and plant sources include fluconazole (49.2 % and 53.6 %), itraconazole (46.4 % and 42.4 %), ketoconazole (43.2 % and 38.0 %), miconazole (36.4 % and 38.0 %), Ciclopirox (17.2 % and 12.0 %) and terbinafine (5.6 % and 3.2 %) respectively. Terbinafine recorded the highest resistant rate for human and plant isolates (94.4 % and 96.8 % respectively).

Minimum Inhibitory Concentrations (MICs) of Conventional Antifungal agents

The minimum inhibition concentration results (Table 1) revealed that voriconazole had the lowest MIC (≤ 1 µg/ml) followed by fluconazole (≤ 8 µg/ml).

MICs of Plant Extract

The plant extract used in this study showed lower minimum inhibitory concentrations than the conventional antifungal agents. These results

showed that the MICs of *A. occidentale* extract was better than those of the conventional drugs. Figs 5A - 5C shows plots of logarithmic concentration of the plant extract against the square of inhibition zone diameters (IZD^2) for human fusarial isolates. However, Figs 6A – 6C represents those of plant isolates to the extract. The lowest MIC for *Anacardium occidentale* leaf extract for the human isolates was 0.0820 µg/ml while those of the plant isolates was 0.0809µg/ml. The corresponding MICs are presented in Fig. 3 and 4 for human and plant isolates respectively. Also the mean IZD of *A. occidentale* against fusarial isolates from human and plant sources was higher compared to other conventional antifungal drugs used(Table 2)

Discussion

The methanol extract of the leaf sample of *Anacardium occidentale* L. was efficacious against all the test organisms with zones of inhibition ranging from 12.0-36 mm. This is in line with the work of Vijayakumar and Kalaichelvan [29], where the acetone extracts of the leaf sample of *Anacardium occidentale* L. was also active against all the test organisms with zones of inhibition ranging from 12.0-28 mm.

The mean inhibition zone diameter (IZD) of the extract against isolates from both human and plant sources were 25.75±0.46 and 24.23±0.85 respectively, while that of vericonazole (most potent) was 15.48±0.69 and 15.24±0.70 for human and plant respectively. This was followed by fluconazole with mean IZD of 13.76±0.78 and 13.99±0.81 for human and plant respectively. This findings further confirmed the potency of *Anacardium occidentale* L.

The active compound present in the plant extract in this study were also mentioned by previous researchers [32,33]. It could be inferred that any of these compounds could be responsible for the efficacious antifungal activity against the considered human and plant pathogenic fusarial strains.

There was a statistically significant difference between the activity of the extract and that of the conventional antifungal agents used (P = 0.000). The significant differences could be due to constant use and abuse of these antifungal agents for human and veterinary purposes. Therefore, the possibility of emergence of resistance to these antifungal agents is not uncommon.

The result of the antifungal sensitivity showed that all species of *Fusarium* tested were more susceptible to voriconazole than other antifungal agents assuming proposed interpretive breakpoints of ≥ 17 mm (susceptible) and ≤ 13 mm (resistant) for voriconazole and ≥ 19 mm (susceptible) and (≤ 14 mm (resistant) for other drugs. This findings is in line with previous studies [34-37], where it was reported that *Fusarium* species responded successfully when treated with voriconazole. Fluconazole was next to voriconazole in activity against the various isolates from humans and plants. This was followed by itraconazole and ketoconazole. Terbinafine was the least sensitive followed by ciclopirox and miconazole. The inhibition of some *Fusarium* species by itraconazole is not consistent with the work of Prajna Lalitha *et al.* [38] and Thomas [39] where none of the *Fusarium* species, was inhibited by itraconazole. No activity *in vitro* of terbinafine was detected against most of the isolates of *Fusarium species*. The observed poor activity of terbinafine confirmed previous report of Prajna Lalitha *et al.* [38], where *Fusarium* species were completely ineffective against terbinafine.

Voriconazole activity was significantly higher than those of other drugs (P = 0.000), with the exception of fluconazole and itraconazole where the differences (P = 0.720 and P = 0.730 respectively), were not significant. The activity of ketoconazole was significantly higher than that of ciclopirox and terbinafine with p = 0.014 and p = 0.001 respectively, same also applies to itraconazole with p = 0.000 for both ciclopirox and terbinafine. Miconazole also had a similar trend with p = 0.003

and p = 0.000 respectively. More so the activity of fluconazole was significantly higher than that of ciclopirox and terbinafine with p = 0.000 for both. Ciclopirox and terbinafine had no statistically significant difference (p = 0.993) in their activities. There was also no statistically significant difference between the activities of itraconazole and miconazole (p = 0.902).

This is consistent with previously repoted works, where voriconazole yielded relatively low MICs for *Fusarium* species(40-44). Other workers further confirmed voriconazole as promising antifungal agent in the treatment of *Fusarium* infections, with MIC values of 0.25- 4 µg/ml [45,46]. *Fusarium* species demonstrating high minimum inhibitory concentration (MIC) values for fluconazole, (MIC > 8 µg/ml) in previous findings [45,46], is not in line with the present study were the MIC was ≤ 8 µg/ml. The relatively high MIC values recorded by other antifungal agents tested are in conformity with the work of previous workers [38, 40, 47].

This present work has shown that plant extract studied is a potential good source of antifungal agents and demonstrates the importance of this plant in medicine and in assisting primary health care in this part of the world. Since this herb appear to have a broad spectrum of action from previous studies and in the present study, it could be useful in antiseptic/ disinfectant formulations and in chemotherapy.

Further purification of *Anacardium occidentale* leaf extract can improve its activity. Possible synergistic or additive interactions between the extract and some commonly used antifungal agents, could be explored.

The secondary metabolites present should be separated individually and tried singly to establish the degree of antifungal activity each possesses. Structural/ chemical elucidation of the antifungal components of the fraction will also be necessary.

The preliminary data presented in this work tenders a strong empirical evidence of the potential applicability of medicinal plants as substitutes in the treatment of antifungal resistant fungal infections.

References

1. Duarte MCT, Figueira GM, Sartoratto A, Rehder VLG, Delarmelina C. Anti-Candida activity of Brazilian medicinal plants. *Journal of Ethnopharmacology*. 2005 Feb;97(2):305–11.
2. Alves TM, Silva AF, Brandão M, Grandi TS, Smânia ED, Smânia Júnior A, Zani CL. Biological screening of Brazilian medicinal plants. *Memórias do Instituto Oswaldo Cruz*. 2000 Jun;95(3):367-73.
3. Sandhu DS, Heinrich M. The use of health foods, spices and other botanicals in the Sikh community in London. *Phytotherapy Research*. 2005;19(7):633–42.
4. Pasqualotto AC, Denning DW. Diagnosis of Invasive Fungal Infections – Current Limitations of Classical and New Diagnostic Methods. European Oncology & Haematology [Internet]. Touch Digital Media, Ltd.; 2005;1. Available from: http://dx.doi.org/10.17925/eoh.2005.0.0.1p
5. Alexander BA. Diagnosis of fungal infection: new technologies for the mycology laboratory. Transplant Infectious Disease [Internet]. Wiley; 2002 Sep;4(s3):32–7. Available from: http://dx.doi.org/10.1034/j.1399-3062.4.s3.5.x
6. LaRocco MT, Burgert SJ. Fungal infections in the transplant recipient and laboratory methods for diagnosis. Revista iberoamericana de micologia. 1997 Dec;14:143-6
7. Nir-Paz R, Strahilevitz J, Shapiro M, Keller N, Goldschmied-Reouven A, Yarden O, et al. Clinical and Epidemiological Aspects of Infections Caused by Fusarium Species: a Collaborative Study from Israel. Journal of Clinical Microbiology [Internet]. American Society for Microbiology; 2004 Aug 1;42(8):3456–61. Available from:

http://dx.doi.org/10.1128/jcm.42.8.3456-3461.2004

8. O'Donnell K, Sutton DA, Rinaldi MG, Magnon KC, Cox PA, Revankar SG, et al. Genetic Diversity of Human Pathogenic Members of the Fusarium oxysporum Complex Inferred from Multilocus DNA Sequence Data and Amplified Fragment Length Polymorphism Analyses: Evidence for the Recent Dispersion of a Geographically Widespread Clonal Lineage and Nosocomial Origin. Journal of Clinical Microbiology [Internet]. American Society for Microbiology; 2004 Nov 1;42(11):5109–20. Available from: http://dx.doi.org/10.1128/jcm.42.11.5109-5120.2004
9. Fleming RV, Walsh TJ, Anaissie EJ. Emerging and less common fungal pathogens. Infectious Disease Clinics of North America [Internet]. Elsevier BV; 2002 Dec;16(4):915–33. Available from: http://dx.doi.org/10.1016/s0891-5520(02)00041-7
10. Marr KA, Carter RA, Crippa F, Wald A, Corey L. Epidemiology and Outcome of Mould Infections in Hematopoietic Stem Cell Transplant Recipients. Clinical Infectious Diseases [Internet]. Oxford University Press (OUP); 2002 Apr;34(7):909–17. Available from: http://dx.doi.org/10.1086/339202
11. Sampathkumar P, Paya CV. Fusarium Infection after Solid-Organ Transplantation. Clinical Infectious Diseases [Internet]. Oxford University Press (OUP); 2001 Apr 15;32(8):1237–40. Available from: http://dx.doi.org/10.1086/319753
12. Torres HA, Bodey GP, Rolston KVI, Kantarjian HM, Raad II, Kontoyiannis DP. Infections in patients with aplastic anemia. Cancer [Internet]. Wiley; 2003 Jun 18;98(1):86–93. Available from: http://dx.doi.org/10.1002/cncr.11478
13. Consigny S, Dhedin N, Datry A, Choquet S, Leblond V, Chosidow O. Successful Voriconazole Treatment of Disseminated Fusarium Infection in an Immunocompromised Patient. Clinical Infectious Diseases [Internet]. Oxford University Press (OUP); 2003 Jul 15;37(2):311–3. Available from: http://dx.doi.org/10.1086/375842
14. Leggiadro RJ. EMERGING DRUG-RESISTANT BACTERIA: THE WAKE-UP CALL HAS COME. Southern Medical Journal [Internet]. Southern Medical Association; 1995 Aug;88(8):884–6. Available from: http://dx.doi.org/10.1097/00007611-199508000-00025
15. Ahmad I, Mehmood Z, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. Journal of Ethnopharmacology [Internet]. Elsevier BV; 1998 Sep;62(2):183–93. Available from: http://dx.doi.org/10.1016/s0378-8741(98)00055-5
16. Martini N, Eloff J. The preliminary isolation of several antibacterial compounds from Combretum erythrophyllum (Combretaceae). Journal of Ethnopharmacology [Internet]. Elsevier BV; 1998 Oct;62(3):255–63. Available from: http://dx.doi.org/10.1016/s0378-8741(98)00067-1
17. Sandhu DS, Heinrich M. The use of health foods, spices and other botanicals in the Sikh community in London. Phytotherapy Research [Internet]. Wiley; 2005;19(7):633–42. Available from: http://dx.doi.org/10.1002/ptr.1714
18. Gupta MP, Solís PN, Calderón AI, Guionneau-Sinclair F, Correa M, Galdames C, et al. Corrigendum to "Medical Ethnobotany of the Teribes of Bocas del Toro, Panama" [Journal of Ethnopharmacology 96 (2005) 389–401]. Journal of Ethnopharmacology [Internet]. Elsevier BV; 2005 Apr;98(1-2):221. Available from: http://dx.doi.org/10.1016/j.jep.2005.01.001
19. Mbaka G. Evaluation of Haematinic Activity and Subchronic Toxicity of Sphenocentrum jollyanum (Menispermaceae) Seed Oil. European Journal of Medicinal Plants

[Internet]. Sciencedomain International; 2011 Jan 10;1(4):140–52. Available from: http://dx.doi.org/10.9734/ejmp/2011/333

20. Elvin-Lewis M. Should we be concerned about herbal remedies. Journal of Ethnopharmacology [Internet]. Elsevier BV; 2001 May;75(2-3):141–64. Available from: http://dx.doi.org/10.1016/s0378-8741(00)00394-9
21. Chan K. Some aspects of toxic contaminants in herbal medicines. Chemosphere [Internet]. Elsevier BV; 2003 Sep;52(9):1361–71. Available from: http://dx.doi.org/10.1016/s0045-6535(03)00471-5
22. Taylor L. The healing power of rainforest herbs: A guide to understanding and using herbal medicinals. New York: Square One Publishers; 2005.
23. Cheersbrough, M. District Laboratory Practice in Tropical Countries Part 2. 2nd ed. Cambridge University Press. 2006. 234-247.
24. Purkayastha S, Dahiya P. Phytochemical screening and antimicrobial activity of some medicinal plants against multi-drug resistant bacteria from clinical isolates. Indian Journal of Pharmaceutical Sciences [Internet]. OMICS Publishing Group; 2012;74(5):443. Available from: http://dx.doi.org/10.4103/0250-474x.108420
25. Mbaka GO, Adeyemi OO. Toxicity study of ethanol root extract of Sphenocentrum jollyanum (Menispermaceae) Pierre. Asian J Exp Biol Sci. 2010;1(4):869-74.
26. Paterson. Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis. . J. B. Harborne. 15 x 23.4 cm, 302 pp. London: Chapman & Hall, 1988. Price f79. ISBN 0-412-57260 (hardback). Plant Pathology [Internet]. Wiley; 1999 Feb;48(1):146–146. Available from: http://dx.doi.org/10.1046/j.1365-3059.1999.00318.x
27. Evans CW. Trease and Evans, Mexican medicinal plants. J. Ethnopharmacol, Pharmacognosy, 13th Edn. Bailliere Tindall, London. 1989
28. Esimone CO, Adikwu MU. Susceptibility of some clinical isolates ofStaphylococcus aureus to bioactive column fractions from the lichenRamalina farinacea (L.) Ach. Phytotherapy Research [Internet]. Wiley; 2002;16(5):494–6. Available from: http://dx.doi.org/10.1002/ptr.963
29. Doss VA, Thangavel KP. Antioxidant and Antimicrobial Activity Using Different Exracts of *Anacardium occidentale L. International Journal of Applied Biology and Pharmaceutical Technology.* 2011 July; 2: (3):436-443
30. Lass-FlÃ¶rl C, Perkhofer S, Mayr A. In vitro susceptibility testing in fungi: a global perspective on a variety of methods. Mycoses [Internet]. Wiley; 2010 Jan;53(1):1–11. Available from: http://dx.doi.org/10.1111/j.1439-0507.2009.01813.x
31. Pfaller MA, Diekema DJ, Rinaldi MG, Barnes R, Hu B, Veselov AV, et al. Results from the ARTEMIS DISK Global Antifungal Surveillance Study: a 6.5-Year Analysis of Susceptibilities of Candida and Other Yeast Species to Fluconazole and Voriconazole by Standardized Disk Diffusion Testing. Journal of Clinical Microbiology [Internet]. American Society for Microbiology; 2005 Dec 1;43(12):5848–59. Available from: http://dx.doi.org/10.1128/jcm.43.12.5848-5859.2005
32. Mbaka G, Adeyemi O, Ogbonnia S, Noronha C, Okanlawon O. The protective role of Sphenocentrum jollyanum (Pierre) root ethanol extract on alloxan -induced diabetic rabbits. Planta Medica [Internet]. Georg Thieme Verlag KG; 2009 Jul;75(09). Available from: http://dx.doi.org/10.1055/s-0029-1234764
33. Tedong L, Dimo T, Djomeni Dzeufiet PD, Asongalem AE, Sokeng DS, Callard P, et al. Antihyperglycemic and renal protective activities of Anacardium occidentale (anacardiaceae) leaves in streptozotocin

34. induced diabetic rats. African Journal of Traditional, Complementary and Alternative Medicines [Internet]. African Journals Online (AJOL); 2005 Dec 15;3(1). Available from: http://dx.doi.org/10.4314/ajtcam.v3i1.31136
35. Madhavan M, Ratnakar C, Veliath AJ, Kanungo R, Smile SR, Bhat S. Primary disseminated fusarial infection. Postgraduate Medical Journal [Internet]. BMJ; 1992 Feb 1;68(796):143–4. Available from: http://dx.doi.org/10.1136/pgmj.68.796.143
36. Chai SJ, Jackson BE, Wilhelmus KR. In vitro effects of moxifloxacin and voriconazole in inhibiting the growth of Fusarium. Investigative Ophthalmology & Visual Science. 2007 May 10;48(13):2679- 2679
37. Vincent AL, Cabrero JE, Greene JN, Sandin RL. Successful Voriconazole Therapy of Disseminated Fusarium Solani in the Brain of a Neutropenic Cancer Patient. Cancer Control [Internet]. SAGE Publications; 2003 Sep;10(5):414–9. Available from: http://dx.doi.org/10.1177/107327480301000511
38. Consigny S, Dhedin N, Datry A, Choquet S, Leblond V, Chosidow O. Successsful Voriconazole Treatment of Disseminated Fusarium Infection in an Immunocompromised Patient. Clinical Infectious Diseases [Internet]. Oxford University Press (OUP); 2003 Jul 15;37(2):311–3. Available from: http://dx.doi.org/10.1086/375842
39. Lalitha P, Shapiro BL, Srinivasan M, et al. Antimicrobial Susceptibility of Fusarium, Aspergillus, and Other Filamentous Fungi Isolated From Keratitis. Archives of Ophthalmology [Internet]. American Medical Association (AMA); 2007 Jun 1;125(6):789-793 Available from: http://dx.doi.org/10.1001/archopht.125.6.789
40. Thomas PA. Current Perspectives on Ophthalmic Mycoses. Clinical Microbiology Reviews [Internet]. American Society for Microbiology; 2003 Oct 1;16(4):730–97. Available from: http://dx.doi.org/10.1128/cmr.16.4.730-797.2003
41. Marco F, Pfaller MA, Messer SA, Jones RN. In vitro activity of a new triazole antifungal agent, Sch 56592, against clinical isolates of filamentous fungi. Mycopathologia. 1998 Feb 1;141(2):73-7.
42. Espinel-Ingroff A. In Vitro Fungicidal Activities of Voriconazole, Itraconazole, and Amphotericin B against Opportunistic Moniliaceous and Dematiaceous Fungi. Journal of Clinical Microbiology [Internet]. American Society for Microbiology; 2001 Mar 1;39(3):954–8. Available from: http://dx.doi.org/10.1128/jcm.39.3.954-958.2001
43. Kappe R. Antifungal activity of the new azole UK-109, 496 (voriconazole). Mycoses [Internet]. Wiley; 1999 Dec;42:83–6. Available from: http://dx.doi.org/10.1111/j.1439-0507.1999.tb00019.x
44. Clancy CJ, Nguyen MH. The combination of amphotericin B and azithromycin as a potential new therapeutic approach to fusariosis. Journal of Antimicrobial Chemotherapy [Internet]. Oxford University Press (OUP); 1998 Jan 1;41(1):127–30. Available from: http://dx.doi.org/10.1093/jac/41.1.127
45. McGinnis MR, Pasarell L, Sutton DA, Fothergill AW, Cooper Jr CR, Rinaldi MG. In vitro activity of voriconazole against selected fungi. Medical Mycology [Internet]. Wiley; 2008 Jul 25;36(4):239–42. Available from: http://dx.doi.org/10.1111/j.1365-280x.1998.00153.x
46. Arikan S, Lozano-Chiu M, Paetznick V, Rex JH. In Vitro Susceptibility Testing Methods for Caspofungin against Aspergillus and Fusarium Isolates. Antimicrobial Agents and Chemotherapy [Internet]. American Society for Microbiology; 2001 Jan 1;45(1):327–30. Available from: http://dx.doi.org/10.1128/aac.45.1.327-330.2001
47. Rotowa NA, Shadomy HJ, Shadomy S. In Vitro Activities of Polyene and Imidazole

48. Antifungal Agents Against Unusual Opportunistic Fungal Pathogens. Mycoses [Internet]. Wiley; 1990 Apr;33(4):203–11. Available from: http://dx.doi.org/10.1111/myc.1990.33.4.203
49. Johnson EM, Szekely A, Warnock DW. In-vitro activity of voriconazole, itraconazole and amphotericin B against filamentous fungi. Journal of Antimicrobial Chemotherapy [Internet]. Oxford University Press (OUP); 1998 Dec 1;42(6):741–5. Available from: http://dx.doi.org/10.1093/jac/42.6.741

Fig 1. Comparison of Mean Inhibition Zone Diameter of Conventional Antifungals and Crude Extracts of *A. occidentale* to *Fusarium* species

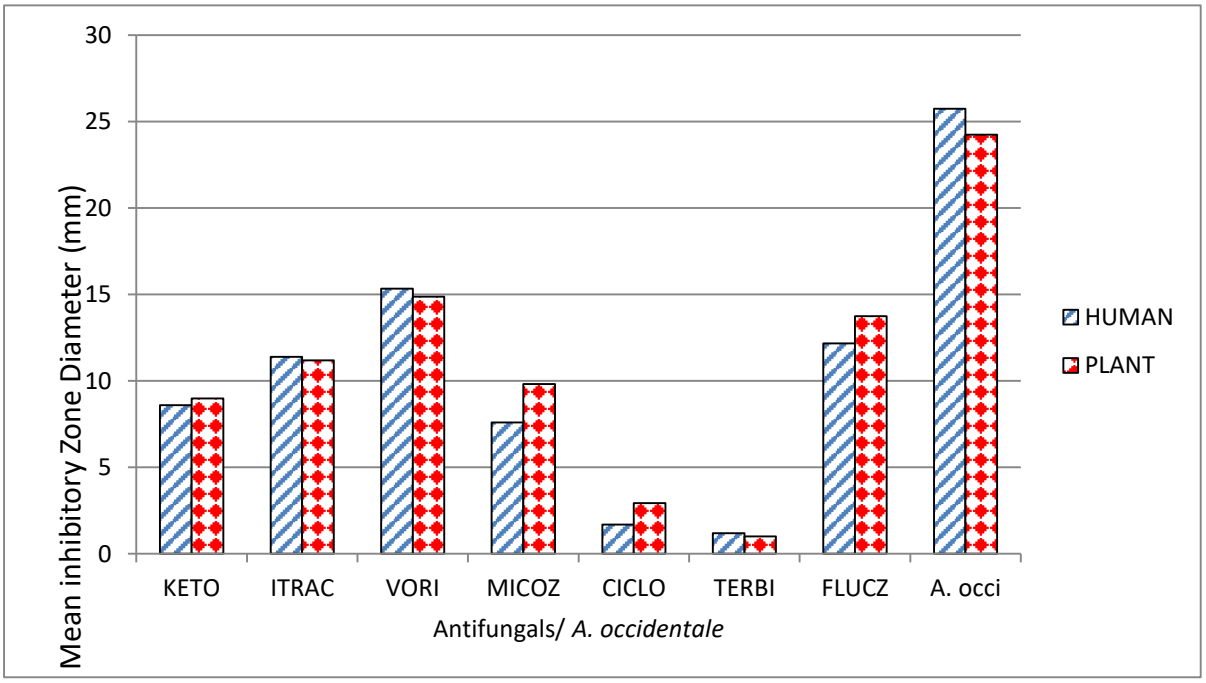

Fig 2. Antifungal Susceptibility of *Fusarium* species Isolated from Human and Plant Sources to Conventional Antifungals

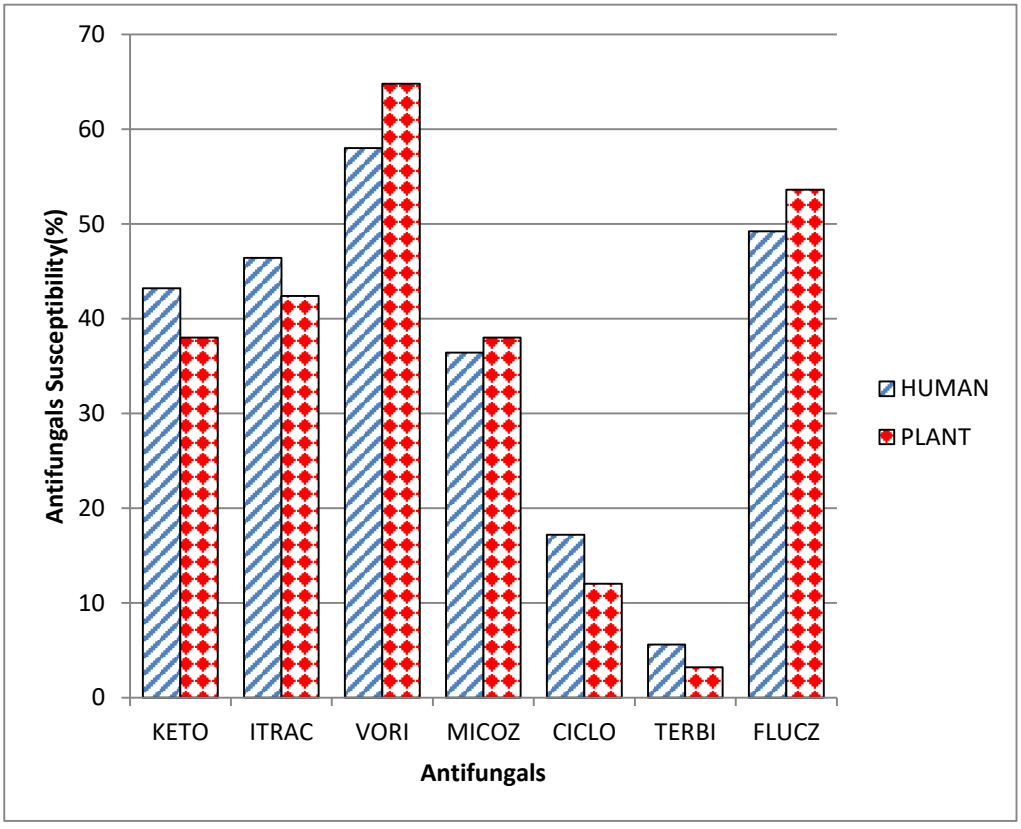

Fig 3. Minimum Inhibitory Concentrations (MICs) of Plant Extract to fusarial isolates from Human

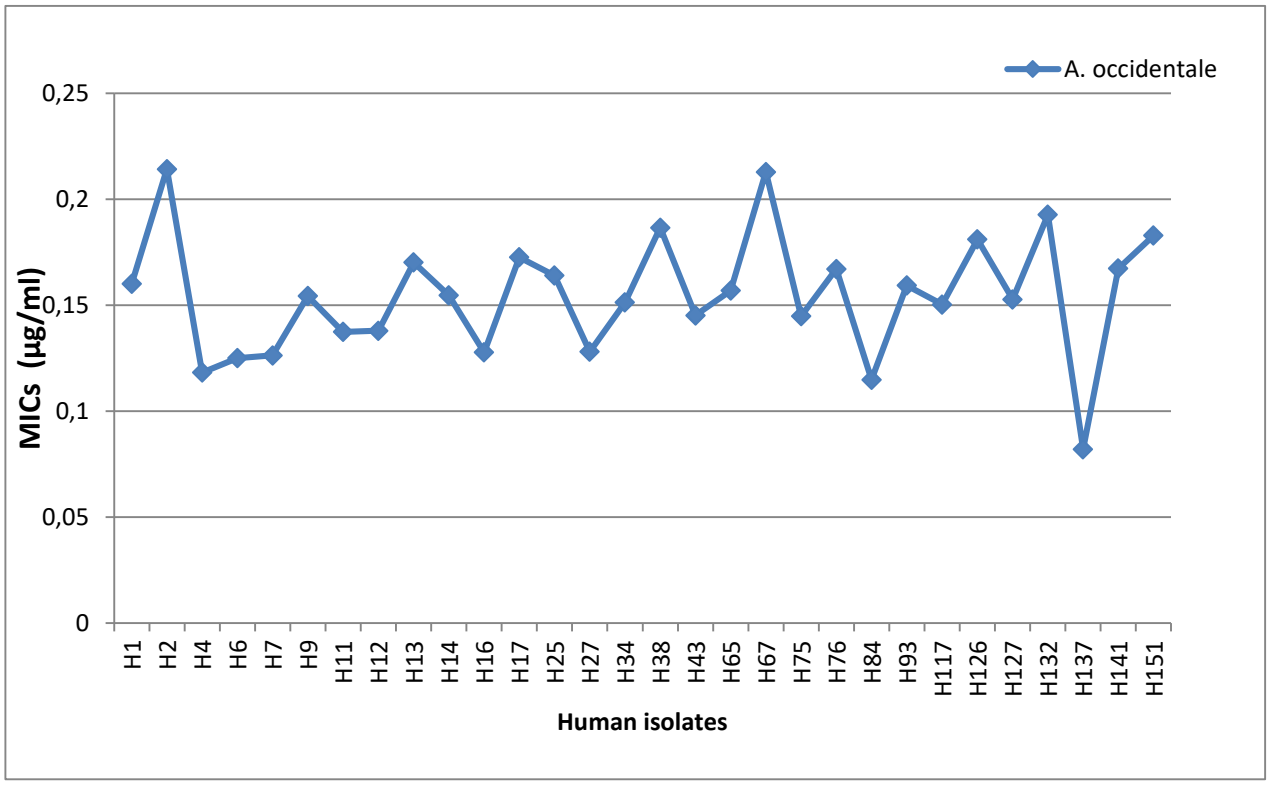

Fig 3B. Minimum Inhibitory Concentrations (MICs) of Plant Extract to fusarial isolates from Plant

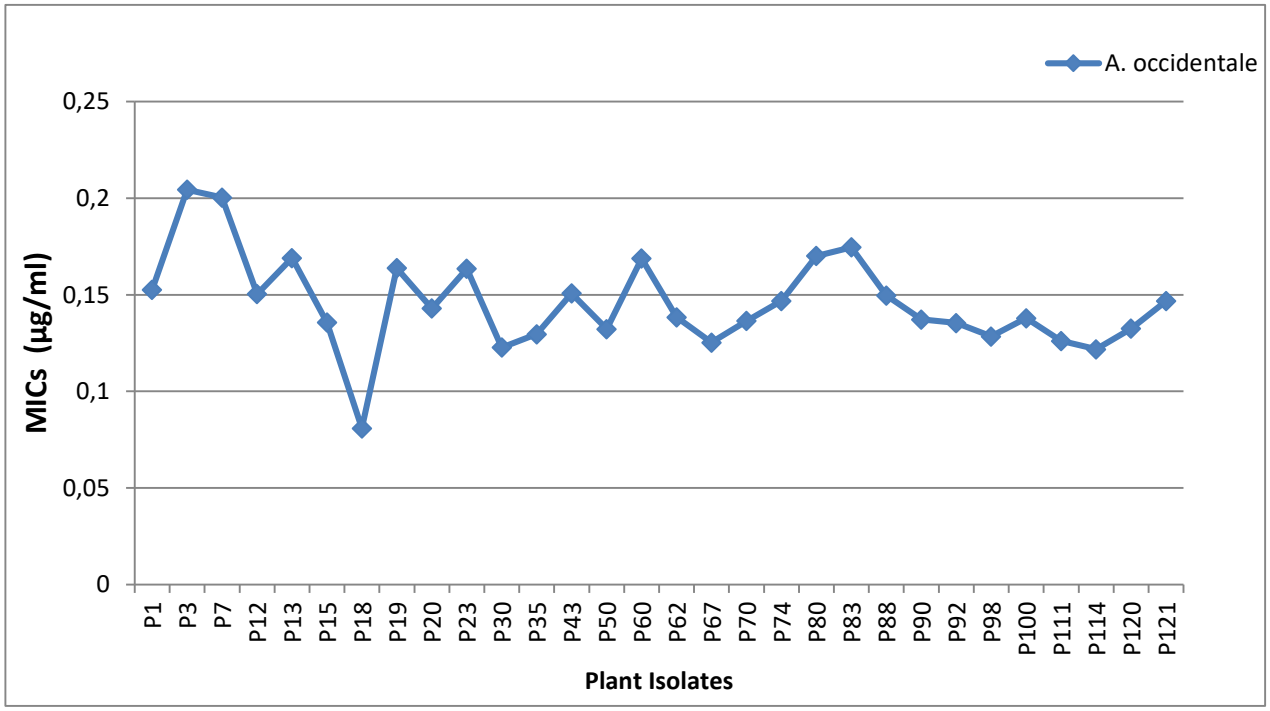

Fig 5A. Log Conc. of Plant Extract vs. IZD² for Human isolates H1 – H14

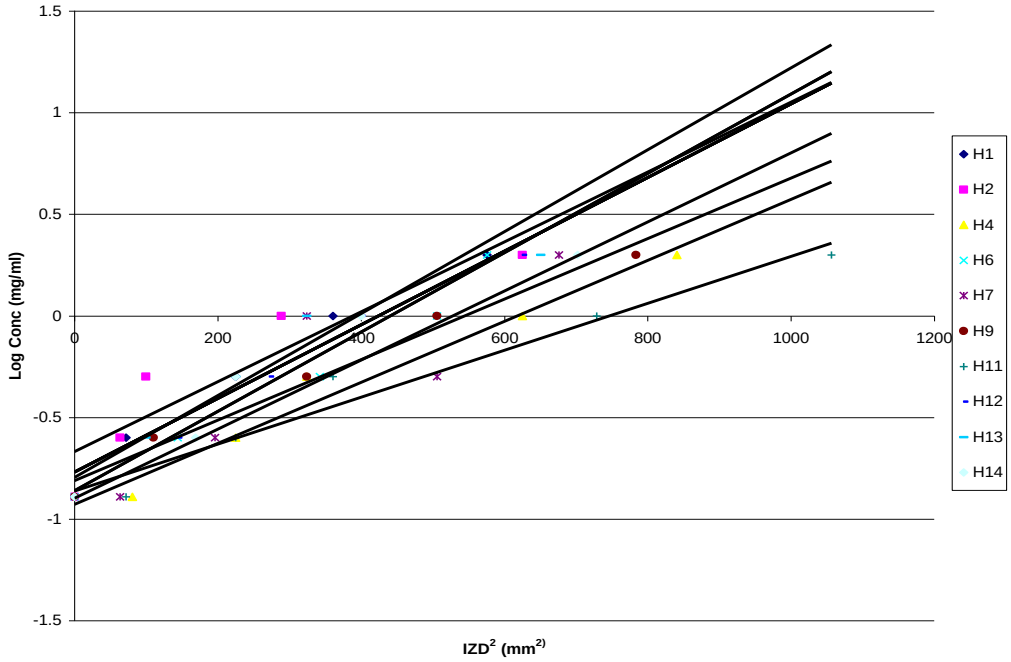

Fig 5 B. Log Conc. of Plant Extract vs. IZD² for Human isolates H16 – H75

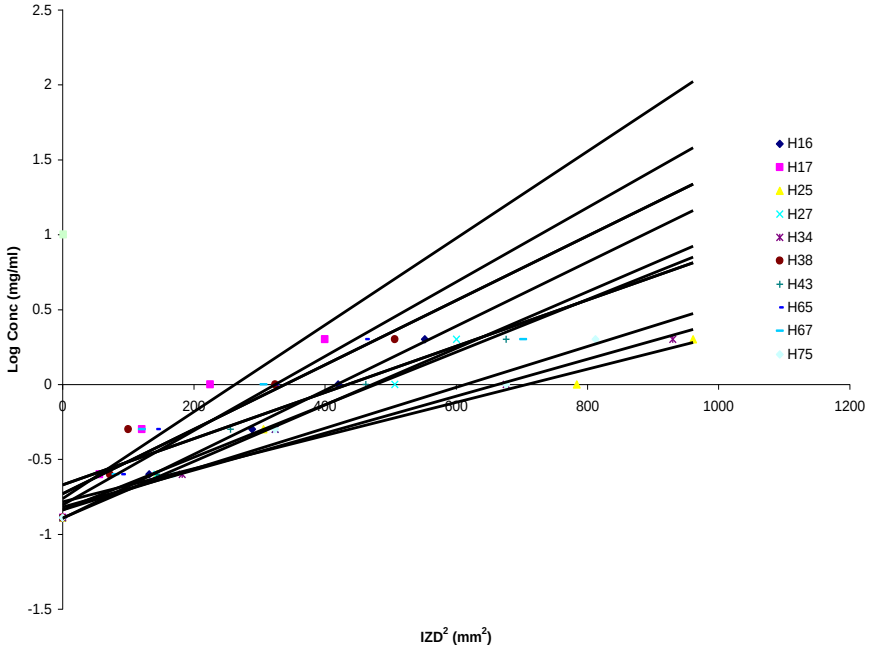

Fig 5C. Log Conc. of Plant Extract vs. IZD² for Human isolates H76 – H151

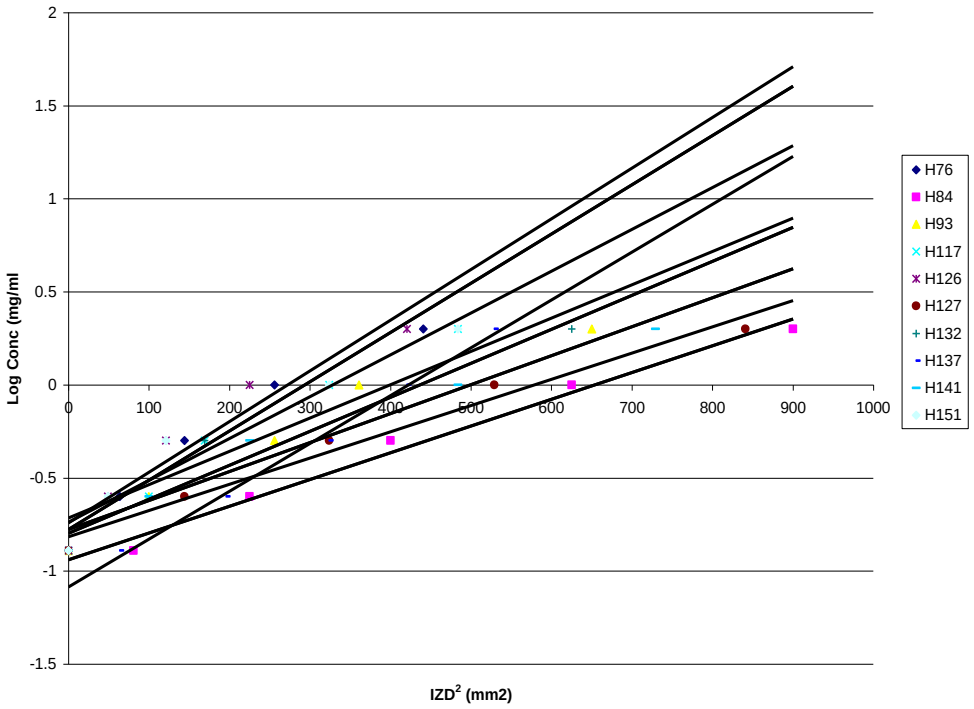

Fig 6A. Log Conc. of Plant Extract 1 vs. IZD² for Plant isolates P1 – P23

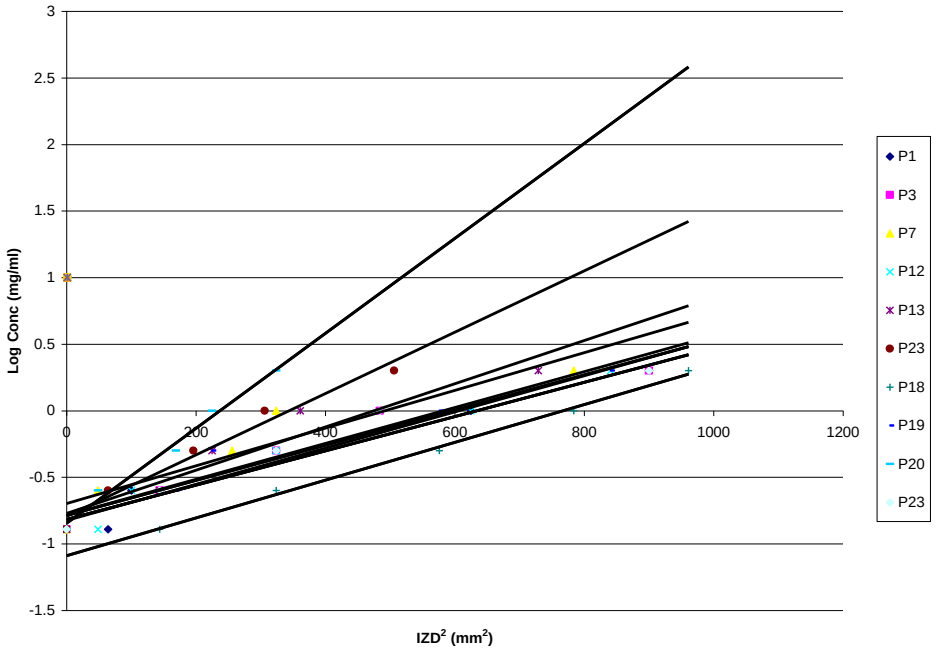

Fig 6B. Log Conc. of Plant Extract 1 vs. IZD² for Plant isolates P30 – P80

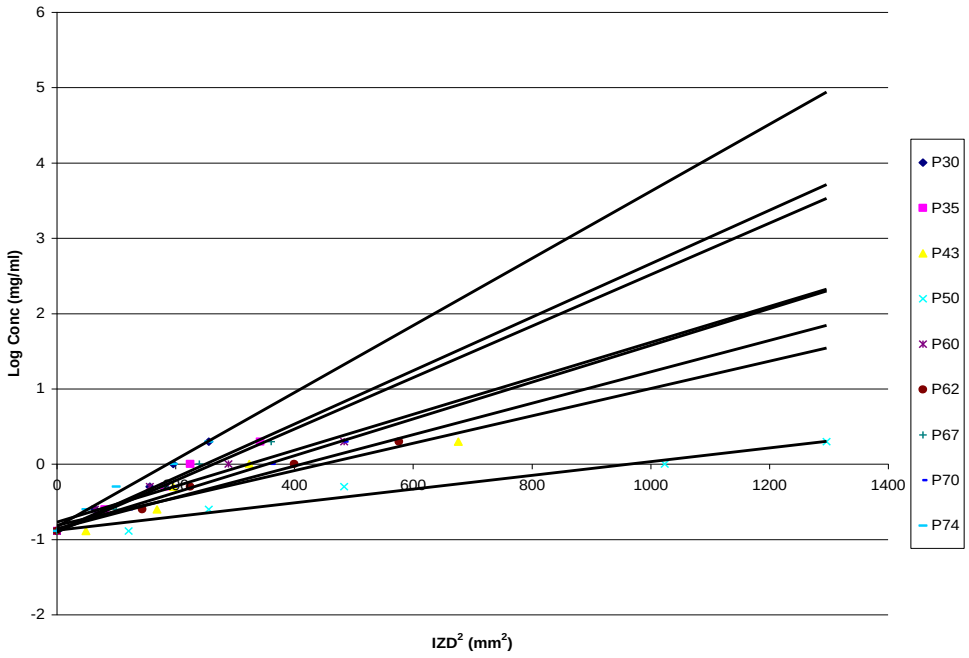

Fig 6C. Log Conc. of Plant Extract 1 vs. IZD² for Plant isolates P83 -p121

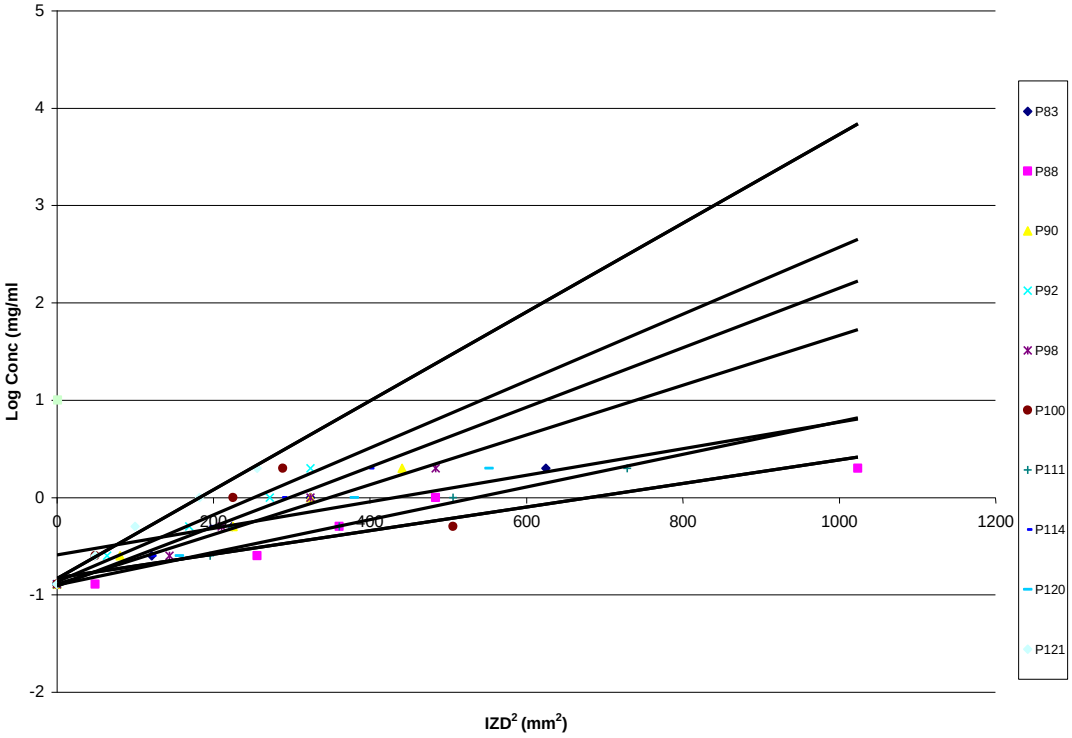

Table 1. Minimum Inhibitory Concentrations (MICs) of Antifungal agents (µg/ml) to Human Fusarial Isolates

	MICs (µg/ml)						
Isolates	KETO	ITRAC	VORI	MICOZ	CICLO	TERBI	FLUCZ
1	≤ 8	≤ 8	≤ 1	≤ 8	≥ 64	≥ 64	≤ 8
2	≤ 8	≤ 8	≤ 1	≤ 8	≥ 64	≥ 64	≤ 8
3	≤ 8	16	≤ 1	≤ 8	≥ 64	≥ 64	≤ 8
4	32	16	≤ 1	16	≥ 64	≥ 64	≤ 8
5	16	≤ 8	≤ 1	16	≥ 64	≥ 64	≤ 8
6	≤ 8	≤ 8	≤ 1	≤ 8	≤ 8	≥ 64	≤ 8
7	≥ 64	≥ 64	≥ 4	≥ 64	≥ 64	≥ 64	≥ 64
8	16	≤ 8	≤ 1	16	≥ 64	≥ 64	≤ 8
9	≤ 8	16	≤ 1	16	≥ 64	≥ 64	≤ 8
10	≤ 8	≤ 8	≤ 1	16	≤ 8	16	≤ 8
11	≥ 64	≥ 64	≥ 4	≥ 64	≥ 64	≥ 64	≥ 64
12	≥ 64	≥ 64	≥ 4	≥ 64	≥ 64	≥ 64	≥ 64
13	≤ 8	≤ 8	≤ 1	16	≥ 64	≥ 64	≤ 8
14	≥ 64	≥ 64	≥ 4	≥ 64	≥ 64	≥ 64	≥ 64
15	≥ 64	≥ 64	≥ 4	≥ 64	≥ 64	≥ 64	≥ 64
16	≥ 64	≥ 64	≥ 4	≥ 64	≥ 64	≥ 64	16
17	≥ 64	≥ 64	≤ 1	≥ 64	≥ 64	≥ 64	64
18	≥ 64	≥ 64	≥ 4	≥ 64	≥ 64	≥ 64	64
19	≤ 8	≤ 8	≤ 1	≥ 64	≥ 64	≥ 64	≤ 8
20	≥ 64	≥ 64	≥ 4	30≤ 8	≥ 64	≥ 64	64
21	≥ 64	≥ 64	≤ 1	≥ 64	≥ 64	≥ 64	≤ 8
22	≥ 64	≥ 64	≤ 1	≥ 64	≥ 64	≥ 64	64
23	16	19.5	≤ 1	21≤ 8	21	≥ 64	≤ 8
24	≥ 64	≥ 64	≥ 4	≥ 64	≥ 64	≥ 64	32
25	≥ 64	≥ 64	≥ 4	≥ 64	≥ 64	≥ 64	64
26	≥ 64	≥ 64	≥ 4	≥ 64	≥ 64	≥ 64	64
27	≥ 64	≥ 64	≥ 4	32	≥ 64	16	64
28	22.5	≥ 64	≥ 4	16	≥ 64	≥ 64	≤ 8
29	16.5	≥ 64	2	16	≥ 64	32	≤ 8
30	≥ 64	≥ 64	≥ 4	≥ 64	≥ 64	≥ 64	64

Fluconazole - S, MIC ≤ 8 µg/ml (≥ 19 mm), SDD, MIC 16 to 32 µg/ml (15 to 18 mm),R, MIC ≥ 64 µg/ml (≤ 14 mm).
Voriconazole - S, MIC ≤ 1 µg/ml (≥ 17 mm), SDD, MIC = 2 µg/ml (14 to 16 mm), R, MIC ≥ 4 µg/ml ((≤ 13 mm).

Table2. Mean Inhibition Zone Diameter (IZD) of Plant Extracts and Conventional Antifungals to Selected *Fusarium* species from Humans and Plants

Mean IZDs (mm)±SEM								
	A.occidentale	KETO	ITRAC	VORI	MICOZ	CICLO	TERBI	FLUCZ
Human	25.75±0.46	8.60±0.625	11.39±0.66	15.48±0.69	7.59±0.61	1.70±0.41	1.20±0.31	13.76±0.78
Plant	24.23±0.85	8.99±0.63	11.18±0.65	15.24±0.70	9.81±0.66	2.94±0.41	1.0±0.30	13.99±0.81

SEM= Standard Error of Mean

KETO= Ketoconazole; ITRAC= Itraconazole; VORI= Voriconazole ; MICOZ= Miconazole; CICLO= Ciclopirox; TERBI= Terbinafine; FLUCZ= Fluconazole