Screening For Antibiotics of Endophytes from Wild Grass (*Cyperus rotundus* L.)

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DECLARATION

No portion of the work referred to in this dissertation has been submitted in support of an application for another degree of qualification of this or any other university or institution of higher learning.

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ABSTRACT

In this present study the presence of antimicrobial activity in endophytic fungi of wild weed (*Cyperus rotundus*) was carried out. Stem samples of about 2.0 cm length were obtained from the bushes located at UNIMAS West Campus. The samples were separately placed in unused plastic bags and then brought to the laboratory for processing, surface sterilising and culturing on Potato Dextrose Agar (PDA). Isolated endophytes were tested for antibacterial and antifungal activities against four species of bacteria, namely *Enterobacter aerogenes, Staphylococcus aureus, Eschericia coli, Salmonella typhi,* and one fungus, which was *Fusarium sp.* All samples were found to be colonized with endophytes, resulting in isolation of nine fungal endophytes. Three displayed both antibacterial, Gram-negative and Gram-positive and at least one of the fungal endophytes could inhibit *Fusarium* sp in antifungal activity. Antibiotic activity screening was carried out using concentration of crude metabolite extract of endophytes at 10 µl. Two methods were used to detect antibiotic activity, which were (i) modified disc diffusion (Kirby-Bauer) and (ii) agar cup method. Result from this study showed that, five from eight fungal endophyte isolates showed antimicrobial activity inhibiting at least one test bacteria. Among the five of antibiotic producing isolates of fungal endophytes, five crude metabolite extracts showed positive against *E. aerogenes.* In addition, four other methanol extracts showed positive results against *S. aureus.* The species were identified as *Pestalotiops* sp. and as *Fusarium* sp..

Key words: Endophytic fungi, *Cyperus rotundus,* antibacterial, antifungal, antibiotic activity.

ABSTRAK

Kajian telah dilakukan untuk menentukan kehadiran aktiviti antimikrob dalam endofit dari rumput liar (Cyperus rotundus) dari kampus UNIMAS Sarawak. Dalam kajian ini, rumput tumbuh liar di dalam semak diambil sebagai sampel dengan memotong sekitar 2.0cm panjang batang. Sampel dibawa ke makmal untuk pengenalan spesies, pemprosesan, dan mensterilkan permukaan dan diinkubasi pada Potato Dextrose Agar (PDA). Endofit di pencilkan untuk ujian antibakteria dan antikulat terhadap empat jenis bakteria, iaitu Enterobacter aerogenes, Staphylococcus aureus, Escherichia coli, Salmonella typhi, dan satu cendawan Fusarium sp. Semua sampel rumput liar yang di pencilkan menunjukkan kehadiran endofit. Secara keseluruhan sembilan endofit cendawan di pencilkan daripada rumput liar. Tiga dipaparkan positif ujian antibakteria untuk gram-negatif dan gram positif dan satu endofit cendawan boleh menghalang aktiviti Fusarium sp. dalam ujian antikulat. Ujian antibiotik telah dilakukan dengan menggunakan kepekatan ekstrak sebanyak 10 µl menggunakan kaedah Kirby-Bauer dan telaga agar yang diubahsuai. Sebanyak lima pencilan endofit cendawan menunjukkan aktiviti antimikrob menghalang bakteria sekurang-kurangnya satu bakteria ujian. Di antara lima pencilan yang menghasilkan antibiotik, lima ekstrak metabolit menunjukkan positif terhadap E. aerogenes. Sementara itu, empat ekstrak metanol menunjukkan hasil yang positif terhadap S. aureus. Spesies dikenalpasti sebagai Pestalotiops sp. dan Fusarium sp..

Kata kunci: Endofit, Cyperus rotundus, antibakteria, antikulat, aktiviti antibiotik.

1.0 INTRODUCTION AND OBJECTIVES

The need for discovery of new and useful drugs to provide effective treatment and relief in all aspects of humankind is increasing due to arise of many drug resistance bacteria, the appearance of life-threading viruses, fungal infection, environmental degradation and loss of biodiversity (Strobel &Daisy, 2003). According to Monnet (2005), antibiotic resistance to microorganisms is one of the main concerns to pharmacists nowadays. A study by Livermore (2003), the emergence and spread of new diversity of microorganisms that is resistant to antibiotics is as a result of genetic changes in microorganisms. In addition, many antimicrobial chemical compounds are too toxic to human use such as polymyxin B nonapeptide tends to be toxic for human systemic use (Danner *et al.*, 1989) and the nonacylated cyclic decapeptide gracimidin S is also quite toxic, causing erythrocyte lysis at concentration only treefold higher than minimum inhibitory concentration for many bacteria (Hancock & Chapple, 1999).

Consequently, new antimicrobial agent with wide ranges of biocides activity should be developed. Endophytes, that living within a plant tissues, are potential sources of novel natural products for exploitation in antimicrobial agents. Endophytes are known to have mutualistic role with its host plant. The plant has been thought to provide nutrients to the endophytes, while the endophytes may produce factors that protect the host against herbivory, insect attack or tissue invading pathogen (Yang *et al.*, 2006). A previous study by Mohanta *et al.* (2008), has been shown significant antimicrobial activity in an endophytic isolate of *Fusarium* sp..

Beside endophytic fungi, endophytic streptomycete has also been shown to produce useful antibiotics, which was a novel antibiotic named kakadumycins. This antibiotic is structurally related to a quinoxaline antibiotic, echinomycin. Kakadumycins has wide spectrum antibiotic activity, especially against Gram positive bacteria and it showed better bioactivity than echinomycin, where kakadumycin has minimum inhibitory concentrationains than echinomycin (Castillo *et al.,* 2003). In addition, endophytes are likely to be rich and reliable source of genetic diversity and biological novelty, and experience has shown that novel endophytic microbes usually produce novel natural products (Strobel, 2003).

According to Ezra *et al.,* (2004), one of the promising endophytic microorganisms to be isolated would be an actinomycete, or specifically a streptomycete since these organisms often shown to produce antibiotics. Endophytic streptomycetes that isolated from a medicinal plant, *Kennedia nigriscans* consistently yielded a yellowish-orange culture of *Streptomyces* sp. which was subsequently to produce a unique family of functionalized peptide antibiotics, the munumbicins (Castillo *et al.,* 2002).

Antibiotics have played a major role in the pharmaceutical industry. After antibiotics such as penicillin were first discovered by Alexander Fleming on 1928, the pharmaceutical industry began to grow rapidly into the huge businesses industry today (Monnet, 2005). Antibiotic also can be used in livestock production such as therapeutics for managing clinically apparent disease (Petterson & Burkholder, 2003), as a growth promoter (Jukes, 1975) and as a prophylactic to prevent disease (Sawant *et al.,* 2005*)*. Therefore antibiotics are necessary to society. The commercial importance of antibiotics is

not only for treating infections in humans but also for agriculture and keeping animals disease free.

Since, it has been found that endophytic fungi has potential as new metabolites with abroad usage in biocides in the recent past studies, research priority should be directed to study them (Strobel & Daisy, 2003). Even though plants has been widely used as a natural source of antibiotics over the past years, potential prospect of finding new drugs for treating newly developing diseases in humans from endophytes should be improved. One of the sources for isolating endophytes is wild grasses. Popay and Rowan (1994), Bacon (2000), and Riverra (2005) have described the discovery of antibiotics from endophytes that have been isolated from wild grasses. Thus, the aim of this study is to reveal the potential of antimicrobial activity in endophytes from wild grass.

The hypothesis of this study is that there is an antimicrobial activity from the endophytic fungi towards the target microorganisms. The objectives of this study are:

1. To isolate from wild grass endophytes with potential capacity to produce novel antimicrobial agents.
2. To identify the species of isolated endophytes.
3. To screen the antimicrobial activities in endophytes.

2.0 LITERATURE REVIEW

2.1 Antibiotics

The word antibiotic comes from Greek *anti-, "against," and biotos, "the means of life."* Antibiotic has had different meanings through the centuries. Ancient philosophers may have used a similar word to mean resistance, in the sense of dealing with the vicissitudes of life. In the 19th century, the word "antibiotic" referred to a belief opposed to the possibility of life, as on other planets (Haubrich, 2003).

Antibiotics are substances that destroy or inhibit the growth of microorganisms, particularly disease-producing bacteria and fungi. Antibiotics are obtained from microorganisms especially molds (Hine and Martin, 2004). Penicillin for example, is one of the earliest discovered, from the mold *Penicillium notatum* (Monnet, 2005) that have been discovered by bacteriologist Alexander Fleming in 1928 (Bellis, 2010). In 1941, penicillin has been introduced as important chemotherapeutic agent, suitable for therapy (Waksman, 1947) and active against a wide variety of bacteria (Allen *et al.*, 2008).

The discovery of penicillin led to the finding other antibiotics of a host of new in particular from members of bacteria of the genus *Streptomyces* and *Bacillus* that yield many clinically important antibiotics (Ming, 2007). Apart from penicillin, antibiotics was as well yielded by fungi are cephalosporins with highly reactive beta-lactam ring (O'callaghan *et al.,* 1971), and griseofulvin produced by *Penicillium griseofulvum* with less toxicity and widely employed for treating a variety of fungal disease (Dasu & Panda, 2000).

2.2 Emerging of Infectious Disease

Despite of widespread advances in medical science, many infectious diseases continue to have devastating consequences for human in populations in many parts of the world. In 1993, Dobson reported tuberculosis killed 2.7 million people and infected another 8.1 million people worldwide up to 1994. A study by Dobson (1996) showed the present tuberculosis epidemic is expected to grow worse mostly in developing countries, by the evolution of multidrug resistant strains tuberculosis.

Infectious disease are illness caused by the presence and activity of one or more pathogenic agents including bacteria, fungi, viruses, protozoa, multicellular parasites and abnormal proteins called prions. The World Health Organization (WHO) have described there are over 100 infectious disease affecting human and animals as recently increasing or threatening to increase (Jorge, 2009). Infectious disease remain among the leading causes of death and disability worldwide and have for centuries ranked as major challenges to human progress and survival (David *et al.,* 2004). The causes of emergence are many and vary widely between diseases. New strains of old diseases with different immunological characteristics, increased virulence and different responses to antibiotics are often responsible cause for new outbreaks of disease. According to Jorge (2009), microbes may emerge suddenly as disease threats by acquiring new capacities for initiating infections or by altering human natural ability to amount an immune response.

Thus, it is extremely crucial that the search for newer and most effective antibiotics should be continued taking into consideration the dangers at hand. Microorganisms have long met the demand and the search has been extended to include an environment which

holds a great potential and will make an enormous impact on the antibiotics discovery process (Ming, 2007).

2.3 Endophyte

Ainsworth (1971) defined endophyte as a plant that lives inside another organism. Further, endophyte could simply refer to the location of the organism, which endo means within and phyte means plant. Thus, an endophyte is an organism which lives inside a plant. This is contrasted to epiphyte, which refers to the organisms living on the outside of the plant. Fahey *et al.,* (1991) defines the organisms commonly associated with the term endophyte are fungi and bacteria. While Wilson (1995) considered endophytes as any fungus or bacterium found inside plant tissues.

Previous research has revealed that endophytes are an endosymbiont and are known to have mutualistic association to their host. Endophyte that occur intercellularly within the leaves, stems and reproductive organs of grasses have impressive effects on the physiology, ecology and reproductive biology of its host plant. They often protect their host plants against a wide range of insect and mammalian herbivores, through the production of toxic alkaloids (Clay, 1990). In addition, endophyte also increased resistance of host plant to pathogens and various abiotic stresses, while the host plant provide nutrient and protective shelter to the endophyte (Saikkonen *et al.,* 1998).

A study by Johri on the species of *Curvularia* as endophytes showed that endophyte help plants to withstand high constant soil temperature of 50ºC and intermittent temperatures as high as 65ºC (2006). While according to Webber (1981) the endophyte *Phomopsis oblonga* gives protection to elm trees against the beetle *Physocnemum*

brevilineum. There are reports to document that there is an association between improved plant varieties with endophytic microorganisms that act efficiently in pest control but do not cause any harm to cattle fed on these plants (Saha *et al*., 1987).

2.4 Biological Activity of Endophytes

Endophytes are the chemical synthesizers inside plants (Owen & Hundley, 2004). Many of them are capable of synthesizing bioactive compounds that can be used by plants for defense against pathogens and some of these compounds have been proven useful for novel drug discovery. Previous studies have reported that hundreds of natural products including substance of alkaloids, terpenoids, flavonoids, steroid, etc. from endophytes ware isolated (Guo *et al.*, 2006).

Currently, endophytes are viewed as an outstanding source of bioactive natural products (Strobel & Daisy, 2003). Furthermore, fungal endophytes have been recognized as repository of novel secondary metabolites for potential therapeutic use (Tan and Zou, 2001). A study by Guo *et al.*, (2006) most of the natural products from endophytes are antibiotics, anticancer agents, biological control agents and other bioactive compounds by their different functional roles.

According to Demain (1981), antibiotics compound is low-molecular-weight organic natural products made by microorganisms that are active at low concentration against other microorganisms, are the most bioactive natural products isolated from endophytes. In addition, Strobel and Daisy have summarized the discovery of penicillin to

most of the novel antibiotics isolated from endophytes. Many of them are proved to be important (2003).

Already at the beginning of the 90's, in a review on endophytic fungi from the genus *Acremonium*, Van-Heeswijck and McDonald (1992) has launched the idea of using engineered endophytic fungi in the control of insects and diseases affecting *L. perenne*. Additionally, several genes from endophytic fungi and related to toxin production are now being cloned and studied in depth. Wang et al. (1999) cloned a gene coding for a dimetilail tryptophane synthase, that is responsible for the first step in the synthesis of ergot by the fungus *Claviceps purpurea*. Genes related to the latter were identified in the endophytic fungi *Balansia obtecta* and *Neotyphodium* spp., known to produce, respectively, ergobalansine and ergovaline. The investigations on *Colletotrichum* sp. a fungal endophyte of *Artemisia annua* by Lu *et al.*, (2000), has led to the isolation of five ergosterol derivatives all of which were effective against *Bacillus cereus, Staphylococcus aureus, Pseudomonas* sp. and some fungal phytopathogens. A previous research on *Fusarium* sp. as endophyte from several plant species has been reported the presence of antimicrobial potential (Wang *et al.*, 2007).

In addition, a study by Bacon (2000), grasses infected with *Neotyphodium* fungal endophytes was found to have a diverse array of biologically active secondary metabolites. Secondary metabolite that emerge from the metabolism of diverse carbon and amino acid sources, can led to the production of a secondary metabolite with antimicrobial capabilities if the microorganisms can metabolize it (Riverra, 2005). Furthermore, the secondary metabolites help defend the host grass against a range of grass herbivores through their antibiotic producing ability and distribution in plant (Popay and Rowan, 1994).

2.5 *Cyperus rotundus*

The family Cyperaceae includes approximately 3000 species, of which about 220 species are identified as weeds. Nearly 42% of these weeds are in genus *Cyperus.* Purple nutsedge, *Cyperus rotundus* was identified as the world's worst weed where it was reported as a common, serious or principal weed (Bendixen & Nandihalli, 1987). In a study by Stoller and Sweet (1987), purple nutsedge exhibit prolific vegetative activity which produces a complex underground system consist of basal bulbs, rhizomes, and tubers. The shoots arise from basal bulbs as a fascicle. Basal bulbs are a primary site for prolific vegetative growth because they contain the meristems for leaves, rhizomes, roots and flower stalks. The tubers contain quiescent buds and function like the seeds. They germinate under the appropriate environmental conditions to produce plants that perpetuate the infestations.

According to Negbi (1992), tubers of *C. rotundus* and *C. esculentus* were used in the ancient eastern Mediterranean as food, medicine and perfume. *C. rotundus* tubers, a dietary staple in a Stone Age Egyptian community, were used much later in perfumes and medicine by Egyptians, Mycenaeans and Greeks. Furthermore, a study by Kilani *et al.*, (2005) show that extracts from *C. rotundus* exhibits significant antioxidant and antimutagenic activities.

Previous research has shown that *B. epichloe* and *B. henningsiana* grow endophytically within host tissues of *C. rotundus.* In contrast, species *Atkinsonella hypoxylon* and *Balansia cyperi* occur as epiphytes around meristems and young leaves of *C. rotundus* (Leuchtmann & Clay, 1988).

3.0 MATERIALS AND METHOD

3.1 Preparation of Culture Media

Potato dextrose agar (PDA) was prepared by dissolving 9.75 g PDA powder in 250ml distilled water in Duran bottle. The solution was heated to mix it thoroughly on the hot plate. After autoclaving at 121 °C for 15 minutes, 250ml of PDA media was poured into 20 Petri plates.

After all agars had solidified, the lid of the plate was placed and the plates were kept in refrigerator at 4 °C for storage until used.

3.2 Collection of Wild Grass

Wild grasses around UNIMAS West Campus were taken randomly and cut off with an ethanol disinfected knife. Each plant sample was wrapped with sterile wet paper tissue and packed separately in a sterile polythene bags to avoid moisture loss. The samples were brought to the laboratory for fungal isolation.

3.3 Isolation of Endophytic Fungi

The plant samples were cut into pieces of about 3cm and surface then surface-sterilized for 5 minutes in 5% sodium hypochloride solution, then in 70% ethanol for another 5 minutes. Following sterilization the samples were thoroughly washed with sterile distilled water. The excess water was dried at room temperature with sterile filter paper. After drying, each branch was further cut into smaller segments of about 1.5 cm and their outer tissue were removed with sterile scalpel to expose their inner tissue and carefully placed on potato dextrose agar (PDA) (Radu & Kqueen, 2002). The plates were then incubated at room

temperature (about 28 ^{o}C) for 2 days for fungal growth. Hypal tips of fungi growing out from the plated segments were subcultured into fresh PDA plates. Further subculturing done by transferring a piece of agar at the edge of the fungal colony into new PDA plates.

The isolates were repeatedly subcultured on fresh PDA agar medium until the pure culture was obtained. After pure cultures were obtained, the fungus and bacteria were grown in slant agar in bijou bottles for 5 to 7 days. The bottles were stored at 4 ^{o}C until used.

3.4 Test Microorganisms

Test microorganisms for antibacterial and antifungal activity screening were made up of four bacteria and one fungal species (Table 1). The test bacteria were streaked from stock cultures onto nutrient agar (NA) and later grown in nutrient broth (NB) medium for antibacterial testing.

The test fungus for antifungal screening was obtained from Mycology Laboratory, FRST. The target microorganisms that were used as described in Table 1. The fungus was grown on Potato Dextrose Agar plate during antifungal test.

Table 1: List of microorganisms used in antimicrobial screening as indicator strains

Test microorganisms	Host	Gram
1. Bacteria		
Staphylococcus aureus	Human	+
Enterobacter aerogenes	Human	-
Eschericia coli	Human	-
Salmonella typhi	Human	-
2. Fungi		
Fusarium sp.	Plant	

3.5 Antibacterial Activity Evaluation

3.5.1 Media Preparation

Soft Nutrient Agar (75 %) was prepared by dissolving 7.5 g of Nutrient Agar (NA) powder in 1L distilled water. After autoclaving at 121 °C for 15 minutes, 1L of 75 % soft Nutrient Agar media was poured into Petri plates. The solution was heated to mix it thoroughly on the hot plate.

After all agars had solidified, the lid of the plate was placed and the plates were kept in the oven at 60°C for storage until used. The test bacteria were grown in Nutrient Broth (NB). NB was prepared by dissolving 8 g of NB powder 1L of distilled water. The solution was mixed thoroughly using hot plate. The media were then sterilized in the autoclave at 121 °C for 15 minutes. Using 1000 µl sterile micropipette, 3 ml of NB is transferred into the Bijou bottles each. The broth is kept inside refrigerator at 4 °C until used.

3.5.2 Fungal Inoculation

Fungal inoculums were prepared by punched the fragment of the endophytic fungi at the edge of the fungal growth about 5 mm in diameter using sterile tube. The segment then was inoculated on sterile PDA growth medium. The plate was incubated for 2 days at room temperature to ensure the optimum time for fungal growth before inoculate the test bacteria.

3.5.3 Preparation of Test Bacteria Inoculums for Indicator Strains

Single colony of target bacteria from stock agar plate was isolated with a sterile wire loop and transfer to a sterile Nutrient Broth (NB). The broth was incubated at 37 ºC for 24 hours until it achieves the turbidity of 0.6 McFarland standards. After incubation, about 100 µl of test bacteria were pipette into 2 ml of soft Nutrient Agar kept at 50 ^{0}C. The target bacteria were then inoculated on endophytic fungal inoculated plates by evenly overlaid the NA soft agar on dried surface of the plate. The plates were incubated at room temperature for 24 hours.

After 24 hours, the plates were examined to evaluate the antibacterial activity of endophytic fungi towards the target bacteria by examined their inhibition zone. The zone of inhibition was examined and measured.

3.6 Antifungal Activity Evaluation

Fungi inoculum was prepared by punching 5mm in diameter agar disc from the edge of the endophytic fungi using sterile tube. The fungi inoculums' agar disc was then carefully transferred and arranged evenly on the surface of the fresh Potato Dextrose Agar plate (24 mm from one inoculum to another). The plate was incubated for 2 days at room temperature to ensure the optimum time before applying the test fungus. After 2 days,

about 5mm in diameter of test fungal from stock agar plate was isolated with a sterile wire loop and cultivated on the endophytic fungi inoculated plate. The plate was then incubated at room temperature up to 3 days.

After 4 days, the plates were examined to evaluate the antifungal activity of endophytic fungi towards the indicator strain by examined their antagonism.

3.7 Identification of Endophytic Fungi

The fungal isolates were identified based on their morphological and reproductive characters using standard identification manuals (Barnett and Hunter, 1972) through light microscope. The fungal cultures that failed to sporulate were categorized as sterile mycelia.

3.7.1 Fungal Slide Culture

In order to accurately identify the fungi, a simple method of slide culturing allows fungi to be studied with as little disturbance as possible. The blocks of agar of the selected fungi was cut at the edge of the actively growth fungi by using a sterile straight wire inoculation, and placed on the slide in a Petri dish. Then sterile cover glass is placed on the agar block. The slide is incubated at room temperature for 4-7 days until growth and sporulation have occurred.

3.8 Methanol Extraction of Metabolites

Each of the potential fungal endophytes was cultivated on fresh Potato Dextrose Agar plate by placing agar blocks of actively growing edge endophytes. The plates were then incubated at room temperature for 5-7 days. After the incubation period, the cultures were dried inside the fume hood. The plates were placed upside down and partially lid opened.

After the cultures are fully dried, the cultures were taken out and were grind by using sterile pastel and mortar. The fungal metabolites were then extracted by solvent extraction procedure where the cultures were dissolved in 20-30 ml of methanol in 250 ml Erlenmeyer flasks. Next, the cultures were filtered through sterile filter paper. The filtrate was allowed to dry and methanol was allowed to evaporate to obtain the crude extract.

3.9 Antibiotics Sensitivity Test

Endophytic fungi susceptibility test was test by a modification of Kirby-Bauer method described by (Benson, 1998) and agar cup disc diffusion method (Hugo and Russell, 1992) was followed. The indicator strains used as described in Table 1.

3.9.1 Disc Diffusion Method

The test bacteria for indicator strains were inoculated in Muller-Hinton Broth (MHB) at 37 ^{0}C for overnight. After overnight incubation, optical density value of 0.16 was obtained using spectrophotometer at wavelength 550 nm. After incubation, the bacteria were then inoculated on dried surface of a Mueller-Hinton Agar plate by streaking the sterile swab over the entire sterile agar surface. The inoculums is incubated for 10-15 minutes to ensure any excess surface moisture to be absorbed and to ensure confluent lawn of bacteria growth before applying the test disc. Next, six sterile paper discs of 6 mm in diameter were placed and arranged evenly on the surface of the agar plate using a sterile forceps. Each of the sterile paper disc was impregnated with 10 µl of the crude metabolites extracted from endophytes. Distilled water was included as negative control, whiles a positive control by using *Penicillium streptomyces*. The plate was then incubated at 37 ºC for 24 hours.

3.9.2 Agar Cup Diffusion Method

Similar inoculation procedure was followed with the disc diffusion of Kirby-Bauer method, whereby the MHA's agar plates were inoculated with each indicator strains suspension. The plates with the inoculated organisms were evenly spread out with sterile cotton swabs. Agar cups were prepared by scooping out the media with a sterile tube (7mm in diameter). The cups were then filled with 10µL of the crude extract. The plates were then incubated at 35℃ overnight.

After the incubation, antibiotic sensitivity was expressed as the diameter of the inhibition zone (mm) produced by the extracts.

4.0 RESULTS

The wild grass plants around UNIMAS West Campus were selected for this endophytic study was identified as *C. rotundus* (Family Cyperaceae) (Table 2). All wild grasses samples that were cultured were found to be colonised by endophytes. Altogether eight fungal endophytes were isolated from the wild grass samples using Potato Dextrose Agar medium.

Table 2: Wild grass plant selected for endophytic studies.

Plant name	Family	Local Name	Medicinal Properties
Cyperus rotundus	Cyperaceae	Purple nutsedge	Used in treatment of fever, stomachache, diarrhea, treat bacterial infection, pain reduction and muscle relaxtion.

4.1 Antibacterial Activity Screening of Endophytic Fungi

A total of eight endophytic fungi were isolated from the grass samples. From the eight endophyte strains that were screening for antibacterial activity, five strains displayed antimicrobial activity inhibiting at least one indicator strains. Among the five strains, three displayed both antibacterial activities against Gram-negative and Gram-positive bacteria.