

CHAPTER ONE

1. INTRODUCTION

1.1. Introduction

In spite of great advances of modern scientific medicine, traditional medicine is still the primary form of treating diseases of majority of people in developing countries. The number of people using one form or another of complementary or alternative medicine is rapidly increasing worldwide (Thomas, 1995).

In African and other developing countries traditional medicine from plants contributes to form the basis of rural medicine care. In general traditional medicines are easily available and cheaper (WHO, 2002; Dole, 2004).

In Sudan, there are vast area and diverse flora, and most of inhabitants rely solely on medicinal plants particularly for various diseases.

The number of medicinal plants around the world is ranging between 250.000 to 300.000 and more than two third of these plants is growing in developing countries, actually just about 51.000 strains were studied for medicinal values (Begum *et al*, 2002).

In recent years, the use of plants with preventive and therapeutic effects contributes to health care needs (Hotez *et al*, 2002).

According to World Health Organization (WHO, 2002) plants are source of compounds that have the ability to combat disease, and have antimicrobial, antiviral and antifungal activities (Abeysinghe, 2010).

Fungal infection of the paranasal sinuses is an increasingly recognized entity, both in normal and immuno-compromised individuals (Lulu, 1996).

Invasive infections can cause tissue invasion and destruction of adjacent structures (e.g. orbit, Central Nervous System (CNS)). However, the more serious infection commonly occurs in patients with diabetes or in individuals who are

immunocompromised and is characterized by its invasiveness and tissue destruction (Devyani, 2015).

On the other hands, antimicrobial resistance that have been reported to be on increase due to inappropriate or wide spread overuse of antimicrobials (Olila *et al*, 2001 and Pawar and Nabar 2010).

The available treatment of fungal sinusitis includes azoles (Itraconazole) and (voriconazole) and polyenes (amphotericin B) (David *et al*, 2012).

1.2. Rationale

Exposure to *Aspergillus species* can cause adverse human health effects, although conventional antifungal drugs available include azoles groups (itraconazole and voriconazole) and polyene amphotericin B in considerable quantities, increase resistance to these drugs can result in treatment failure.

Therefore, there is a crucial need for discovery of new safer, and more effective antifungal agents from traditional Sudanese plants, and to verify the claimed activity of these plants to treat the infectious diseases.

1.3. Objectives

1.3.1. General objective

To determine antifungal activity of *Commiphora myrrha* against *Aspergillus species* isolated from fungal sinusitis among Sudanese patient.

1.3.2. Specific objectives

1. To isolate and identify the *Aspergillus species* from fungal sinusitis.
2. To determine the antifungal activity of *Commiphora myrrha* methanol, petroleum ether, volatile oil and aqueous extracts against *Aspergillus species*.
3. To determine the minimum inhibitory concentrations (MICs) and minimum cidal concentration (MCCs) of selected plant and to compare their activity with the commonly used antifungal agents in Sudan.
4. To identify the major chemical components of the most effective extracts of the tested plants.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. The medicinal plants and their traditional uses

In spite of great advances of modern scientific medicine, traditional medicine is still the primary form of treating diseases of majority of people in developing countries; even among those to whom western medicine is available, the number of people using one form or another of complementary or alternative medicine is rapidly increasing worldwide (Yadu *et al*, 2012).

Ancient Chinese and Egyptian papyrus writings describe medicinal uses for plants as early as 3000 BC. Indigenous cultures (such as African and Native American) used herbs in their healing rituals, while other developed traditional medical systems (such as Ayurveda and Traditional Chinese medicine) in which herbal therapies were used. The researchers found that people in different parts of the world tended to use the same or similar plants for the same purposes (Steven and Ehrlich, 2011).

During the 8th until the 11th centuries; Arab physicians upgraded the existing knowledge about herbs and their potential medical efficacy and safety. Their greatest contributions to modern medicine were the immune system and introduction of microbiological science (Azaizeh *et al*, 2008).

The World Health Organization (WHO, 2002) estimates that about 80% of the populations living in the developing countries rely almost exclusively on traditional medicine for their primary healthcare needs (Yadu *et al*, 2012).

In Africa the application of herbs in internal and external uses has been a major factor in the medicine. In Sudan the stimulation is very similar to that in African countries, the overall effect of any scientific will help variously to establish and confirm the credibility of the use of herbs and medicinal plants as an effective source of both traditional and modern medicine (Almagboul *et al*, 1985).

In Ethiopia, medicinal plants have been used as traditional medicine to treat different human ailments by the local people from time immemorial. These medicinal plants are estimated to be over 700 species and most of them are confined to the south western regions of the country (Yinger and Yewhalaw, 2007).

In Latin American countries, herbal medicine is deeply rooted, practiced extensively by indigenous groups and frequently used by abroad cross-section of larger society. Often it is an economically inevitable alternative to expensive western medicine (Bussmann and Sharon, 2006).

In recent years, Over 50% of all modern drugs are of natural product origin and they play an important role in drug development programs of the pharmaceutical industry. Epidemiological evidence suggests that dietary factors play an important role in human health and in the treatment of certain chronic diseases including cancer (Yadu *et al*, 2012).

Some of traditional medicines are still included as part of habitual treatment of various maladies for example: The resins of *Commiphora* species have emerged as a good source of the traditional medicines for the treatment of inflammation, arthritis, obesity, microbial infection, wound, pain, fractures, tumor and gastrointestinal diseases. The resin of *C. mukul* in India and that of *C. molmol* in Egypt have been developed as anti-hyperlipidemia and antischistosomal agents (Taoshen *et al*, 2012). Olive leaf extract has strong antibacterial, antifungal, antimicrobial, antiviral, antiparasitic and antioxidant properties. It is often used as a natural defense against the onset of a cold or flu with good results, and has also been demonstrated to have blood sugar lowering effects. Henna- though most commonly used as natural hair and skin dye, Henna also contains a compound called lawsone, which demonstrates antibacterial and antifungal activity (Safaa, 2014).

2.2. Myrrha gum *Commiphora myrrha* (Almurr alhigazy)

Family: *Burseraceae*

Scientific Name(s): *Commiphora myrrha* (T. Nees) Engl., *Commiphora abyssinica* (Bevg.) Engl., or *Commiphora molmol* Engl.

Common Name(s): African myrrha (*C. habessinica*), Somali Myrrha (*C. molmol*), Arabian and Yemen myrrha (*C. abyssinica*), myrrha , myrrhe , gum myrrha , bola , bal , bol , heerabol_(Blumenthal *et al*, 1998).

2.2.1. Distribution:

Commiphora species are native to Africa, Eastern Mediterranean countries and South Arabia (Evans, 1989).

2.2.2. Botanical description

The *Commiphora* species that serve as source of myrrh, are thorny shrubs or small trees that grow to up to 3 meters high. A pale yellow-white viscous liquid exudes from natural cracks or fissures in the bark or from fissures cut intentionally to harvest the material. When air-dried, this exudates hardens into a reddish-brown mass that often contains white patches. These tears are approximately the size of a walnut and from the basis of myrrh resin. Myrrh is usually collected in the summer months (Leung, 1980; Evans, 1989; Michie and Cooper, 1991).

2.2.3. Chemical constituents

Myrrha is an oleo-gum-resin obtained from the stem of *C. molmol* (Evans, 1989; Michie and cooper, 1991). It contains 2% to 10% of a volatile oil composed predominantly of sesquiterpenes, sterols, and steroids. Water soluble gum portion (30%- 60%) contains polysaccharides and proteins as well as ethanol-soluble resins (25%-40%). After undergoing hydrolysis the gum produces a variety of sugars. Furanosesquiterpenes are responsible for myrrhs odor and are believed to exert anesthetic, antibacterial, antifungal, and hypoglycemic effect (Hanus *et al*, 2005;

Zhu *et al*, 2003). When oleo-gum-resin is mixed with water it forms an emulsion (El Ashry *et al*, 2003).

2.2.4. Antimicrobial activity of *Commiphora myrrha*

Extracted, purified, and characterized 8 sesquiterpene fractions from *C.molmol* were a mixture of furanodiene-6-one and methoxyfuranoguaia-9-ene-8-one, which showed antibacterial and antifungal activity against standard pathogenic strains of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*, with minimum inhibitory concentrations ranging from 0.18 to 2.8 mg/ml. These compounds also had local anaesthetic activity, blocking the inward sodium current of excitable mammalian membranes. (Dolara *et al*, 2000).

The antifungal activity of Myrrha (*Commiphora molmol*) essential oil was tested against three s fungi, *Aspergillus flavus*, *Aspergillus niger* and *Penicillium citrinum*, Results showed inhibitory effect of the oil against tested fungi with the increasing concentrations. Minimum inhibitory concentration (MIC) using agar dilution method was 4% (v/v) for *A. niger* and *A. flavus* and above 4% (v/v) for *P. citrinum* (Batool, 2007).

2.2.5. Medicinal uses

The Arabic term "murr" means "bitter" and describes myrrh taste and balsamic odor (Michie and Cooper, 1991; El Ashry *et al*, 2003). Myrrha has been used for centuries as incense (Evans, 1989), and for medicinal purposes (Michie and Cooper, 1991), medicinally, it has been used as an astringent, antiseptic, anti parasitic, antitussive, emmenagogue, and antispasmodic agent. It was commonly included in mixtures used to treat worm, wounds, sepsis, cough in children, skin and mouth infections, and as suppository form to treat proctitis (Michie and Cooper, 1991).

Myrrh has also been reported to treat gout, headache, jaundice, throat ailments indigestion, fatigue, and paralysis (Greene, 1993). It has also been used in variety of

infectious diseases, including leprosy and syphilis and to treat cancers (El Ashry *et al*, 2003).

2.3. Genus *Aspergillus*

Aspergillus species are ubiquitous saprobes in nature (Geo *et al*, 2007). *Aspergillus species* are highly aerobic and are found in almost all oxygen-rich environments, where they commonly grow as molds on the surface of a substrate, as a result of the high oxygen tension (Geiser, 2009). The mold is a multi-cellular organism measuring 2-10 μm in diameter. These organisms grow by branching into structures termed hyphae. Another important component of the fungal organism is the spore. The spore is a reproductive structure that can be produced in the presence of unfavorable conditions. These spores can withstand many adverse conditions, and are dispersed widely throughout the environment. Once these spores are exposed to a favorable environment, they begin to grow. Inhalation of spores is thought to be the primary means by which fungal organisms gain access to the sinonasal tract. (David *et al*, 2012).

2.3.1. Identification of *Aspergillus species*

Generally basic and essential tool for identification of *Aspergillus species* are macroscopic characteristics such as colony diameter, conidial color, exudates, colony reverse and microscopic characteristics including conidiophore, vesicle, metulae, phialides and conidia (Raper and Fennell, 1965; McClenny, 2005; Diba *et al*, 2007 and Domsch *et al*, 1980). Although molecular methods continue to improve and become more rapidly available, microscopy and culture remain commonly used as essential tools for identification of the fungi like *Aspergillus species* (Diba *et al*, 2007).

A survey by the American Society for Microbiology (ASM) documented that 89% of laboratories performing mycological examination (morphology based), 16% of them use serological tests and fewer than 5% use molecular test for identification of

microbial pathogens (ASM, 2004). Only 3% of reporting laboratories use home molecular testing for microbial pathogens (Warris *et al*, 2001).

2.3.2. Generic description

Colonies usually growing rapidly, powdery, white, green, yellowish, brown or black. Conidiophore erect, unbranched, with swollen apical vesicles and without septum at the base; the part of the supporting hyphae which is continuous with the stipe is known as the foot cell. Phialides borne directly on the vesicle (uniseriate) or on subtending metulae (biseriate); phialide openings without collarettes. Conidia produced in dry chains, forming columns (columnar) or diverging (radiate), from flask-shaped phialides, 1-celled, smooth walled or ornamented, (sub) hyaline or pigmented (Hoog *et al*, 2000).

2.3.3. Classification of *Aspergillus*

Aspergillus is a member of the Deuteromycetes fungi, which is a group with no known sexual state. With DNA evidence forthcoming, all members of the genus *Aspergillus* likely are closely related and should be considered members of the Ascomycota (Geiser, 2009).

Aspergillus divided into eighteen groups viz., *Aspergillus clavatus*, *Aspergillus glaucus*, *Aspergillus ornatus*, *Aspergillus cervinus*, *Aspergillus restrictus*, *Aspergillus fumigatus*, *Aspergillus ochraceous*, *Aspergillus niger*, *Aspergillus candidus*, *Aspergillus flavus*, *Aspergillus wentii*, *Aspergillus cremeus*, *Aspergillus sparsus*, *Aspergillus versicolor*, *Aspergillus nidulans*, *Aspergillus ustus*, *Aspergillus flavipes* and *Aspergillus terreus* (Hina *et al*, 2013).

2.3.4. Fungal sinusitis

The pattern of fungal infection of the sinuses has been traditionally divided into invasive and noninvasive, based on the presence of fungal hyphae in the tissue with associated granulomatous reaction or tissue necrosis. Classifieds sinus aspergillosis into four types: noninvasive extra mucosal disease, which includes allergic fungal

sinusitis and aspergilloma, and invasive mucosal disease, which includes indolent, chronic sinusitis and fulminant (acute) sinusitis. This classification has provided a useful morphologic basis for diagnosis and has been found to have excellent prognostic and therapeutic correlations (Hartwick and Batsakis, 1991).

2.3.5. Prevalence and transmission

Aspergillus species produces abundant small conidia that are easily aerosolized. Following inhalation of these conidia, atopic individuals often develop severe allergic reactions to the conidial antigens. In immunocompromised patients especially those with leukemia, stem cell transplant patients, and individuals taking corticosteroids, the conidia may germinate to produce hyphae that invade the lung and other tissues (Geo *et al*, 2007).

In immune competent host, various factors may cause fungi to settle in mucus plug of sinuses, sinus obstruction with impaired ventilation and prolonged exposure to large inocula of spores, dusty arid conditions facilitate growth of saprophytic fungi. Climate appears to be an important factor as higher incidence of fungal sinusitis has been reported from areas which have warm and dry climate. Chronic invasive sinusitis is endemic in Sudan (Midhat *et al*, 2012).

2.3.6. Clinical manifestation

Fever is one of the most common signs of initial infection as it is seen in 90% of cases of fungal sinusitis. Other signs and symptoms include rhinorrhea, nasal congestion, facial pain, facial numbness, diplopia, headaches, seizures, cranial nerve deficits, and ulcerations of the nasal, facial, or palatal mucosa. As the disease can result in mortality in days, it must be recognized early. Any immunocompromised patient with fever and one other sinonasal symptom should undergo evaluation for fungal sinusitis (David *et al*, 2012).

2.3.7. Treatment

The treatment involves a combination of medical and surgical therapy. Systemic antifungals should also be started. amphotericin B used but Close monitoring of renal function is imperative at this time as 80% of patients will suffer nephrotoxicity. A lipid-based form of amphotericin B is available, but is more expensive. However, it has less side effects, and higher concentrations of the drug can be maintained. Less toxic antifungals such as voriconazole and itraconazole can be used (David *et al*, 2012).

2.4. Antifungal susceptibility testing

Antimicrobial susceptibility testing methods for fungi have recently been standardized by Clinical and Laboratory Standard Institutes (CLSI), formerly known as the National Committee for Clinical Laboratory Standards (NCCLS, 2000). The CLSI first published methods for antifungal susceptibility testing of *Candida species* and *Cryptococcus neoformans* in 1997, where as the methods for filamentous fungi were approved in 2002. A macro dilution method for susceptibility testing was approved first, quickly followed by a micro dilution method that is simpler and equally effective. It is important to note that only non propriety methods are standardized by the CLSI. Thus, some methods including colorimetric or E-test methods, although shown studies to compare with the micro dilution methods will not be standardized by the CLSI. Although in some instances proprietary tests may be more costly, they are often simpler and more easily implemented in laboratories with little experience working with fungi. Recently, a disk diffusion method for testing yeast has also been approved by CLSI (NCCLS, 2004).

Several important factors have been evaluated and standardized in these published guidelines including the use of synthetic media, temperature of incubation, duration

of incubation, and endpoint definition. Several review articles have been published, providing more details than can be discussed here (Rex *et al*, 2001).

2.4.1. Antifungal susceptibility testing methods

A. Broth dilution

The aim of broth and agar dilution methods determined the lowest concentration of assayed antimicrobial agent (minimal inhibitory concentrations, MICs) which, under defined test conditions, inhibits the visible growth of *Aspergillus* colonies were being investigated. MICs values are used to determine susceptibilities of fungi to drugs and also to evaluate the activity of new antimicrobial agents. The most commonly used method in dilution techniques is the broth dilution technique, which described by Rahman *et al*, (2001).

Broth dilution methods for antifungal can be performed by macro dilution or micro dilution methods (NCCLS, 2002). The macro dilution method is prepared in test tubes in 1 ml volumes. This method has been established as the basis for comparing all other methods of susceptibility testing for yeast.

The macro dilution method has largely been replaced by the micro dilution method that is performed in a 96-well micro dilution plate in volumes of 200 µl per well. Both methods use a starting inoculum on $0.5-2.5 \times 10^3$ colony-forming units CFU/ml for *Candida spp*. The plates are incubated at 35°C for 48 or 72 hr for *Candida species* and *C.neoformans* respectively. The minimum inhibitory concentration (MIC) end point differs among the antifungal agents currently approved. The endpoint for Amphotericin B is defined as the lowest concentration resulting in no visible growth, whereas the end point for Azole antifungals is when either 80 or 50% reduction in fungal growth reduction occurs for the macro dilution and micro dilution methods, respectively. Trailing has been described when micro dilution methods are performed on *Candida species* to evaluate the MIC of the azole antifungals. Trailing is reduced but persistent growth of organism even in the

presence of very high concentration of azoles. This persistent growth can cause confusion when reading the MIC endpoint for azoles against *Candida species*. Not all *Candida* display these phenomena and it may not be apparent at 24 hr but may be visible when the endpoint is read at 48hr. the trailing effect can be minimized by decreasing the pH of the culture medium or the addition of dextrose and starting with higher inoculums. The methods approved for testing common filamentous fungi include *Aspergillus species*, *fusarium species*, *Rhizopus species*, *Pseudallescheria byodii*, and *Sporothrix schenkii* (NCCLS, 2002).

B. Colorimetric testing

Colorimetric tests have been developed for both yeast and filamentous fungi (Espinel-Igroff *et al*, 2009). Other methods are spectrophotometric testing, disk diffusion and flow cytometry (Espinel-Igroff *et al*, 2009).

2.4.2. Fungicidal testing

The ability of a compound to kill a pathogen as opposed to simply inhibiting its growth is an apparently desirable quality, particularly in the setting of decreased immunity. Although several studies have characterized the fungicidal activity of antifungal agents, there is no standardized method for doing so the NCCLS has published guidelines for evaluating the cidal activity of antibacterial agents (NCCLS, 1998).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Study design

It was a descriptive cross sectional study.

3.2. Study area

Ear, Nose and Throat (ENT) Hospital, Khartoum.

3.3. Study population

All clinically suspected cases of fungal sinusitis attended the ENT hospital, Khartoum during period of the study from April to October 2015.

3.3.1. Inclusion criteria

Patients with fungal sinusitis.

3.3.2. Exclusion criteria

Patients with history of bacterial or viral sinusitis were excluded.

3.4. Sampling

Non-probability sampling

3.4.1. Sample size

A total of seventy (n= 70) samples were collected.

3.5. Study variables

Screen of *Aspergillus species* as dependent variables. Age, gender, history of disease, and operation number as independent variables.

3.6. Data collection

By questionnaire that contains all study variables (Appendix 1).

3.7. Ethical clearance

Permission of this study was obtained from the local authorities in the area of the study, the objectives of the study clearly and simply explained to all individuals participating in the study, verbal inform consent was obtained (Appendix 1).

3.8. Data analysis

All collected data were analyzed using SPSS (Statistical Package of Social Science) soft program version 16.0.

3.9. Experimental Work

3.9.1. Specimen collection and sampling technique

Nasal biopsy and BronchoAlveolar Lavages (BAL) were collected from the sinuses by endoscopic sinus surgery.

3.9.2. Method of collection

A portion of surgically excised specimen was received in sterile container containing normal saline and sent to Mycology Laboratory on Central health public laboratory (ESTAC) for *Aspergillus species* isolation, identification and screening of antifungal activity of *C.myrrha*.

3.9.3. Microscopic examination of specimen

Specimens were examined microscopically by KOH for fungal hyphae of *Aspergillus species*.

3.9.3.1. Wet preparation

On a clean slide, a drop of 20% KOH mount preparation and piece of specimen placed and covered with cover glass and examined under microscope using x10 and x40 for fungal hyphae of *Aspergillus species* (Gamba *et al.*, 1986; Manning *et al.*, 1997). And suspected to find hyphae of *Aspergillus species* are hyaline, septate and uniform in width (about 4µm) and branch dichotomously (Geo *et al.*, 2007).

3.9.4. Culture:

The tissue specimens were minced into small pieces (0.5-1 mm in diameter) using sterile scalpel. Culture was done on Sabouraud's Dextrose Agar (SDA) (HIMEDIA Lab, India) with Chloramphenicol 0.05 mg/ml (Alexandria Co. Egypt) and incubated at 37⁰ C for 2weeks for growth of *Aspergillus species* (Gamba *et al.*, 1986; Manning *et al.*, 1997).

3.9.5. Identification of *Aspergillus* species

3.9.5.1. Macroscopic examination

The cultures were examined for rate of growth, colonial morphology, pigment production, texture, and color of surface or reverse (Alessandro, 2010).

3.9.5.2. Microscopic examination

The microscopic examination was done by Needle mounts technique.

3.9.5.2.1. Needle mounts technique

A drop of 70% alcohol was placed on a slide then a small amount of the colony surface was immersed in a drop of alcohol (Alessandro, 2010), then one drop of lactophenol cotton blue (LPCB) mountant was added, the preparation covered with cover glass avoiding air bubbles then this preparation was microscopically examined (Astrid, 1999).

3.10. Extraction of *C. myrrha*

3.10.1. Collection and preparation of *C. myrrha* sample

The myrrha gum (*Commiphora myrrha*) samples used in this study were collected from local market in Khartoum State, identified and authenticated by the Medicinal and Aromatic Plant Research Institute (MAPRI). The dried myrrha was cleaned then separately crushed to a powder form using mortar and pestle.

3.10.2. *C. myrrha* extraction

3.10.2.1. Preparation of the crude extract

Extraction was carried out according to method described by Sukhdev *et al*, (2008). One hundred grams of the sample were grinded using mortar and pestle and extracted with hexane and methanol 350 ml and Petroleum ether 350 ml using soxhelt extractor apparatus. Extraction was carried out for about six hours till the solvent returned colorless at the last siphoning times. Solvent was evaporated under reduced pressure using rotary evaporator apparatus. Finally the extract was allowed

to dry by air in Petri dishes till complete dryness and the yield percentage was calculated as follows:

Weight of extract obtained / weight of plant sample X 100

3.10.2.2. Distillation of Volatile oil:

One hundred grams of the *C.myrrha* was placed in 1000 ml rounded bottom capacity flask. 1000 ml of distilled water on Clevenger receiver (Duran West Germany) and condenser attached to the top of the flask. The system was heated at 100° C for about four hours till the volume of oil above water layer at the receiver constant due to the oil was lighter than water .Oil was pipetted, dried over sodium sulphate anhydrous and stored in a dark container in a refrigerator till used. Yield percentage was calculated as followed:

Volume of oil / weight of plant sample X 100

3.10.2.3. Preparation of the aqueous extract:

Fifty grams of the plant sample was soaked in 1000 ml hot distilled water, and left to cool down with continuous stirring at room temperature. Extract was then filtered and free-zed in a deepfreezer at -20°C. Freezed extract was dried using freeze dryer at - 40°C till powdered extract obtained. Yield percentage was calculated.

3.10.3. Preparation of fungal suspension:

The fungal cultures were maintained on SDA, incubated at 37 °C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspended in sterile normal saline, the suspension was stored at 4°C until used and matched with McFarland standard (contain 10⁸ spore/ml).

3.10.4. Quality control

Each isolate was sub-cultured at least twice on SDA for 24 hours, then used prior to screening of antifungal activity of *C. myrrha*. This was to obtain pure culture of each isolate. The quality control strains used was *Aspergillus niger* ATCC 9763 (MAPRI).

Methanol and petroleum ether as negative control tested against isolated and standard *Aspergillus species* .

3.11. *In vitro* Antimicrobial activity of *C.myrrha* extracts

Antimicrobial activity was measured *in vitro* in order to determine:

a. The potency of an antimicrobial agent in solution. Sensitivity tests were also used to evaluate new antimicrobial agents by testing them against large number of organisms of known susceptibility and to compare these results with drugs already available (Garrod *et al*, 1981).

b. Its concentration in body fluids or tissue.

C. The sensitivity of a given microorganism to a known concentration of the drug.

3.11.1. Measurement of antifungal activity

Determination of these quantities was undertaken by one of two methods:

Diffusion or dilution. Using an appropriate standard test organism. These methods can be employed to estimate either the potency of antifungal in the sample or the sensitivity of microorganism.

3.11.1.1. Diffusion methods

In the diffusion technique, the tested organisms were exposed on SDA, and then sterile filter paper disc (by oven 180°C for 1 hour) was mounted on the agar, the disk was impregnated by diffusion gradient of chemotherapeutic drug arising from a reservoir (Anderson, 1970; Garrod, 1981).

3.11.1.2. Dilution methods:

Dilution tests on solid media involved addition of series concentrations of *C. myrrha* extracts (50, 25, 12.5, 6.25, 3.125mg/ml) to 20 ml of SDA 2x2 which had been melted and cooled to 45-50°C, the resultant mixtures are then poured into test tubes. Standardized inoculum was seeded onto the surface of the medium and MIC was read after incubation period at 37°C for 48 hours (Garrod *et al*, 1981).

3.11.2. Determination of Minimum Inhibitory Concentrations (MICs) by agar plate dilution method:

The principle of the agar plate dilution is the inhibition of the growth on the surface of the agar by the plant extracts incorporated into the medium. Plates were prepared in the series of decreasing concentrations of the plant extraction in the following order 50, 25, 12.5, 6.25, 3.125 mg/ml. The bottom of each plate was marked off into 5 segments. The organisms tested were grown in broth to contain 10^8 spore/ml for 48 hours. Loop-full of diluted culture is spotted with a standard loop that delivers 0.001 ml on the surface of each segment and then incubated at 37 °C for 48 hours.

The end point (MIC) is the least concentration of antimicrobial agent that completely inhibits the growth. Results are reported as the MIC in mg/ml.

3.11.3. Determination of Minimum Cidal Concentration (MCCs):

To determined MCCs: after the first incubation period all tubes were sub cultured on SDA, then the plates were placed in an incubator at 37°C for 24-48 hrs, after incubation period the lowest concentration of plant extracts which gave sterile culture was regarded as MCCs.

3.11.3.1. MICs

The lowest concentration of plant extract that did not permit any visible growth of the inoculated test organism in broth culture was regarded as MIC in each case.

3.11.3.1. MCCs

The lowest concentration of plant extract which gave sterile culture after incubation period was regarded as MCCs.

3.12. Gas chromatography screening

The general analysis by Gas chromatography screening for the active constituents was carried out for the most effective methanol extract of *Commiphora myrrha* using the Gas Chromatographic (GC).

In gas chromatography, the mobile phase (or moving phase) was a carrier gas, usually inert as an unreactive gas such as nitrogen. The stationary phase was a microscopic layer of liquid or polymer on an inert solid support inside a piece of glass or metal tubing called column (a homage to the fractionating column used in distillation).

The instrument used to perform gas chromatography was called chromatograph (or Aerograph, gas separator). The gaseous compound being analyzed interacts with the walls of the column, which was coated with stationary phase.

This causes each compound to elute at a different time, known as retention time (RT) of the compound. The comparison of retention times is what gives GC its analytical usefulness (Safaa, 2014).

CHAPTER FOUR

4. RESULTS

In the present study *Aspergillus flavus*, *Aspergillus fumigatus*, and *Aspergillus terreus* were isolated and identified.

4.1. Identification of molds

4.1.1. Growth on Sabouraud's Dextrose Agar (SDA)

Aspergillus flavus was identified by growth as green colonies (Fig1), *A.fumigatus* by growth as blue-grey colonies (Fig 2), *A.terreus* by growth as pale yellow (beige) (Fig 3), and *A.niger* by growth as black (Fig 4) and (table 1).



Fig 1: Growth of *Aspergillus flavus* on SDA



Fig 2: Growth of *A. fumigatus* on SDA



Fig 3: Growth of *A. terreus* on SDA



Fig 4: Growth of *A.niger* on SDA

Table 1: Microscopic features of *Aspergillus species* on LPCB mount at X40

<i>Aspergillus</i> species	Stipes colour	Surface Of stipe	Vesicle serration	Conidial head	Shape	Conidia surface
<i>Aspergillus</i> <i>flavus</i>	Pale brown	finely roughened	Biseriate	Radiate to columnar	Globose or ellipsoid	finely roughened
<i>Aspergillus</i> <i>fumigatus</i>	Grayish	Smooth walled	Uniseriate	Columnar	flask shape	Smooth
<i>Aspergillus</i> <i>terreus</i>	Uncolored	Smooth walled	biseriate	Compact columns	Globose	Smooth walled
<i>Aspergillus</i> <i>niger</i>	Slightly brown	Smooth walled	Biseriate large in size	Radiate	Globose	Very rough irregular

4.2. Frequency of sinusitis and percentage of sampling according to Age of the patients

Out of 70 specimens, 16 samples were taken from patients with an age of 1-20 years, 29 samples were taken from patients with an age of 21-40 years, 19 samples were taken from patients with an age of from 41-60 years, and 6 samples with an age of 61-80 years, as shown in Fig 5.

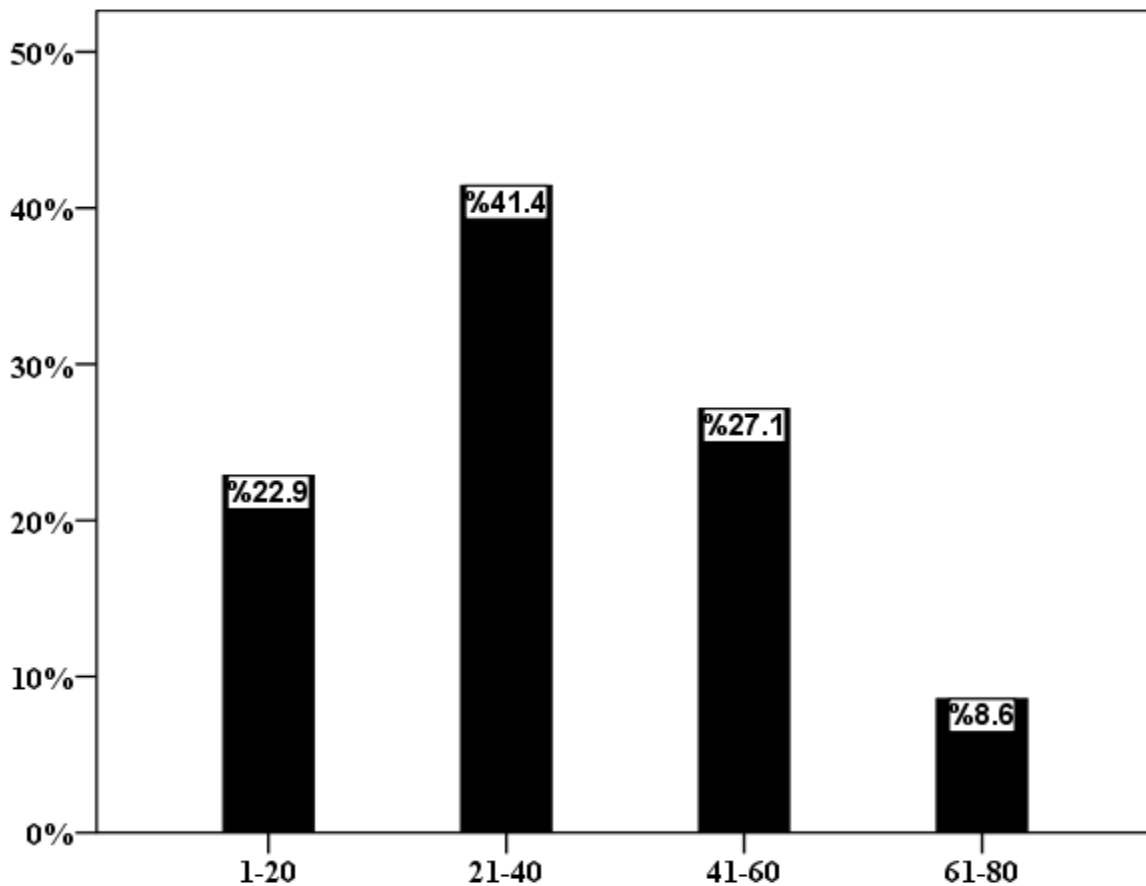


Fig 5: Frequency of sampling according to age of the patients

4.3. Frequency of sampling according to Gender

Out of 70 specimens, 32(45.7%) were males, whereas 38(54.3%) were females, as shown in table 2.

Table 2: Frequency of Sampling according to Gender

Gender	Frequency	Percentage
Male	32	45.7%
Female	38	54.3%
Total	70	100.0%

4.4. Frequency and percentage of *Aspergillus* growth

Out of 70 specimens, 30 (42.9%) showed no growth, while 40 (57.1%) showed the growth of *Aspergillus species*, as shown in table 3.

Table 3: Frequency and percentage of *Aspergillus* growth

Isolate	Frequency	Percentage
No growth	30	42.9%
Growth	40	57.1%
Total	70	100.0%

4.5. Frequency and percentage of tested *Aspergillus species*

Three different *Aspergillus species* were isolated from nasal biopsy. *Aspergillus flavus* 33(47.1%), *Aspergillus fumigatus* 3(4.3%), and *Aspergillus terreus* 4(5.7) as shown in Fig 6.

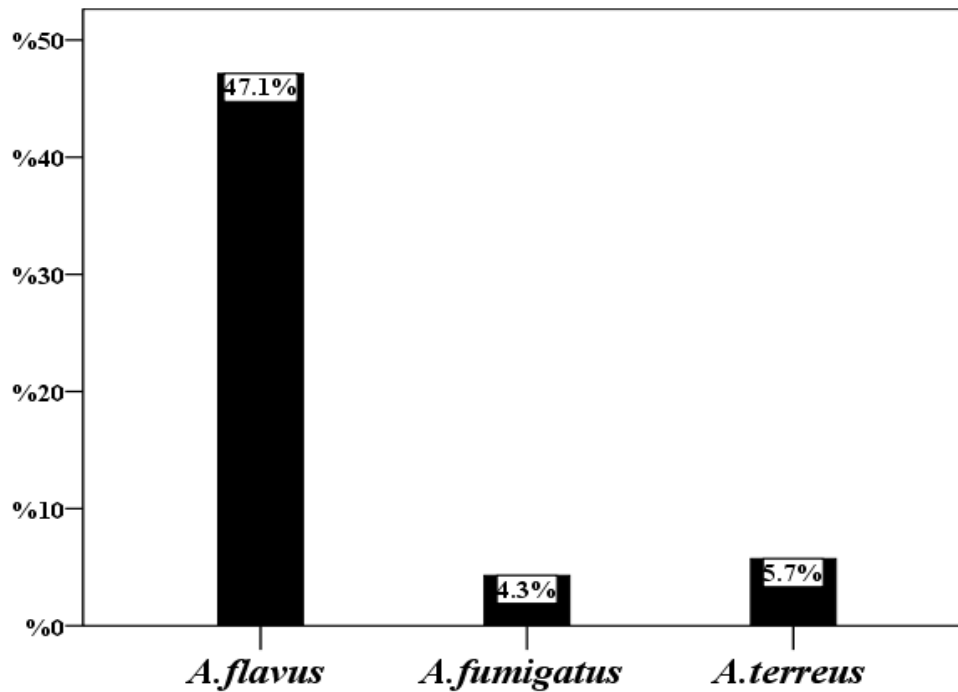


Fig 6: Frequency and percentage of tested *Aspergillus species*

Table 4: Frequency and percentage of *Aspergillus species* according to the type of sample

Sample	Isolated fungi				Total
	<i>A.flavus</i>	<i>A.fumigatus</i>	<i>A.terreus</i>	Negative	
Nasal biopsy	33(51.6%)	3(4.69%)	4(6.25%)	24(37.5%)	64(100.0%)
Broncho alveolaer lavage	0(0%)	0(0%)	0(0%)	6(100%)	6(100.0%)
Total	33(47.1%)	3(4.29%)	4(5.71%)	30(42.9%)	70(100.0%)

4.6: Frequency and percentage of tested *Aspergillus species* according to Gender

Out of 40 isolated *Aspergillus species*, 18 were isolated from males, and 22 were isolated from females as shown in table 5.

Table 5: Frequency and percentage tested of *Aspergillus species* according to gender

Gender	Isolated fungi			Total
	<i>A.flavus</i>	<i>A.fumigatus</i>	<i>A.terreus</i>	
Males	15(83.3%)	1(5.5%)	2(11.1%)	18(100.0%)
Females	18(81.8%)	2(9.0%)	2(9.0%)	22(100.0%)
Total	33(82.5%)	3(7.5%)	4(10%)	40(100.0%)

4.7: Frequency and percentage tested of *Aspergillus species* according to history of disease

Out of 40 isolated *Aspergillus species*, *A.flavus* was isolated 7times from 1 year and 2 month, 5 time from 2 year, 3 time from 10 year , 2 times from 3 years, 4years, 7 months and 8 years ,*A. fumigates* was isolated 2 time from 3 years and 1time from 8years, *A.terreus* was isolated 1 time from 1 year,3 years, 7 years and 9 years, as shown in table 6.

Table 6: Frequency and percentage tested of *Aspergillus species* according to history of disease

History of disease	Isolated fungi			Total
	<i>Aspergillus flavus</i>	<i>Aspergillus fumigatus</i>	<i>Aspergillus terreus</i>	
1 year	6(85.7%)	0(0%)	1(14.2%)	7(100%)
2 year	5(100%)	0(0%)	0(0%)	5(100%)
3 year	2(40%)	2(40%)	1(10%)	5(100%)
4 year	2(100%)	0(0%)	0(0%)	2(100%)
5 year	1(100%)	0(0%)	0(0%)	1(100%)
7 month	2(100%)	0(0%)	0(0%)	2(100%)
7 year	1(50%)	0(0%)	1(50%)	2(100%)
8 year	2(66.7%)	1(33.3%)	0(0%)	3(100%)
9 year	1(50%)	0(0%)	1(50%)	2(100%)
10 year	3(100%)	0(0%)	0(0%)	3(100%)
1 month	1(100%)	0(0%)	0(0%)	1(100%)
2 month	6(100%)	0(0%)	0(0%)	6(100%)
5 month	1(100%)	0(0%)	0(0%)	1(100%)
Total	33(82.5%)	3(7.5%)	4(10%)	40(100%)

4.8: Distribution of fungal sinusitis according to operation numbers

Distribution of fungal sinusitis in Sudanese patients according to operation numbers is shown in table 7. There was a significant association ($p=0.001$) between operation numbers and fungal sinusitis in the studied groups, as shown in table 7.

Table 7: Distribution of fungal sinusitis according to operation numbers

Isolated fungi	Operation numbers				Total	P.value
	First	Second	Third	Six		
<i>A.flavus</i>	30(90.9%)	3(9.09%)	0(0%)	0(0%)	33(100%)	0.001
<i>A.fumigatus</i>	2(66.7%)	0(0%)	0(0%)	1(33.3%)	3(100%)	
<i>A.terreus</i>	4(100%)	0(0%)	0(0%)	0(0%)	4(100%)	
Negative	26(86.7%)	3(10%)	1(3.3%)	0(0%)	30(100%)	
Total	61(87.1%)	6(8.6%)	2(2.9%)	1(1.4%)	70(100%)	

4.9. Screening of antifungal activity of *C. myrrha*

Table 8: Weight and yield % of *C.myrrha* extract obtained by using different solvents

Weight of <i>C.myrrha</i> gums	Petroleum ether		Methanol		Volatile oil		Aqueous	
	Weight of extract	Yields %	Weight of extract	Yields %	Weight of extract	Yields %	Weight of extract	Yield %
100 gm	8.53 g	8.53%	25.472 g	25.472%	4.9 g	4.9%	17.089 g	34.178%

Methanol (MeOH), petroleum ether (C₆H₁₄) and volatile oil extracts of *C. myrrha* showed antifungal effect against *Aspergillus flavus*, *A.fumigatus*, *A.terreus* and *A.niger* at the concentration 100 mg/ml are shown in Table 9 and Fig (7-12).

Table 9: The means zone of inhibition (mm) of *C.myrrha* against *Aspergillus* species at concentration 100 mg/ml

Solvent used	<i>Aspergillus flavus</i>	<i>Aspergillus fumigates</i>	<i>Aspergillus terreus</i>	<i>Aspergillus niger</i>
MeOH	22	22	24	19
C ₆ H ₁₄	17	12.5	19	16
Volatile oil	28.5	26	26	25
Aqueous	00.00	00.00	00.00	00.00

Note

C₆H₁₄ (petroleum ether),

MeOH (Methanol),

S (Sensitive).

Concentration used 100mg/ml (10%) at 0.1 ml/disk.

Interpretation of the result

Means Diameter of growth Inhibition Zone (MDIZ) in (mm) of the two replicates was as follow:

If MDIZ

- ≥ 18 sensitive
- 15-18 intermediate
- ≤ 14 resistant

Table10: The means zone of inhibition (mm) of Negative control against *Aspergillus species*

Isolated fungi	Methanol	Petroleum ether
<i>Aspergillus flavus</i>	0	0
<i>A.fumigatus</i>	10	0
<i>A.terreus</i>	13	10
<i>A.niger</i>	0	0

4.10. Antifungal activity of reference drugs against *Aspergillus species*

Itraconazole was effective against *Aspergillus terreus* only at a concentration of 100mg/ml, Amphotericin B was effective against *A.flavus* , *A.fumigatus*, *A.terreus* and *A.niger* at a concentration 100mg/ml, as showed in table 11.

Table 11: The means zone of inhibition of reference drugs against *Aspergillus* species

		Microorganisms and the mean zone of inhibition (in mm) (MDIZ) of drugs used			
Drug Used	Conc mg/ml	<i>Aspergillus Flavus</i>	<i>A.fumigatus</i>	<i>A.terreus</i>	<i>A.niger</i> ATCC9763
Itraconazole	100	0	0	15	0
	40	0	0	11	0
	20	0	0	0	0
	10	0	0	0	0
	5	0	0	0	0
Amphotericin B	100	31	27.5	22	28
	40	25	25	18	25
	20	0	22	15	17.5
	10	0	18	0	14
	5	0	12	0	12

Interpretation of the result

Means Diameter of growth Inhibition Zone (MDIZ) in (mm) of the two replicates was as follow:

If MDIZ

- ≥ 19 sensitive

- 18-15 intermediate
- ≤ 14 resistant

4.11. The Minimum Inhibitory Concentrations (MICs) of *C.myrrha* obtained by the broth dilution method

The MICs of *C.myrrha* oil and extracts obtained by the agar dilution method are shown in table 12 and figure 13. Methanol and Volatile oil extracts inhibited *Aspergillus flavus*, *A.fumigatus*, *A.terreus* and standard *Aspergillus niger* at 6.25mg/ml, while Petroleum ether extract inhibited *Aspergillus flavus*, *A.terreus* and standard *Aspergillus niger* ATCC9763 at a concentration 25mg/ml, as showed in table 12.

Table 12: The MICs of *C.myrrha* extracts obtained by the broth dilution method (%W/V)

Solvent used	Concentration	<i>A.flavus</i>	<i>A.fumigatus</i>	<i>A.terreus</i>	<i>A.niger</i>
C₆H₁₄	50	-	+	-	-
	25	-	+	-	-
	12.5	+	+	+	+
	6.25	+	+	+	+
	3.1	+	+	+	+
MeOH	50	-	-	-	-
	25	-	-	-	-
	12.5	-	-	-	-
	6.25	-	-	-	-
	3.1	+	+	+	+
V.oil	50	-	-	-	-
	25	-	-	-	-
	12.5	-	-	-	-
	6.25	-	-	-	-
	3.1	+	+	+	+

Note

C₆H₁₄ (petroleum ether)

MeOH (Methanol)

V.oil (Volatile oil)

A (*Aspergillus*)

(+): visible growth

(-): no visible growth

Concentration used 100mg/ml (10%) at 0.1 ml/disk.

Interpretation of the result

The MICs were determined as the lowest concentration of oil and soxhlet extracts which inhibiting the visible growth of each organism on a tube.

Table 13: The Minimum Cidal Concentration (MCCs) of *C.myrrha* extracts obtained by the agar dilution method

Solvent used and their MCCs in mg/ml	<i>Aspergillus flavus</i>	<i>Aspergillus fumigatus</i>	<i>Aspergillus terreus</i>	<i>A.niger</i>
C₆H₁₄	25	>50	25	25
MeOH	12.5	12.5	12.5	12.5
V.oil	12.5	12.5	12.5	12.5

Note: **C₆H₁₄** (petroleum ether)

MeOH (methanol)

V.oil (volatile oil)

Concentration used 100 mg/ml (10%) at 0.1disk

Interpretation of the result

The MCCs were determined as the lowest concentration of oil and soxhlet extracts used that gave sterile culture after incubation period was regarded as MCCs in each case.

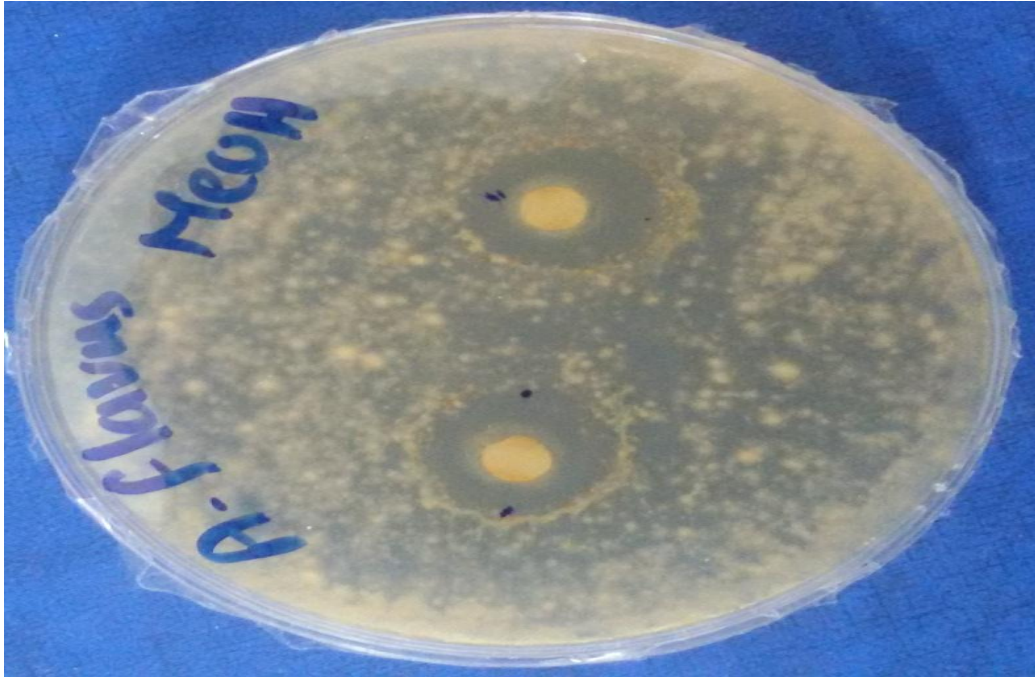


Fig 7: Activity of Methanol extract of *Commiphora myrrha* against *Aspergillus flavus* isolate

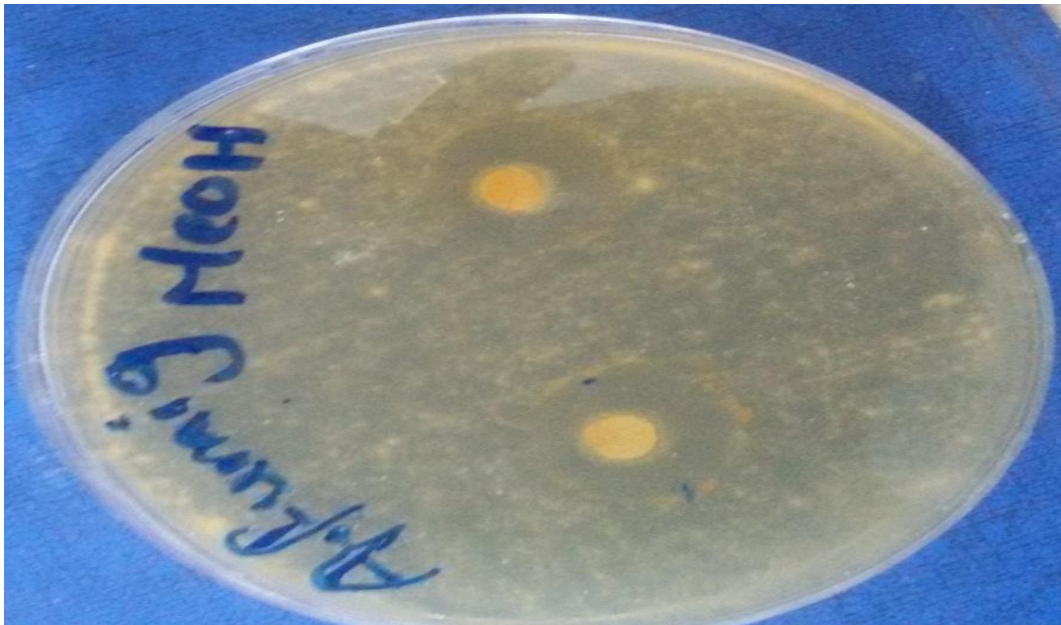


Fig 8: Activity of Methanol extract of *Commiphora myrrha* against *Aspergillus fumigatus* isolate



Fig 9: Activity of Methanol extract of *Commiphora myrrha* against *Aspergillus terreus* isolate

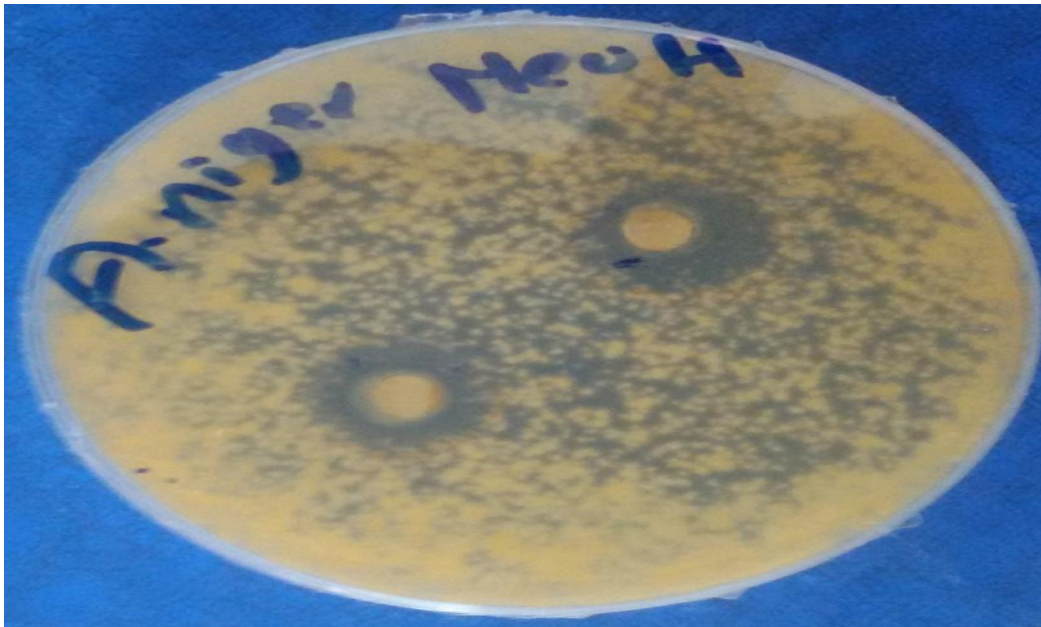


Fig 10: The activity of Methanol extract of *Commiphora myrrha* against *Aspergillus niger*



Fig 11: Activity of Volatile oil extract of *Commiphora myrrha* against *Aspergillus fumigates* isolate

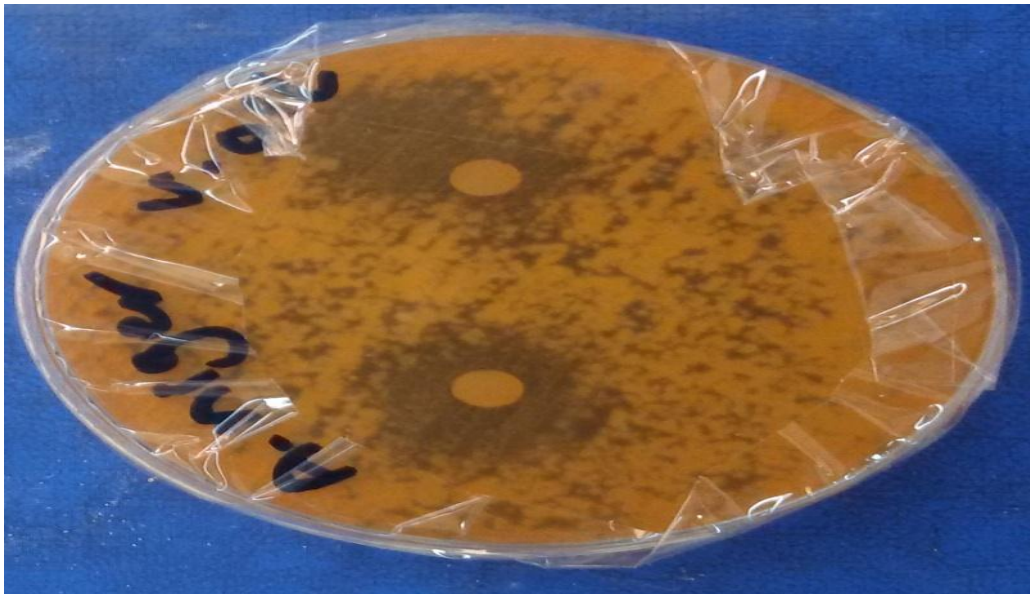
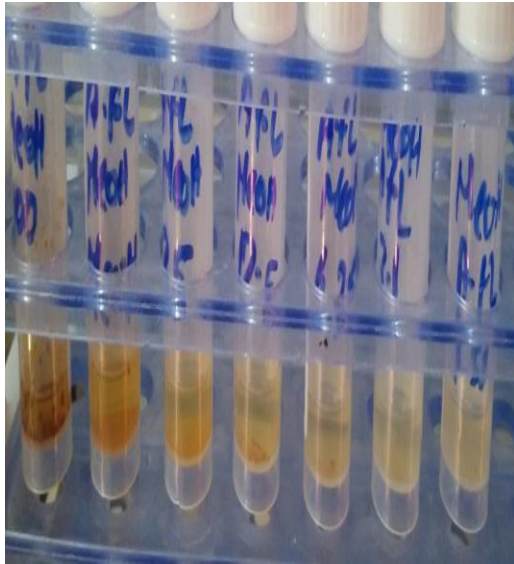
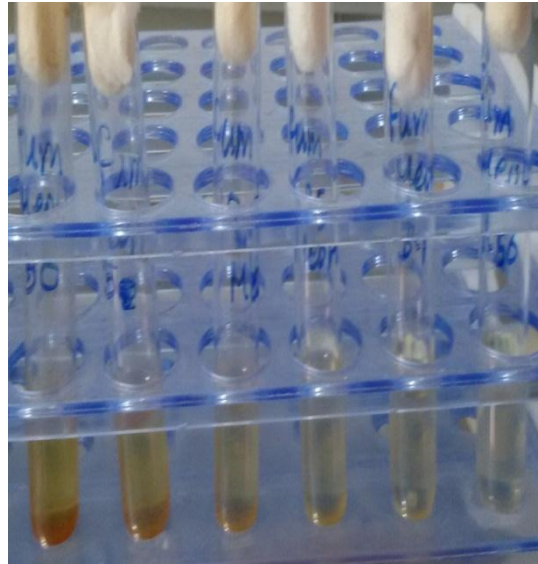


Fig 12: Activity of Volatile oil extract of *Commiphora myrrha* against *Aspergillus niger*



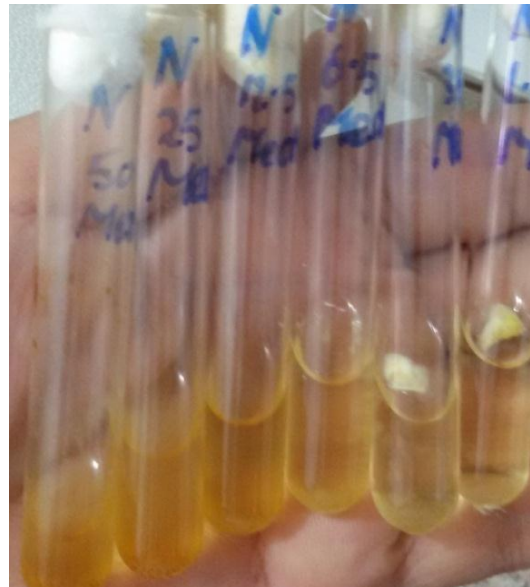
MIC of *Aspergillus flavus*



MIC of *A.fumigatus*



MIC of *A. terreus*



MIC of *A.niger*

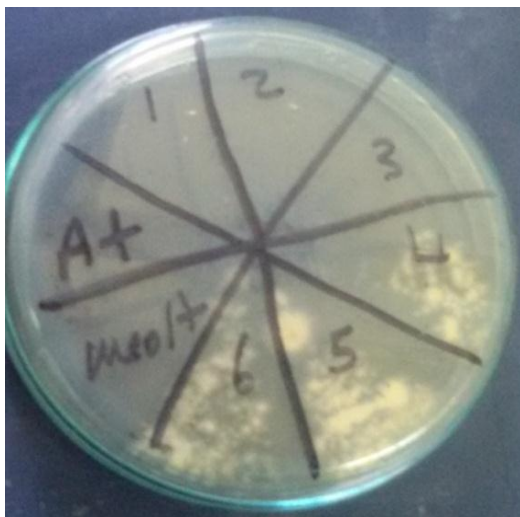
Fig 13: Minimum inhibitory concentration (MICs) of methanol extract of *Commiphora myrrha* against *Aspergillus* species (MICs at 6.25mg/ml) obtained by dilution method



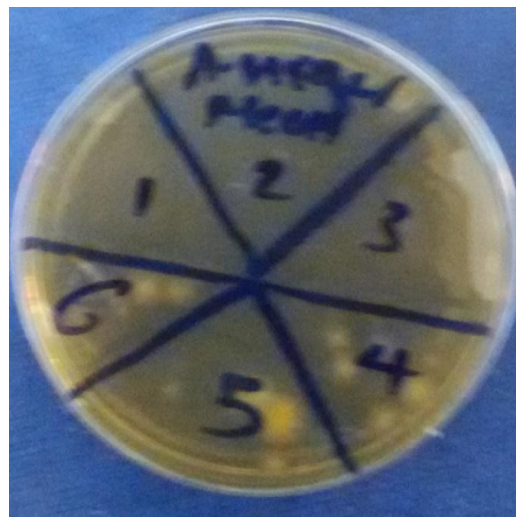
MCC of *Aspergillus flavus*



MCC of *A.fumigatus*



MCC of *A.terreus*



MCC of *A.niger*

Fig 14: Minimum cidal concentration (MCCs) of methanol extract of *Commiphora myrrha* against *Aspergillus* species obtained by dilution method

4.12. Gas chromatography results

Table 14 and (fig15)revealed that *C. myrrha* (methanol extract) contain 10 degraded components. The active ingredients was not evident in this study

Table 14: Gas chromatography analysis of *C. myrrha* methanol extract

Peak	Retention time	Area	Area %	Name
1	24.217	4087126	4.01	Cyclohexane,1-ethenyl-1-methyl-2
2	25.217	1968961	1.93	Germacrene B (CAS)
3	26.906	27976124	27.42	Benzofuran
4	28.409	1613949	1.58	1,5-cyclodecadiene
5	30.050	32828572	32.18	2-(2-nitro-1-p-toly-ethyl)-cyclohexanone
6	30.202	1194833	11.72	2-dimethylene bicyclohexy1-3
7	30.040	2182018	2.14	4-trimethyl1-3-(1-methylethylene)
8	31.380	2480868	2.43	Azuleno [4,5-6] furan-2
9	32.035	13024688	12.77	4-(2,6,6-trimethylcyclohexa-1,3-dienyl) pent-3-en-2-ol
10	35.502	3895966	3.83	Acetic acid 6-(1-hydroxymethyl-vinyl)-4

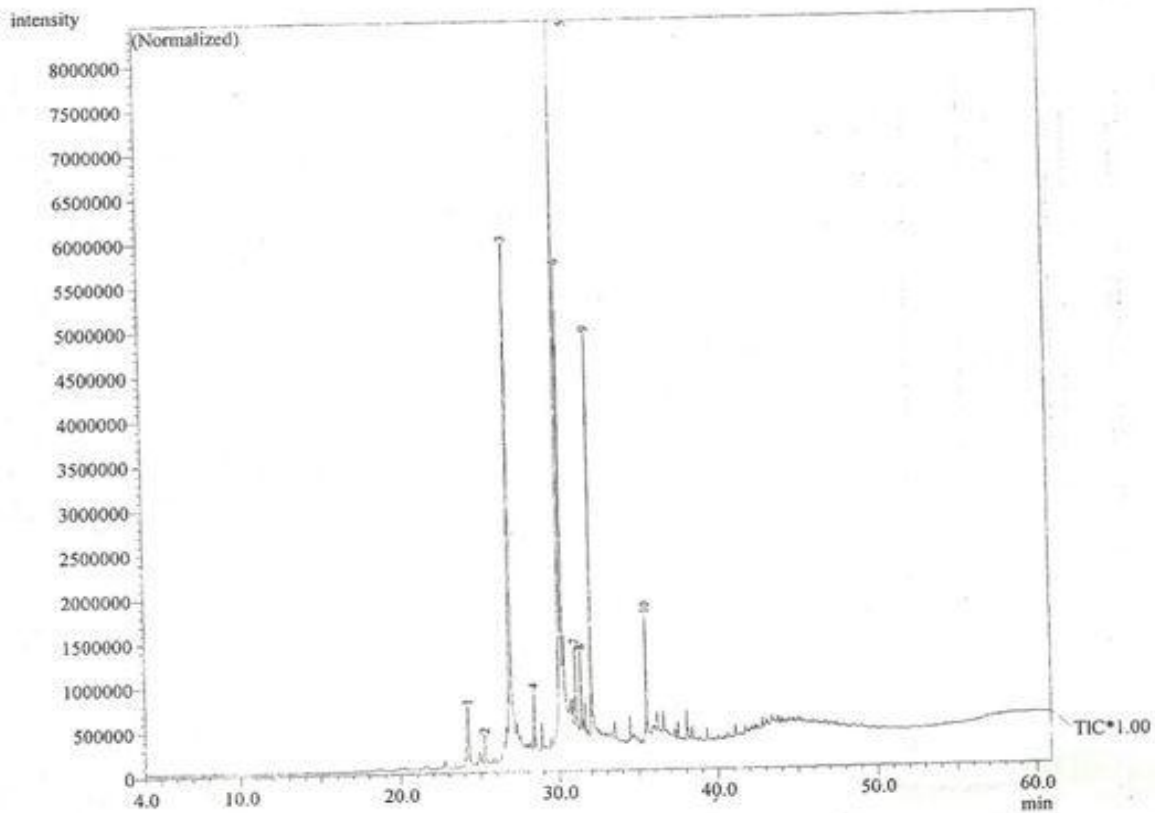


Fig 15: Gas chromatography showing degraded components found in *C.myrrha* methanol extract

CHAPTER FIVE

5. DISCUSSION

5.1. Discussion

In this study the volatile oil, petroleum ether, and methanol extracts of the oleo-gum resins of *C.myrrha* revealed antifungal activity against the standard and clinical isolates of *Aspergillus* species: *A. flavus*, *A.fumigatus*, *A.terreus* and *A.niger*. This result is in agreement with Batool,(2007); Bhanu *et al*, (2012); Hamedi *et al*, (2015) and Abd-Ulgadir *et al*, (2015) findings. However, the aqueous extract had no activity against the standard and clinical isolates of *Aspergillus* species: *A. flavus*, *A.fumigatus*, *A.terreus* and *A.niger*. This result was not agreement with Abd-ulgadir *et al*, (2015) finding. They observed antifungal activity against *A.niger* only. This might be due to of different in the nature of *C.myrrha* that differ from one place to another.

It was observed that the growth of *Aspergillus species* only from nasal biopsy and no growth from BAL that means the disease was invasive. This result in agreement with Hartwick and Batsakis, (1991) findings .

It was observed that Means Diameter of growth Inhibition Zone (MDIZ) was increased with the increase in extract concentration. This result is in agreement with Safaa, (2014) finding .

In the negative control showed that the effective action on tested *Aspergillus species* was from *C.myrrha* not from methanol or petroleum ether.

Amphotericin B exhibited higher activity against the standard and clinical isolates of *Aspergillus* species: *A. flavus*, *A.fumigatus*, and *A.niger* compared to *A.terreus*. This result was in agreement with that of Diba *et al*, (2007) finding. On the other hand, Itraconazole showed Intermediate activity against *A.terreus* isolate only but revealed no activity against *A. flavus*, *A.fumigatus*, and *A.niger*. This result is in agreement

with Manuel *et al*, (1998); Eric *et al*, (1999) and Susan *et al*, (2009) findings . They observed antifungal activity against *A.fumigatus* only.

The methanol extracts of *C.myrrha* inhibited *A.niger* growth this result is in agreement with that of Abd-ulgadir *et al*, (2015) finding.

It was observed that MDIZ were increased with the increase in drug concentration. This result is in agreement with Ioana *et al*, (2012) and Safaa (2014) findings.

The methanol extract of *C.myrrha* inhibited *A.terreus* growth at MIC of 6.25mg/ml, whereas MICs of Itraconazole at 40 mg/ml, and MICs of Amphotericin B at 10 mg/ml against standard and clinical isolate *A.flavus*, *A.fumigatus*, *A.terreus*, *A.niger*, so methanol extract of *C.myrrha* more potent than these antifungal drugs.

The volatile oil of *C. myrrha* extract revealed high antifungal activity against *A. flavus* and *A.niger* this result is in agreement with Batool (2007) and Bhanu *etal*, (2012) findings who observed antifungal activity against *A.niger* ATCC9763.

The Volatile oil extract of *C. myrrha* inhibited *A.terreus* isolate at MICs 6.25mg/ml, whereas MICs of Itraconazole at 40 mg/ml, and MIC of Amphotericin B at 10 mg/ml against standard and clinical isolate *A.flavus*, *A.fumigatus*, *A.terreus*, *A.niger*, so volatile oil extract of *C.myrrha* was more potent than these antifungal drugs.

In this study the petroleum ether extract of *C.myrrha* revealed activity against standard and clinical isolates *A.flavus*, *A.terreus*, *A.niger*. This result is in agreement with Hamedi *et al*, (2015) finding,+\ who observed antifungal activity against *A.flavus* and no activity against *A.fumigatus* This result is in disagreement with Hamedi *et al*, (2015) finding.

Many attempts were carried out world-wide to explore the role of *C.myrrha* in medical field to compact infectious diseases Chandrasekharnath *et al*, (2013) on screening and isolation of bioactive factors from *C.myrrha* and evaluation of their antimicrobial activity. Safaa, (2014) tested the antifungal activity of *C. myrrha*

against *Candida* species isolated from Vaginal Candidiasis of Pregnant woman, El Ashry *et al*, (2003) studied the components, therapeutic value and uses of myrrha, where as Zhu *et al*, (2003) isolated and characterized several aromatic sesquiterpenes from *C. myrrha*.

Comparison of the data obtained in this study with previously published results is problematic. Because , plant oils and extracts are known to vary according to local climatic and environmental conditions. Furthermore, some oils with the same common name may be derived from different plant species (Hammer *et al*, 1999). Also, the method used to assess antimicrobial activity, and the choice of test organism(s), vary. (Ivanova *et al*, 2013) and (Hammer *et al*, 1999).

The method frequently used to screen plant extracts and reference drugs for antimicrobial activity is the agar disc diffusion technique. The usefulness of this method is limited, as the hydrophobic nature of most essential oils and plant extracts prevents the uniform diffusion of these substances through the agar medium. Agar and broth dilution methods are also commonly used.

The results obtained by each of these methods may differ as many factors vary between assays. These include differences in microbial growth, exposure of microorganisms to plant oil, the solubility of oil or oil components, and the use and quantity of an emulsifier. These and other elements may account for the large differences in MICs obtained by the agar and broth dilution methods in this study. (Hammer *et al*, 1999).

Analysis of *C.myrrha* by gas chromatography exhibited 10 compounds of *C.myrrha*. compounds were identified qualitatively by the retention time, and quantitatively by the area under the curve. The active compound which inhibited the growth of fungi may be one or more of these 10 compounds, so further study must be performed to identify the compounds and thier active ingredients.

5.2. Conclusion

- The volatile oil, methanol and petroleum ether extracts of *Commiphora myrrha* revealed high activity against tested *Aspergillus species*, which justify their traditional uses as antiseptic for treatment of fungal sinusitis.
- *Commiphora myrrha* extracts was found more effective than Itraconazole combating the pathogenic *Aspergillus species* studied.
- The efficiency of antifungal activity of extracts was increased by increasing the concentration.

5.3. Recommendations

1. Based on this study and the results obtained it is recommended to identify the active ingredient in the compounds extracts responsible for antifungal activity.
2. Determination of the minimum inhibitory concentrations (MICs) and minimum cidal concentrations (MCCs) for the active ingredients on each fungus including those in this study.
3. Determination of the toxicity of the active ingredients.
4. *In vivo* studies may be required to confirm the validity of some of the results obtained.

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Appendix 1

Sudan University of Science and Technology

College of Graduate studies

Antifungal activity of *Commiphora myrrha* (Almurr Alhigazy) against *Aspergillus species* isolated from Fungal Sinusitis of Sudanese patients

Questionnaire

Age:.....

Gender: male () female ()

History of disease:.....

Operation number (s):.....

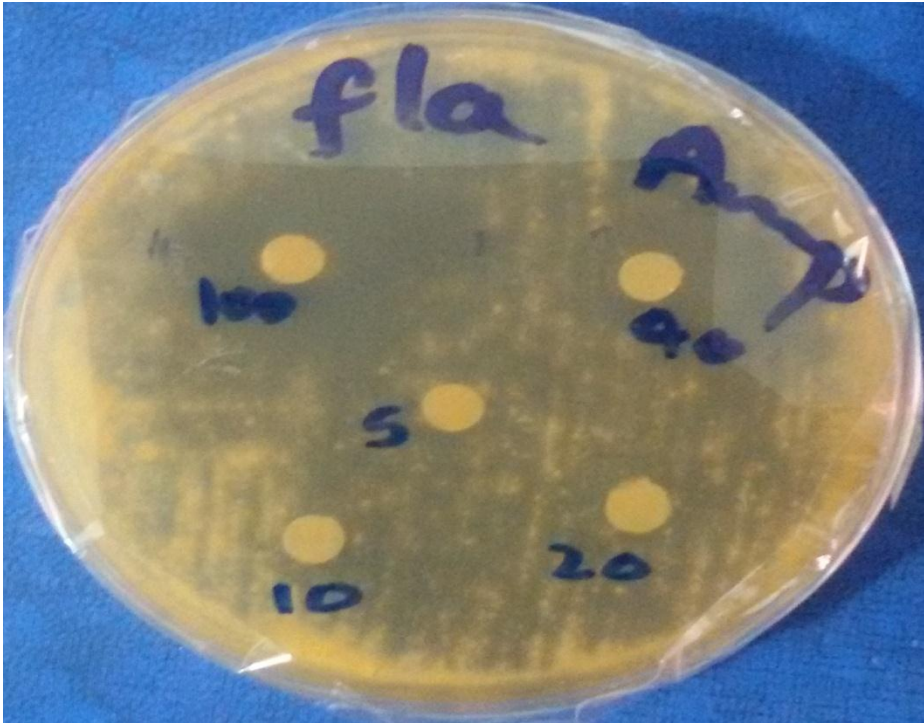
Medication:

.....
.....
.....

Appendix 2:



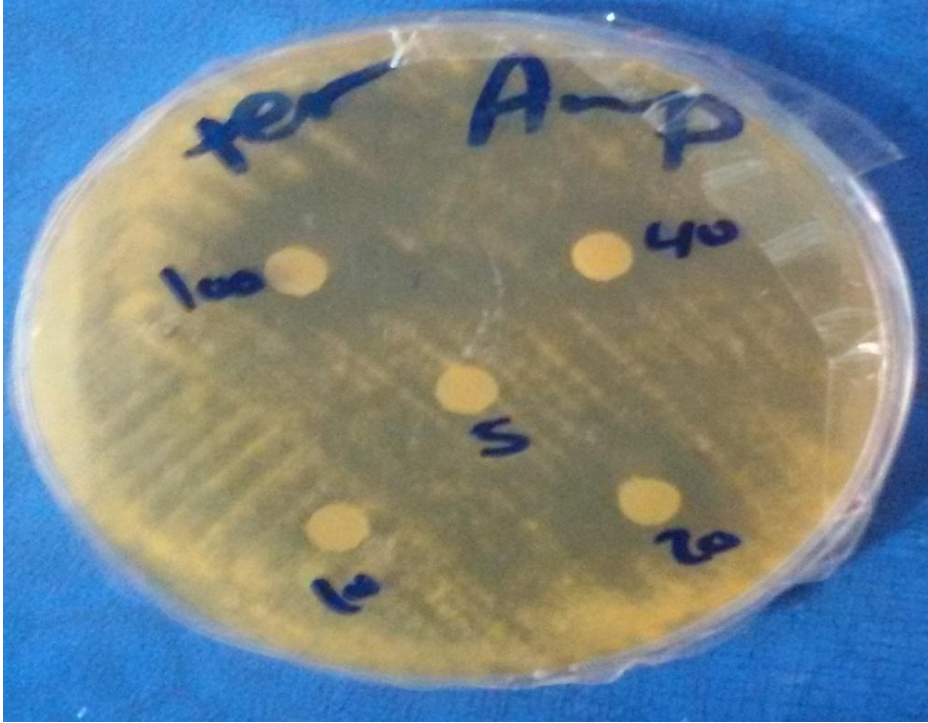
Activity of Itraconazole against *Aspergillus terreus* isolate



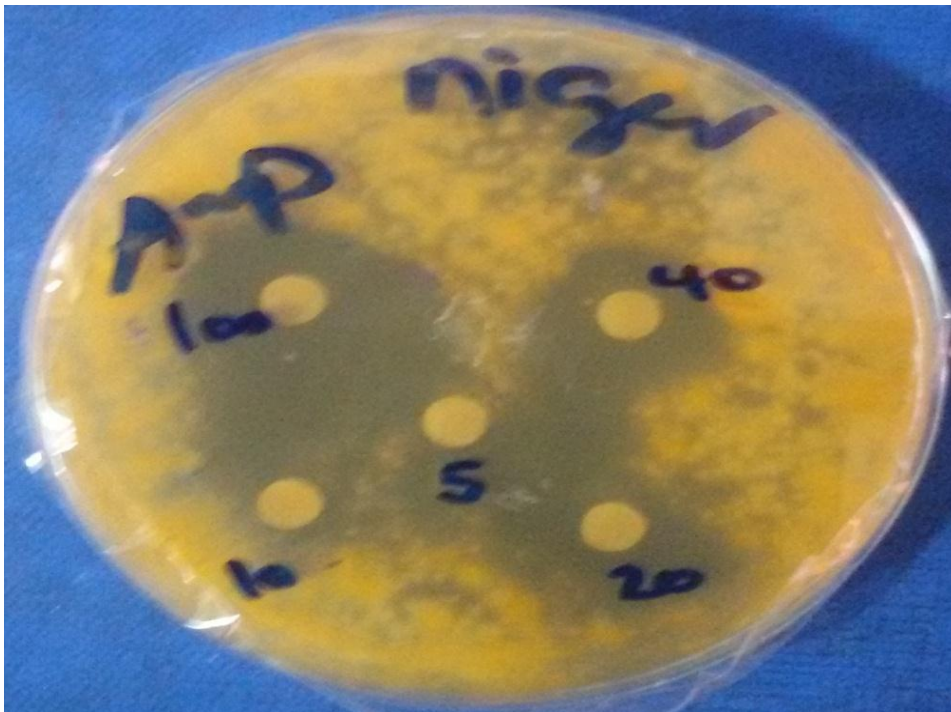
Activity of Amphotericin B against *Aspergillus flavus* isolate



Activity of Amphotericin B against *Aspergillus fumigates* isolate



Activity of Amphotericin B against *Aspergillus terreus* isolate



Activity of Amphotericin B against *Aspergillus niger* ATCC9763