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COMPARISON OF ANTIFUNGAL ACTIVITIES OF METHANOLIC LEAVES EXTRACT OF *SPHENOCENTRUM JOLLYANUM* (SJ) PIERRE (MOON SEED) AND CONVENTIONAL ANTIFUNGAL DRUGS USING *FUSARIUM* ISOLATES

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Abstract

Antifungal activity of crude methanolic leaf extract of *Sphenocentrum jollyanum* (SJ) was screened for antifungal activities against fusarial isolates from humans and plants sources. Isolation and identification of *Fusarium* species were carried out using standard mycological methods. The extract and antifungal agents (miconazole, ketoconazole, terbinafine, fluconazole, ciclopirox, voriconazole, and itraconazole) were screened against 1,260 and 2,140 fusarial isolates from human and plant specimens respectively, using modified agar well diffusion and modified agar disc diffusion methods. Susceptibility test of SJ extract revealed that it possesses potent antifungal activity against both human and plant isolates tested. The mean inhibition zone diameter (IZD) of the extract against isolates from both human and plant sources were 31.97±0.66 and 29.03±0.97 respectively, while that of voriconazole (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. There was significant difference between the activity of the extract and that of antifungal agents (P = 0.000). The lowest MIC for SJ was 0.0679µg/ml while that of antifungal agent was < 1. The IZD and MICs results further proved that extract has potent antifungal activity. In conclusion the results of this study showed that SJ is a rich source of antimicrobial agents.

Keywords: *Sphenocentrum jollyanum*, Antifungal activity, mycotoxicosis, chemotherapeutic

Introduction

Fusarium species is one of most clinically important molds that that causes opportunistic infections[1]. They are ubiquitous hence may be found in the soil, air and on plants causing infection in human, animal and plant [2, 3, 4, 5]. Fusarial mycotoxicosis can occur in humans and animals following ingestion of food contaminated with the fungal agent [6, 7]. Human can also acquired the infection by inhaling fungal conidia or direct contact with objects contaminated with conidia of Fusarium. Localized, focally invasive or disseminated disease has also been reported in human[2, 8]. Immunocompromised persons are frequently affected by *Fusarium* pathogens while immunocompetent individuals are seldomly affected [7, 9]. However, cases of superficial infection such as Keratitis and onychomycosis are frequently reported in immunocompetent persons. Following entrance into a suitable host, conidia germinate and form filaments that invade the surrounding host tissues leading to invasive infection(such as pneumonia, deep cutaneous infections, sinusitis) or disseminated infections[10, 11].

Plant infection by *Fusarium* species are serious problem globally as a result of mycotoxins produced by the fungi[12, 13]. Plant-pathogenic members such as *F. oxysporum* and *F. moniliforme* cause diseases in many agriculturally important crops leading to economical losses yearly[14].

Recently members of *Fusarium* species have become resistance to almost all commonly used antifungal drugs [14]. Thus posing a serious healthcare challenge, and threat to agricultural sectors. Hence there is a yearning need to search for new, cheap and alternative antimicrobial drugs from plant sources for the treatment of fungal infectious diseases

Some Medicinal herbs in our environment serve as a good source of novel antifungal and antibacterial chemotherapeutic agents. Medicinal plant research has yielded many valuable drugs and new compounds with biological activity from plants are discovered almost daily. *Sphenocentrum jollyanum* (SJ) Pierre (Moon seed) belongs to the **Kingdom** Plantae, **Phyllum** Magnoliophyta, **Class** Magnoliopsida, **Order** Ranunculales, Family Menispermaceae, **Genus** *Sphenocentrum*, **Specie** *jollyanum* [15]. A broad spectrum Biological and pharmacological activities SJ has reported by [16] been shown to display a wide spectrum of biological and pharmacological activities. *Sphenocentrum jollyanum* contains flavonoids, tannins, bitter tasting terpenoid, isoquinoline, alkaloids such as palmatine, columbamine, deterpenes and some other alkaloids[17, 18]. Basically, they are used locally for common ailments such as malaria, diarrhea and skin diseases. The root and leaf extracts of SJ have antiviral properties [17]. The leaves decoction has been used for stopping bleeding of wound, sores and cuts. The aim of the present study was to determine the antifungal susceptibility pattern of methanolic leaf extract of *Sphenocentrum jollyanum* (SJ) Pierre (Moon seed) and conventional antifungal drugs on *Fusarium* isolates.

Aims of Present Study

To compare antifungal activities of methanolic Leaves extract of *Sphenocentrum jollyanum* (SJ) Pierre (Moon seed) and Conventional Antifungal drugs using *Fusarium* isolates.

Specific objectives of the present study include:

1. To determine the antifungal properties of methanolic Leaf extract of *Sphenocentrum jollyanum* (SJ)
2. To determine the *in vitro* antifungal susceptibility profile of the various *Fusarium* species isolated to some conventional antifungal agents

Methods

Isolates source

A total of five hundred and twelve isolates of *Fusarium* species were used for this study. This comprises of 252 and 260 from human and symptomatic plants respectively. The isolates were confirmed by standard mycological methods[19, 20].

Plant leaves collection

Fresh Leaves of *Sphenocentrum jollyanum* (SJ) Pierre used for this work were collected from Agulu community, Anambra State, Nigeria. The plant species was 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, University of Nigeria Nsukka.

Crude extract preparation

S. jollyanum leaves were washed with running tap water, shade- dried for 7days and further dried at 40°C in an oven. An electric grinder was used to reduce the dried leaves to a coarse powder and was then kept in an airtight container before methanol extraction [21].

Exactly 750 g of dried powdered leaf sample was weighed and macerated with 2 liters of absolute alcohol (methanol) until complete exhaustion and was placed on orbital shaker at 150 rpm for 48hrs under room temperature. The extracted material was filtered using Whattman No. 1 filter paper. the residue discarded, and the filtrate evaporated to dryness in a steady air current for about 24 hours. The extract was also placed under UV rays for 24 hours for sterilization. The yield, which was 44.2 grams, was stored in sterile screw capped container in a refrigerator (4°C) until needed [22].

Percentage yield of the extract was calculated thus

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

Susceptibility testing of conventional antifungal agents against Fusarial isolates

Plant extract was by standard method and susceptibility test of fusarial isolates to extract of *S. jollyanum* was by modified Agar well diffusion method.

Antifungal susceptibility of selected isolates was established by the modified Agar disc diffusion method [23] 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.

Susceptibility testing of Sphenocentrum jollyanum leaves extract against Fusarial isolates

The sensitivity of selected *Fusarium* isolates to the crude methanolic extract of *Sphenocentrum jollyanum* leaf was evaluated by modified agar well diffusion technique [24, 22, 21]. 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 extract was dissolved in 2 mls dimethyl sulfoxide (DMSO) and the resulting solution diluted to a concentration of 2 mg/ml using sterile distilled water.

Mueller-Hinton Agar supplemented with 2 % glucose and 0.5 µg of methylene blue per ml (MH- MB) was sterilized. This media was pured into a sterile Petri dish up to depth of 4mm and were left to solidify. Inoculation of agar plate's surface was done by dipping a sterile swab into a fungal cell suspension (adjusted to the turbidity of a 0.5 McFarland standard) and was spread evenly over the agar surface.

The agar (19.9 mls each) was seeded with 0.1 ml of standardized inocula and allowed to set. Sterile cork borer was used to bore five wells of a diameter of 8mm each on the media. Two drops (0.02 ml per drop) of the extract were aseptically placed into each of the wells. The well at centre was filled with two drops of 2 fold diluted DSMO and this served as control. The plates were left for 1 hour at room temperature for proper diffusion of the extract before incubating at 28°C for 24, 48 and 72 hours. Inhibition zone diameters (IZD) of the extract at the different incubation periods were measured.

Minimum Inhibitory Concentrations of Conventional Antifungal Agents

The MICs of the antifungal agents for *Fusarium* species were calculated from disk zone diameter measurements using standard method of[25], where The zone diameter in millimeters of the continuous agar gradient around each disk, calibrated with MICs, was calculated by a balanced weight regression analysis using the standard CLSI M27-A2 broth dilution process.

Even though the interpretative breakpoints for *Fusarium* species, have not yet been established, that of Candida species according to [25] 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 and Discussion

Extraction of plant extract

The percentage yields of the methanol extracts of *S. jollyanum* leaves was 5.9%. The phytochemical analysis showed that alkaloids, saponins, terpenoids, , flavonoids, tannins and were present while anthraquinones, phenols, steroids, and cardiac glycosides were absent (Table 1). This finding is in line with earlier studies [26, 27, 28 29]. Saponin one of the active constituent of *S. jollyanum* had been reported by [30], to been a potent antifungal agent. More so, terprenoids had been reported to be biologically active in treatment of infectious and non-infectious diseases[31]. It could be inferred therefore that other constituents of this extract would have had synergic action with saponin and terprenoids to achieve the efficacious antifungal activity against the considered human and plant pathogenic fusarial strains.

Antifungal activity of Sphenocentrum jollyanum leaves extract

The results of antifungal susceptibility testing of the *S. jollyanum* crude methanolic against selected *Fusarium* isolate showed a potent antifungal activity against the *Fusarium* species. The fungicidal action of the plant extract was higher when compared with those of conventional antifungal agents (Fig. 1). The mean inhibition zone diameter (IZD) of the extract against isolates from both human and plant sources were 31.97±0.66 and 29.03±0.97 respectively. The result of this study is in consistence with that of [32] who also recorded mean inhibition zone diameter of 13.33±1.53 against *Candida albicans* using *S. jollyanum* plant extract even though with a lower value.

The Mean IZD of the plant extract was higher than that of all the conventional antifungal agents tested. Voriconazole was the most potent with mean inhibition zone diameter of 15.48±0.69 and 15.24±0.70 for human and plant respectively. This high potency of vericonazole was reported [33] on *Fusarium* and *C. albican*. This was followed by fluconazole with mean IZD of 13.76±0.78 and 13.99±0.81 for human and plant respectively. There was significant difference between the activity of the extract and that of antifungal agents (P = 0.000). Several studies had reported plant extracts to be more potent against some microbes when compared with conventional antimicrobial agents [34, 35, 32]. A mean inhibition zone diameters of 14.33 and 16.0 were reported for Fluconazole and Miconazole against *C. albicans* by [32] respectively. 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.

MICs of plant extracts

The plant extracts used in this study showed lower minimum inhibitory concentrations than the conventional antifungal agents. The lowest MIC for *Sphenocentrum jollyanum* leaves for the human isolates was 0.0679µg/ml, while those of the plant isolates 0.0767µg/ml (Fig. 3A and 3B). These results showed that the MICs of the *S. jollyanum* leaves extract were better than those of the conventional drugs. Fig 4A-4C shows plots of logarithmic concentration of the plant extract against the square of inhibition zone diameters (IZD^2) for human fusarial isolates, while Fig 5A-5C represents those of plant isolates.

Conclusion

The antifungal susceptibility profile results in this study support the utility of this leaf extract in developing novel antifungal agents in traditional medicine to treat various ailments. *Sphenocentrum jollyanum* leaf exhibited apical antimicrobial activities and is thus expected to be a essential source of antimicrobial agents for the future endeavors to combat multi drug resistance organism. However, more studies are required for the purpose of drug development with respect to this plant.

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Table 1. Phytochemical components of methanolic leaf extracts of *S. jollyanum*

Bioactive constituents	Results
Saponin	+
Flavonoid	+
Cardiac glycosides	-
Akaloids	+
Tannin	+
Terpenoids	+
Phenols	-
Steroids	-
Anthraquinones	-

Absent: - Present: +

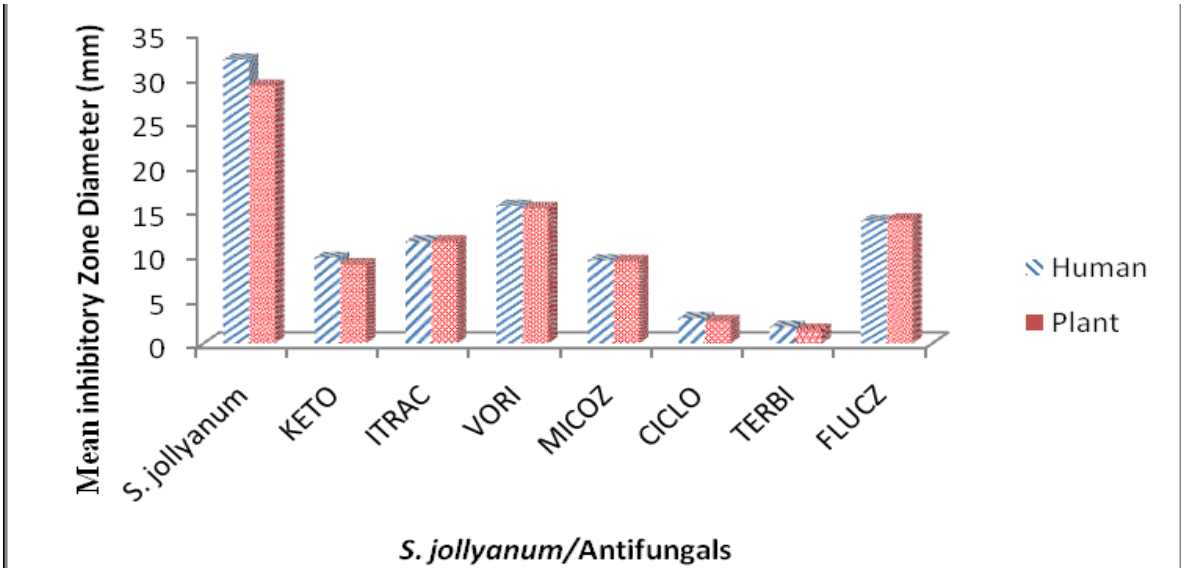

Figure 1. Comparison of Mean Inhibition Zone Diameter of Conventional Antifungals and Crude Extracts of *S. jollyanum* to *Fusarium* species

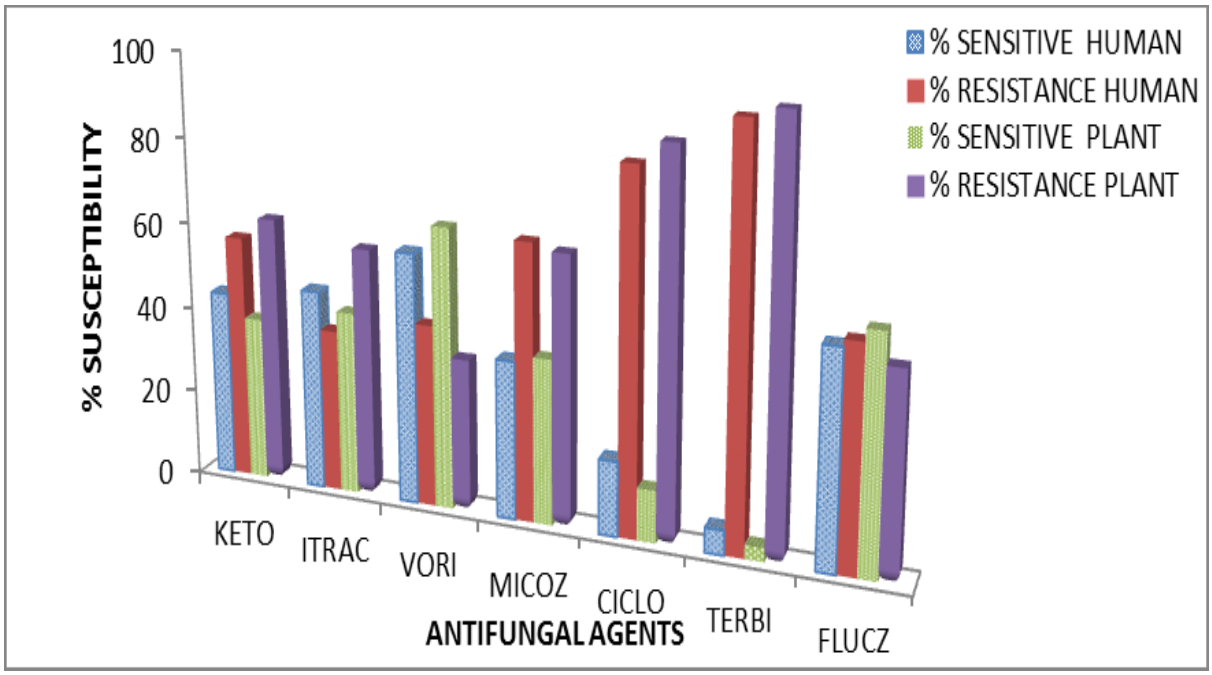

Figure 2. Antifungal Susceptibility of *Fusarium* species Isolated from Human and Plant Sources to Conventional Antifungals

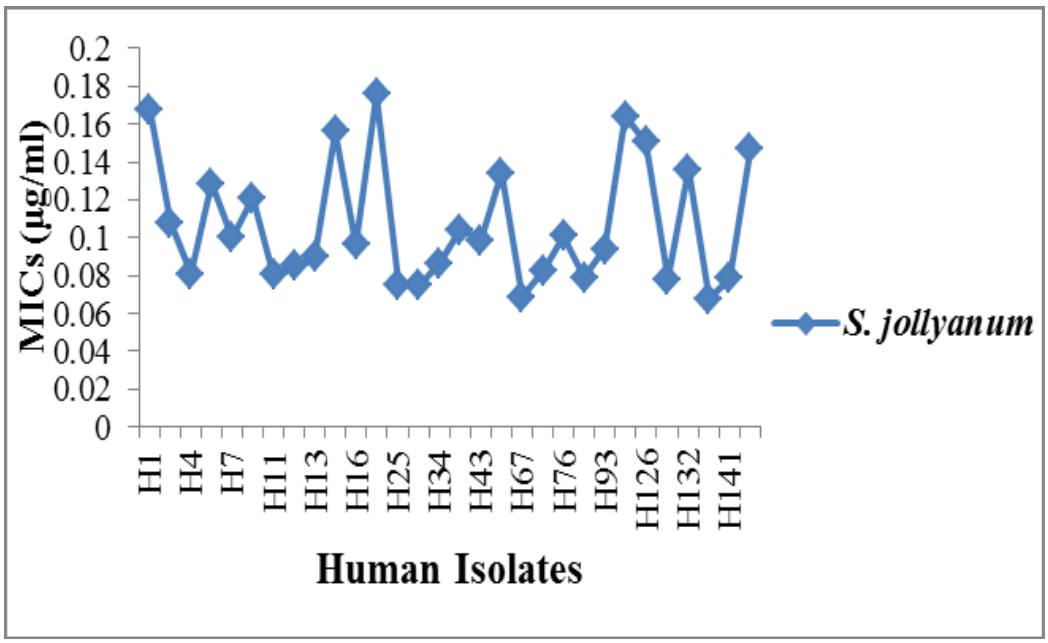

Figure 3A. Plant Extract's Minimum Inhibitory Concentrations (MICs) to fusarial isolates from Human

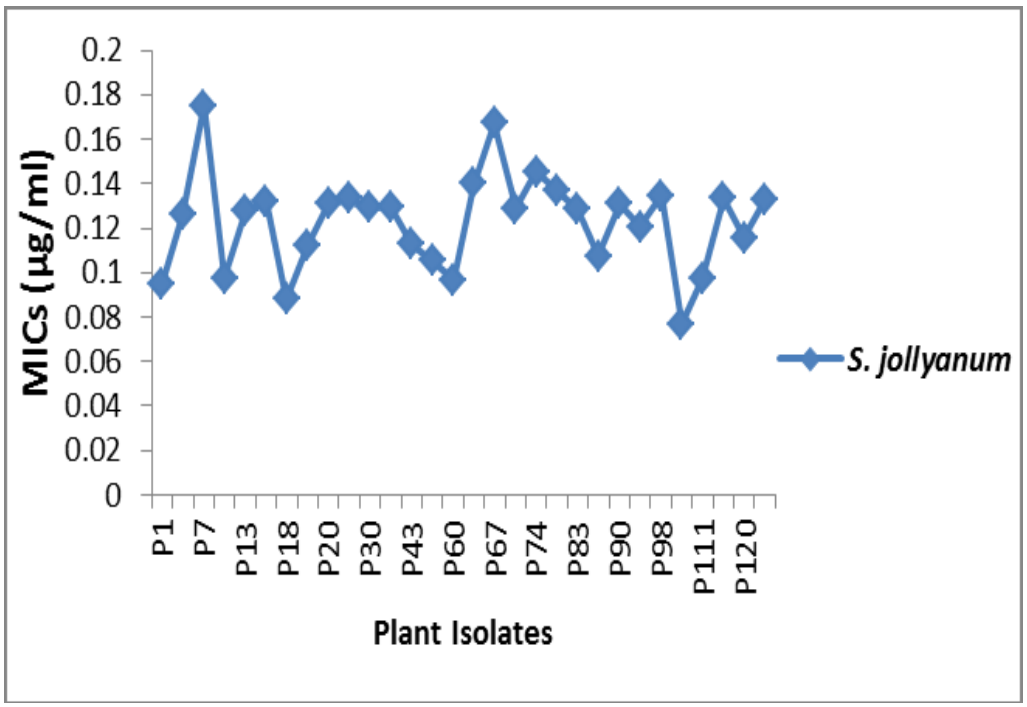

Figure 3B. Plant Extract's Minimum Inhibitory Concentrations (MICs) to fusarial isolates from Plant

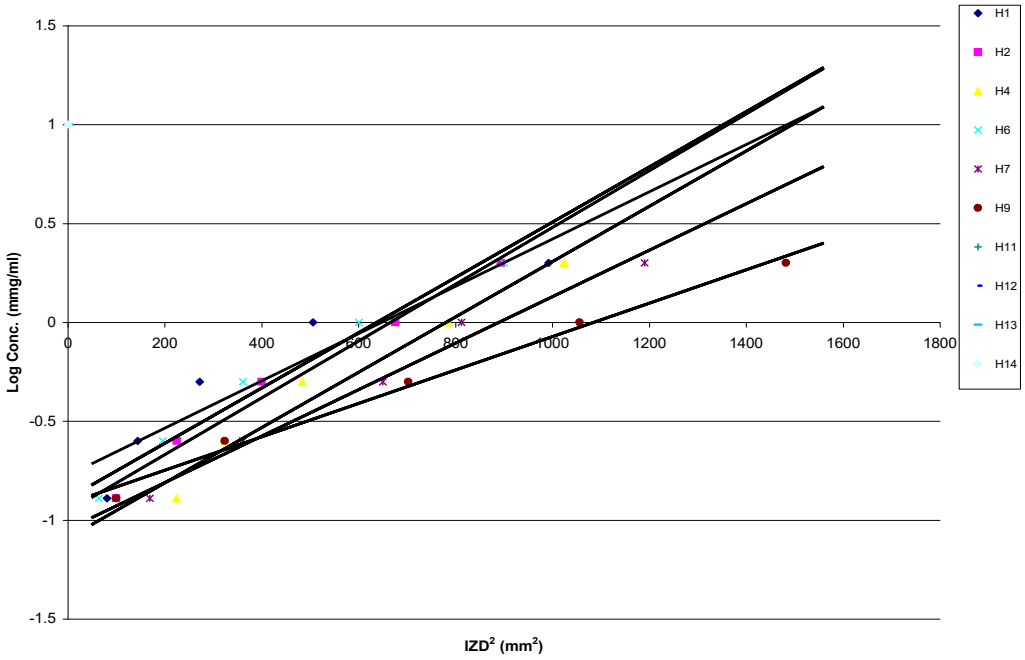

Figure 4A. Log Conc. of Plant Extract vs. IZD^2 for Human isolates H1 – H14

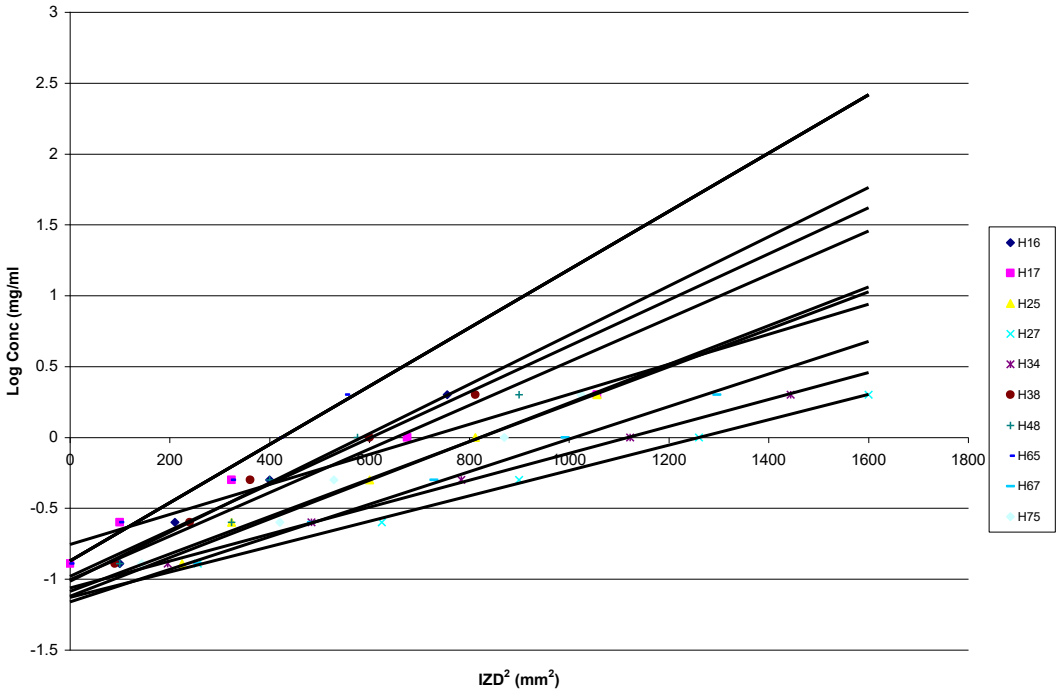

Figure 4B. Log Conc. of Plant Extract vs. IZD^2 for Human isolates H16 - H75

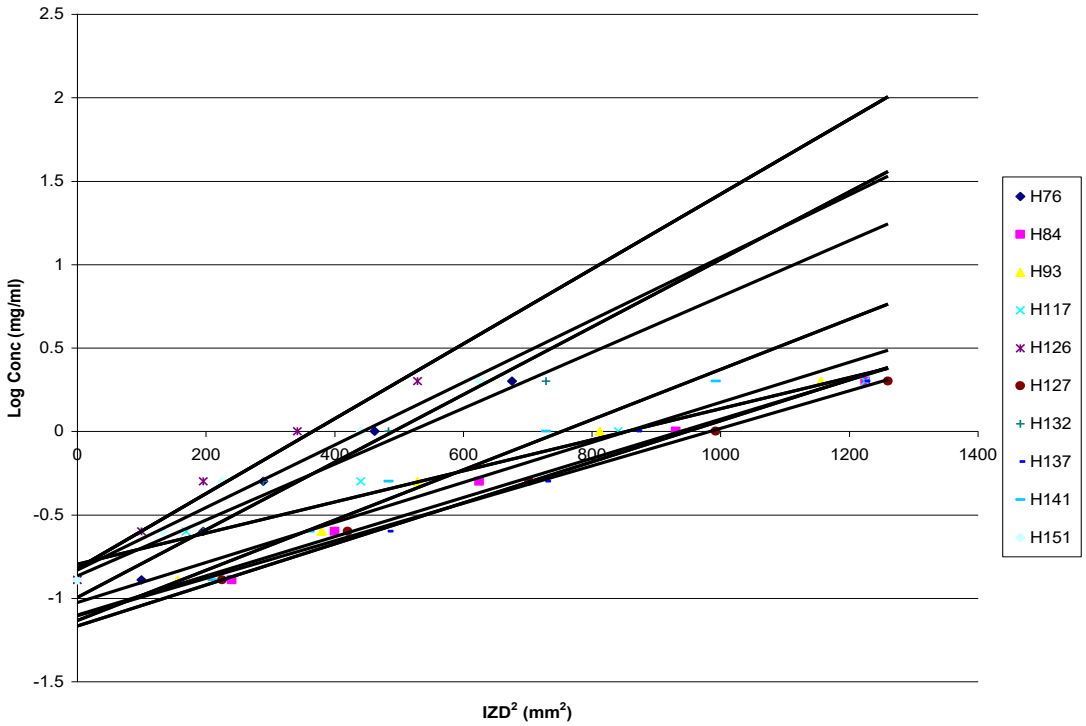

Figure 4C. Log Conc. of Plant Extract vs. IZD^2 for Human isolates H76 -H151

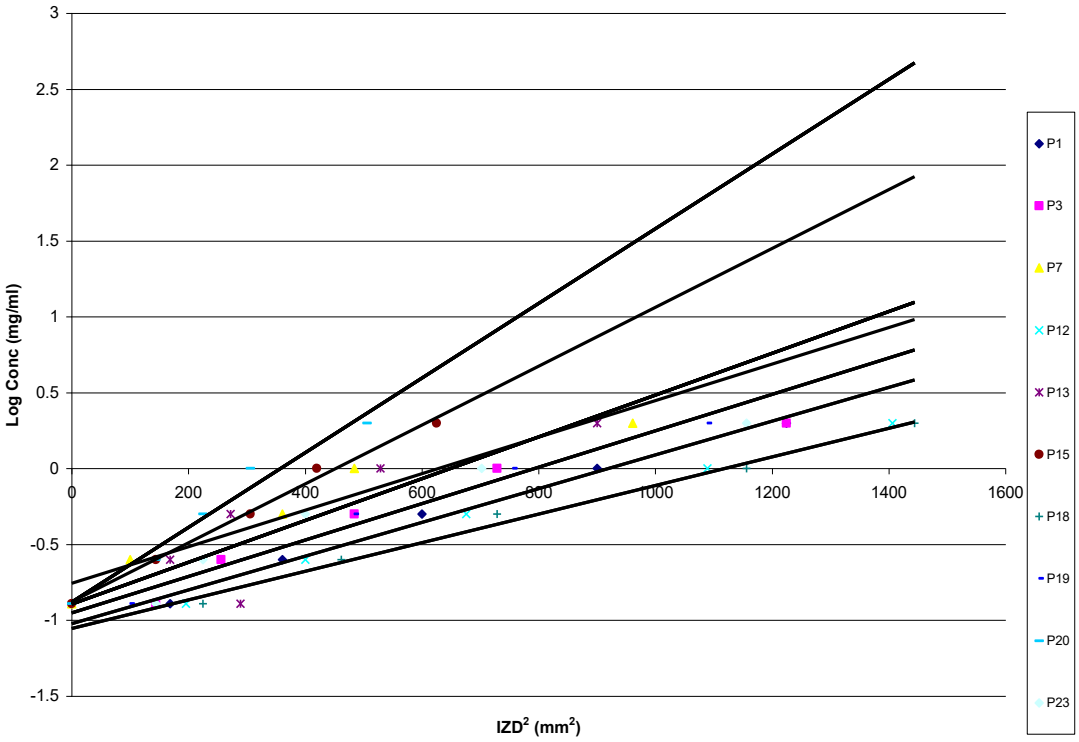

Figure 5A. Log Conc. of Plant Extract vs. IZD^2 for Plant isolates P1 – P23

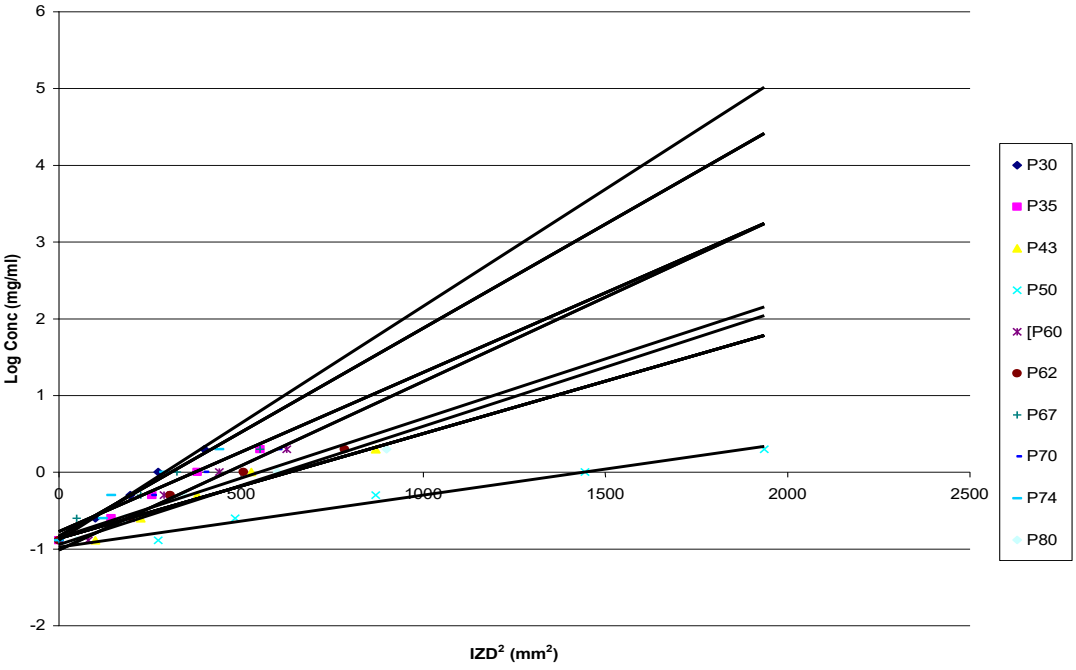

Figure 5B. Log Conc. of Plant Extract vs. IZD^2 for Plant isolates P30 – P80

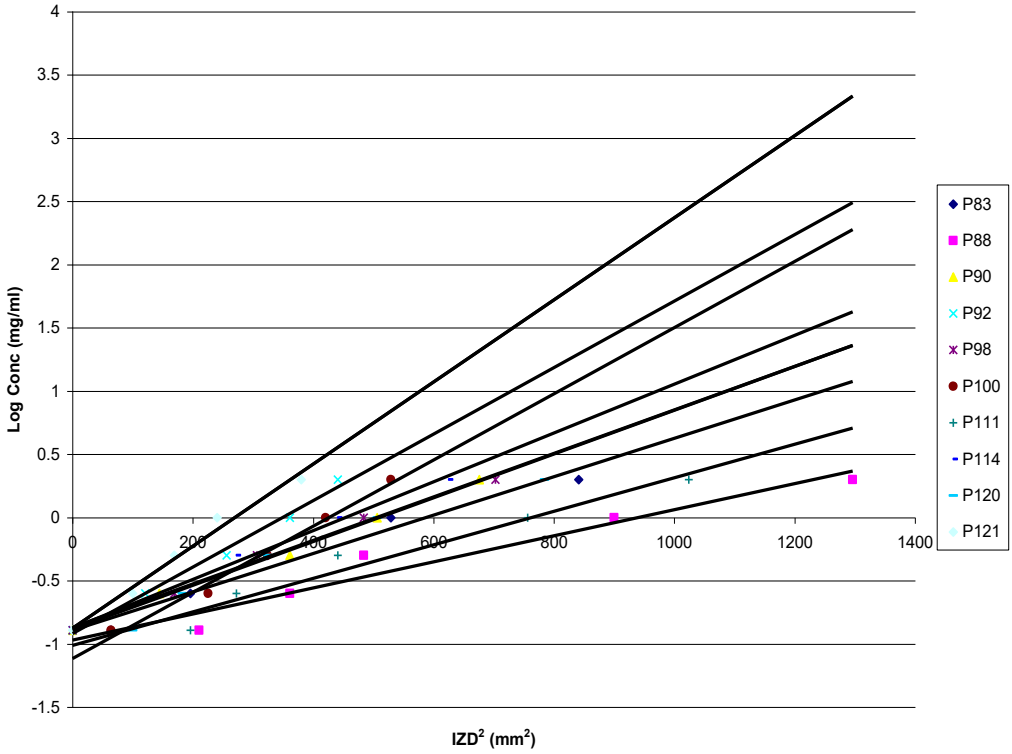

Figure 5C. Log Conc. of Plant Extract vs. IZD^2 for Plant isolates P83 – P121