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Candidiasis in Nablus city: Epidemiological Study

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Dedication
To
My Dear Mother, Sister and Brothers
For Their Patience and Encouragement, with Love and Respect

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List of Abbreviations

ICU	**Intensive Care Unit**
STI	**Sexually Transmitted Infection**
AIDS	**Acquired Immune Deficiency Syndrome**
API	**Analytical Profile Index**
MIC	**Minimum Inhibitory Concentration**
MCC	**Minimum Candicidal Concentration**
SDA	**Sabouraud Dextrose Agar**
DMSO	**Dimethyl Sulfoxide**
CFU	**Colony Forming Unit**
HT29	**Human Carcinoma**
Hep G2	**Hepatic Cells**
L929	**Human Lung Fibroblast**
MEM	**Minimum Essential Medium**
WHO	**World Health Organization**
MTT	**3-(4.5-Dimethyl thiazole-2-yl)-2.5 diphenyl-tetrazolium bromide**
EDTA	**Ethylene Diamine Tetra-acetic Acid**
UTI	**Urinary Tract Infection**
ECACC	**European Collection of Cell Culture**
ELISA	**Enzyme-Linked Immunosorbent Assay**

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Abstract

The current study was aimed at carrying out a comprehensive population-based epidemiological study of candidiasis among women and school children in the city of Nablus. The study investigated the susceptibility of recovered yeast isolates to selected drugs and certain plant extracts and the cytotoxic effects of certain plant extracts on selected human cell lines. The study was conducted during the period of January- May 2002. It involved 119 women, attending a gynecologist private clinic, and 463 school children aged 15-18 years (251 males and 212 females). Of the 119 women complaining from vaginal discharge and suspected for yeast infection, 63 (52.9%) were yeast positive. Associated symptoms included itching (22.5%), dysparenia (8.9%), burning and dysurea (8.9%), urinary tract infection (7.9%), bad odor (7.9%) and other symptoms (17.5%). The highest rate of infection was observed among women aged (21-30); women residents in villages (61.9%) and in pregnant women (58.1%).

The infection rates of 36.3% and 19.8% were found among male and female school children, respectively. The most prevalent symptom among both males and females was interdigital (92.3% and 57.1%), respectively. Male refugee camp inhabitants showed the highest infection rates (85%), compared to city (66.2%) and village (40.0%) residents. Whereas female population showed the highest rate of infection among village residents (100%) compared to city (68.9%) and refugee camp (38.1%) inhabitants. Other mycotic infections showed the highest percentage of associated disorders (19.8%) among males, whereas eczema was the predominant associated disorder among females (9.5%). Other practices such as use of common facilities did not seem to indicate an association between such practice and infection in both males and females, while contact with domestic animals showed a significant association with infection in both males (58.2%) and females (80.9%). Feet drying practice showed a convenient positive association with infection especially among females who reported not to dry their feet (64.3%), whereas it was contradictory in males who claimed to dry their feet but showed a positive yeast infection (57.1%).

With respect to anticandidal activity of plant extracts, all tested plants showed pronounced activity to various degrees. *Allium sativum* (Garlic) showed the highest effect (100%) followed by *Pistacia lentiscus* (3.8%), *Salvia dominica* (2.25%) and *Petrosilinum sativum* (2.25%) compared to reference antibiotics (Nystatin and Econazole).

Studies on the cytotoxic effects of plant extracts (*Anagalis arvensis, Anthemis tictoria* L. and *Parieteria diffusa*) against human cell lines showed that all extracts were cytotoxic at ≥ 500 μg against HT29 human cell line, more pronounced effects were observed for *Parieteria diffusa* and *Anagalis arvensis,* respectively. *Anthemis tinctoria* L. showed the most pronounced effect against human cell line L929, however, extracts of *Anagalis arvensis* and *Parieteria diffusa* showed effects at ≥ 500 μg. All three extracts showed cytotoxic effects at ≥ 5000 μg against cell line Hep G2, however, *Anagalis arvensis* showed the same effect at ≥ 500 μg. *Anthemis tinctoria* L. showed a pronounced cytotoxic effect at ≥ 5000 μg against human pulp fibroblasts, however, *Anagalis arvensis* and *Parieteria diffusa* showed reduced effects using the same concentration compared to their effects at 500 μg.

CHAPTER ONE
INTRODUCTION

1.1 Epidemiology

1.1.1 Introductory remarks

Yeast-like fungi of the genus *Candida* are colonizers of human mucosa or epidermis (Mackowiak, 1982). In patients with certain underlying conditions, *Candida* colonization is enhanced, and may in addition, involve other fungal opportunists. The colonizing fungi have to come to some arrangement with the resident bacterial biota.

The colonzation of *Candida spp.* could be endogenous or exogenous (Voss *et al.*, 1994; Pittet *et al.*, 1991). The infection could be arised from invasion by the patients own endogenous colonizing flora, or from the exogenous acquisition of the infecting yeast strains as reported in several outbreaks. Several studies have documented that 60-70% of patients in Intensive Care Units (ICUs) are colonized with *Candida spp.* (Voss *et al.*, 1994; Pittet *et al.*, 1991).

Candida is one of the most common causes of vaginal infections (Sobel, 1990), and the incidence of vulvovaginal candidiasis continued to increase during the last decade (Horowitz *et al.*, 1991). On the other hand, *onychomycosis*, whether primary or secondary is a universal problem, constituting about 30% of superfecial fungal infections (Haneke, 1991).

Candidal infections are commonly seen in compromised patients and manifest both as superfecial and systemic diseases. However; the superfecial *Candida* infections are by far the most prevalent form of the disease.

1.1.2 Age

In healthy individuals, candidal infections are usually due to impaired epithelial barrier functions and occur in all age groups, but are most common in the newborn and elderly, because of less of activity of the immune system (Murray *et al.,* 2000).

1.1.3 Etiological agents and source of infection with *Candida*

Candida is a part of human flora. It becomes pathogenic when certain conditions are present and becomes opportunistic infection (Kown-Chung *et al.,* 1992). The major etiological agent is *Candida albicans*, whereas different *Candida* species can cause a variety of infections (Bodey, 1984), including *C. tropicalis, C. parapsilosis, C. krusei, C. guillermondii, C. glabrata,* and *C. kefyer* which represent many clinical forms of candidiasis. Some of these species are encountered as secondary infection to another species, for example; *C. parapsilosis* is second infection only to *C. albicans* as a cause of *Candida* endocarditis (Hickey *et al.,* 1983). Still other species of *Candida* have been occasionally isolated from clinical isolates like *C. catenulata, C. intermedia, C. lambica*, and *C. zeylanoides* (Crozier *et al.,* 1977; Odds, 1988; Strom *et al.,* 1985). These species are therefore not considered as agents of opportunistic infections.

1.1.4 Incidence and prevalence

In recent years, the incidence of life-threatening mycoses caused by opportunistic fungal pathogens has increased dramatically (Barnett *et al.,* 1990). Many studies have showed that the prevalence of infection increased with age

(Heihkila *et al.,* 1995). According to earlier reports, *C. albicans* was the cause of 80-95% of cases of symptomatic fungal vulvovaginitis, whereas other *Candida* species such as *C. glabrata*, *C. parapsilosis*, and *C. tropicalis*, were responsible for the remaining cases (Vincent *et al.,* 1995; Nolla-Salas *et al.,* 1997). In many low-income countries, concerted efforts are being made to establish programmes for the control of reproductive-tract infections, including sexually transmitted infections (STIs) (Grosskurth *et al.,* 1995; Cohen *et al.,* 1997). The incidence of vulvovaginal candidiasis contiued to increase during the last decade (Vincent *et al.,* 1998).

The world-wide incidence of invasive fungal infections, particulary due to *Candida spp*., has increased substantially in patients requiring intensive care (Vincent *et al.,* 1998). In Europe the prevalence of fungal infections, in an intensive care study, rated the fifth among most frequent nosocomial pathogens, such as, *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Staphylococcus spp*. (Vincent *et al.,* 1995).

Members of the genera *Candida* are the most regularly encountered species, which belongs to ascomycetous-like fungal species. Several *Candida* species have been implicated in human disease and their incidence is rising relative to that for *C. albicans* (Hazan, 1995). This is most likely a reflection of a true species shift generated by the increased awareness of microbiologists (Sullivan *et al.,* 1995). The organism causes a wide variety of infections ranging from superficial disease to life threatening mycoses (Pfaller *et al.,* 2001). Other

than epidemiological importance, possible causes and documenting; this new trend may have therapeutic implications. This is clear from the findings on non-albicans species, which appear to be less responsive to azole therapy than *C. albicans* (Sobel *et al.,* 1997; Redondo-Lopez *et al.,* 1990; Vazquez *al.,* 1994). The previously mentioned factors stimulated further research to evaluate relative prevalence of non-albicans spp. might be involved in vaginal infections in different clinical settings (Horowitz, 1991).

1.1.5 Predisposing factors

Invasive candidiasis is a life threatining infection in immuno-compromised hosts such as bone marrow and organ transplant recepients, in patients receiving intensive chemotherapy treatment and in AIDS patients (Lyles *et al.,* 1999). Moreover, systemic *Candida* infections are observed in patients with extensive surgery or burns, intensive antibiotic therapy, indwelling catheters, patients with diabetes mellitus, oral contraception, pregnancy, local wormth and moisture, skin irritance, trauma, recurrent diseas and in elderly patients (Dean *et al.,* 1996; Wenzel, 1995).

The significance of *Candida* in the vagina of asymptomatic women between episodes of recurrent vulvovaginal candidiasis is not clear. Prevalence studies indicates that 10% to 55% of healthy women who are completely asymptomatic, have vaginal cultures positive for *Candida albicans* (Linden *et al.,* 1978; Sobel *et al.,* 1993). The finding of this organism during the symptom free periods could indicate previous inadequate treatment, resistance of the

organism to complete eradication by drugs, insuffecient use of antifungal medication, or recolonization. Clinical groups and or predisposing factors for invasive candidiasis include: neutropenia (especially more than 7 days); hematological malignancy; solid tumor malignancy; post surgical intensive care patients; broad-spectrum or multiple antibiotic therapy; diabetes mellitus; parental nutrition; severe burns; neonates; corticosteroid therapy; intravenous drug abuse; and excessive exposure to moist, heat, friction and maceration of the skin (Murray *et al.,* 2000).

Candidal infections usually remain superfecial and respond readily to treatment. Systemic candidiasisis is usually seen in patients with cell-mediated immune defeciency, and those receiving aggressive cancer treatment, immunosuppressants, or transplatation therapies. *Candida* is considered as an opportunistic pathogen (Lamagni *et al.,* 2001).

It requires host dysfunction to become pathogenic such as the defects caused by administration of broad spectrum antibiotics, or in the cases of neutropenia, disruption of protective barriers including catheterisation, and taking advantage of impaired immunity in a debiliated patient to establish the disease (Davis *et al.,* 2000).

1.1.6 Pathogenesis

The life cycle of *Candida* is characterized by budding in which the parent noncapsulated oval blastophore gives rise to filamentous mycelium (Friedrich, 1988). The mycelium is composed of non- branching hyphae whose growth is

initiated by germ- tube formation. Germ- tube formation is associated with adherence of *Candida* to epithelial cells and occurs optimally at pH less than 5.5 and at a temperature greater than 33°C. At least 18 different strains of *Candida albicans* have been identified, but no significant differencce in pathogenesity has been found between these strains (Friedrich, 1988). It may be of importance that they are phenotypic variations which can switch back and forth at high frequency (Soll *et al.,* 1987). This switching can occur at the site of infection and may be of advantage to the survival of *Candida* to escape its environment constraint imposed by pH and temperature; it may evade host surveillance by changing antigenicity; it may conceivably alter resistance to antifungal agents.

The mechanism by which *Candida* damages the genital epithelium is uncertain. The association of filamentous forms of *C. albicans* in the deeper layer of the mucous membrane with active disease, and their greater adhesiveness to epithelial cells than to blastophores, suggests that filamentous forms are important in the pathogenesis of candidiasis (Oriel, 1977).

The vaginal epithelium shows a marked inflammatory response, unlike the cervical epithelium, which is largely unaffected. In the vagina there is active phagocytosis by polymorphonuclear leukocytes and monocytes, with penetration of tissue by the hyphae. Although the local and serum antibodies can be demonstrated, their significance is uncertain.

In healthy individuals intradermal tests for *Candida* antigens give a strong delayed hypersensitivity reaction which is lost in immunocompromised patients, such as in those with AIDS.

1.2 Clinical manifestations

In healthy individuals, candidiasis occurs as a result of dysfunction in epithelial barrier of normal flora (Murray *et al.*, 2000). The clinical manifestations can be acute, subacute, chronic to episodic. The location of infection is usually locallized to the mouth, throat, skin, scalp, vagina, finger, nails, bronchi, lungs, or the gastrointestinal tract, or becomes more complicated in systemic septicemia, endocarditis and meningitis.

1.2.1 Oropharengeal candidiasis

Severe immunological impairments which caused by certain diseases like diabetes mellitus, leukemia, lymphoma, malignancy, neutropenia, inhaled steroids and HIV are the main cause of acute oral candidiasis. This type of infection is rarely seen in healthy adults but they may occur in up to 5% newborn and 10% of elderly people (Murray *et al.*, 2000).

1.2.2 Cutaneous candidiasis

Also called intertriginous candidiasis, this type of candidiasis is commonly seen in the axillae, groin, intra-and sub-intra mammary folds, intergluteal folds, interdigital spaces, and umbilicus. Infants under unhygienic conditions are subjected to diaper candidiasis which resulted from the skin maceration with

ammonitic irritation due to irregularly changed unclean diapers and thus erythematous lesions are developed (Murray *et al.*, 2000). *Candida spp*. are also well known as a primary or secondary causative of foot infections (Perea *et al* 2000 and Kamihama *et al.*, 1997).

Continuous subjection of hands or foot to humidity especially with sugar solutions and flour, resulting in maceration of the nail folds and cuticle. This leads to a condition called Paronychia of finger nails, which causes painfull erythema swelling around the infected nails. If this case is not treated, chronic infection will progress causing detachment of the nail with cuticle. The case is called onychomycosis, which causes complete destruction of the nail and usually develops in immunocompromised patients.

1.2.3 Vulvovaginal candidiasis

1.2.3.1 Vaginal secretions - discharge

The vagina is the muscular passageway from the external opening of the vagina to the uterus (Merck Manual, 2001). A normal function of the vaginal walls and the cervix (opening of the uterus into the vagina) is to produce secretions that are typically watery, mucousy or milky white. These secretions help to maintain healthy conditions inside the vagina and provide lubrication during sexual intercourse. The secretions produced by the vagina and cervix can sometimes be noticed outside of the vagina (this is referred to as 'vaginal discharge').

Normal vaginal secretions and discharge change from time to time; sometimes clear, almost like water, and at other times, mucousy and whitish in color, sometimes scant and, at other times, larger in amount. These are normal variations. Normal vaginal secretions and discharge may vary from one woman to the next depending on: the stage of the menstrual cycle, menopause, whether a woman is taking birth control or hormone replacement medications, whether the woman is pregnant or not and state of sexual arousal. It is normal for vaginal secretions and discharge to contain the fungus called *'Candida albicans'* in controlled amounts. The growth of candida in the vagina is normally kept in check by the amount of 'non-harmful' bacteria (normal flora) that are also present in the vagina. Abnormal vaginal secretions and discharge may be caused by a number of conditions, including: Bacterial infection - examples of bacteria that can cause vaginal infections are sexually transmitted diseases such as chlamydia and gonorrhea. The abnormal secretions and discharge associated with bacterial infection vary, depending on the type of bacteria, but they are generally colored (yellowish, grayish or greenish, as opposed to clear or white) and they usually have a foul odor. Itching and skin irritation may also be present. Other main causatives are *Candida* or 'yeast' infection - while *Candida* is normally found in vaginal secretions and discharge, if the balance of *Candida* and bacteria is upset, *Candida* growth can become excessive. This is referred to as a *Candida*, or 'yeast', infection. The symptoms of *Candida* over-growth include thick, white, cottage-cheese like vaginal discharge and itching and/or irritation of the vagina and tissues surrounding the opening of the vagina. The

symptoms are often worse during the week before a menstrual period. Factors that may contribute to the over-growth of *Candida* include taking antibiotics or birth control medications, pregnancy, having diabetes and having a weakened immune system. *Trichomonas* infection - this type of infection is caused by a single celled parasite and results in the production of large amounts of white, grayish-green, or yellowish discharge. Cancer of the vagina, cervix, or uterine lining (endometrium) may cause a watery discharge that contains blood. Forgotten tampon, contraceptive sponge or other foreign object in the vagina usually produces a foul odor and may also produce a thick colored discharge (Merck Manual, 2001).

Vulvovaginal candidiasis is associated with many predisposing factors. Low pH in the vagina is one of the most important. The use of oral contraceptives and sexual activity are other factors. Vulvovaginal candidiasis is one of the most common infections in women. It becomes more complicated in HIV patients, in this case a combination of oral and vulvovaginal candidiasis can arise (Murray *et al*., 2000).

1.2.4 Chronic mucocutaneous candidiasis

Occurs in patients with various metabolic disturbances to cell-mediated immunity (Murray *et al.*, 2000). Other clinical manifestations are: neonatal and congenital candidiasis, oseophageal candidiasis, gastointestinal candidiasis, pulmonary candidiasis, perotinitis, urinary tract candidiasis, meningitis,

hepatosplenic candidiasis, endocarditis, candidemia (septicemia), ocular candidiasis, osteoarticular candidiasis, and many other forms.

1.3 Identification of *Candida species*

Identification to the species level of yeasts isolated from clinical specimens is often problematic for diagnostic laboratories, but it has become increasingly necessary (Koehler, 1999). Greater number of immunocompressed patients, a widening range of recognized pathogens, and the discovery of resistance to antifungal drugs mean that the common practice of identification or exclusion of *C. albicans* alone is no longer adequate.

Reference procedures that use biochemical, morphological studies are not practicable for the clinical laboratory because they are labor-intensive and run over several weeks. Packaged kit systems (API 20 C, API 20 C AUX) are widely used, but they are expensive, and limited by the sizes of their databases, while automated systems have many of the same limitations (Koehler, 1999).

1.4 Susceptibility

Anticandidal activity screening of natural products is usually performed using disk diffusion method and broth dilution method to test for susceptibility of selected recovered *Candida spp*. isolates against commonly used anticandidal drugs and plant extracts (Murray *et al.*, 1995). Susceptibility of *Candida spp*. to antifungal agents shows intra and interspecific variability and increased variation in minimum inhibitory concentration (MIC) values.

Susceptibility of *Candida spp.* to several plant extracts is also employed to study the action of medicinal plants as a part of folk medicine which comprises numerous herbal and plant prescriptions for therapeutic purposes.

1.4.1 The anticandidal activity of plant extracts

1.4.1.1 Medicinal plants

Medicinal plants have been used for centuries as remedies for human illnesses (Bisignano *et al.,* 1996). This use is based on the fact that plants have a high therapeutic value which has led to the acceptance of traditional medicine as an alternative choice for health care.

Medicinal plants are integral component of research developments in the pharmaceutical industry (Gorman, 1992). Such research focuses on the isolation and direct use of active medicinal constituents or in the development of semi-synthetic drugs (Gorman, 1992). Plants have been a rich source of medicines because they produce a host bioactive molecules, most of which probably evolved as chemical defenses against infection (Frank, 1996).

Folk or traditional medicine is the use of these plants for treatment of physical, mental or social abnormalities (Sofowara, 1982; Bruneton, 1995). The choice of the plant depends mainly on the combination of knowledge and practice relying on past experience and observation handed down from generation to another (Sofowora, 1982). Folk medicine comprises numerous herbal and plant prescriptions for therapeutic purposes, which include healing of

wounds, treatment of inflammation and skin ulcers (Tanira *et al.,* 1994) pneumonia and bullet wounds (Desta, 1993), dermatomucosal, skin and candidial infections (Caceres *et al.,* 1991, 1993a).

Many plant species have been used in folkloric medicine in Palestine to treat various ailments of man (Palevitch, 1991; Ali-Shtayeh, *et al.,* 2000, Ali-Shtayeh and Jamous, 2003). Eighteen of these plants (Table 2.2) are used to treat dermatomucosal infections and other ailments, were selected and used in the present work for their anticandidal activities.

To study the biological effects of traditional medicinal plants, many comprehensive screening programs have been established for isolating the active components of these plants to treat human illness (Boulos, 1983; Kottob, 1983). World Health Organization also encourages the inclusion of medicinal plants in programs for developing countries because of the great potential of such plants in combating various diseases (Noumi *et al.,* 1999). In Palestine, the screening of the flora for pharmacological active compounds started in the late sixties (Silva *et al.,* 1991). The abundance of species (about 3000) condensed on a very small geographical area is a major characteristic of the Palestinian flora. This richness is due to the diversity of habitats created by soil and climatic conditions, in addition to the lack of medical care, and economics.

Many plant species (>700) have been used in folkloric medicine in Palestine to treat various ailments of man (Palvitch, 1991; Shtayeh and Hamad, 1995; Ali-Shtayeh, *et al.,* 2000). Folk remedies used are prepared as powders,

poultices, ointments, baths, decoctions, infusions and teas. Decoctions is the most popular form of home remedies.

Decoctions, infusions and teas are usually prepared just before application and filtered through a cloth, cotton or wool. Most plants are stored for use in the dry state, which permits their utilization throughout the year; sometimes fresh plants are used (Sezik *et al.*, 1991).

1.4.2 Screening methods for anticandidal activity of natural products

Anticandidal activity screening of natural products is usually performed using agar diffusion and dilution methods (Rios *et al.*, 1988, Woods *et al.*, 1995; Silva, 1996). The followings are some recommended methods (Rios *et al.*, 1988).

Principal diffusion method: It is a technique in which a homogeneous dispersion in water is not required, the agar diffusion method (Murray *et al.*, 1995) using a disk, hole or cylinder as reservoir. The reservoir containing the sample to be tested is brought into contact with an inoculated medium and after incubation, the diameter of clear zone is measured. The zone size is inversely proportional to the minimum inhibitory concentration (MIC), the least concentration of the extract that completely inhibits the growth of the test organism (Rios *et al.*, 1988; Waxler *et al.*, 1991; Woods and Washington, 1995).

The advantages of this method are the small size of the sample needed in the screening and the possibility of testing five or six compounds against a single microorganism (Rios *et al.*, 1988).

Dilution method: A homogeneous dispersion of the sample in water is needed in this technique (Rios *et al.,* 1988). Dilution susceptibility testing methods are used to determine the minimum concentration of an antimicrobial agent required to inhibit the growth or kill a microorganism. MIC determined by this technique, is defined as the lowest concentration that inhibits the visible growth of an organism (Rios *et al.,* 1988).

In this method, turbidity resembles the indication of candidal density. The degree of inhibition (indicated by turbidity) is measured by spectrophotometer (Woods *et al.* 1995). Flexibility, is the major advantage of dilution testing methods. Further advantages include the detection of certain resistance patterns that may not be defected by disk diversion methods (Rios *et al.,* 1988).

Another important advantage of this method, is that it is the only technique that is used for determination of minimum candicidal concentration (MCC), which is defined as the lowest concentration of the extract that does not permit any visible colony of *Candida* to grow on the agar plate after the period of incubation (Irobi and Daramala, 1994). Subculturing the tube with no turbidity on solid media indicates complete inhibition whether on agar plate or in liquid medium (Rios *et al.,* 1988).

1.4.3 Extraction Techniques

Anticandidal activity of plant is usually assessed after extracting plant material with water, ethanol, petrol, chloroform and other organic solvents, in

order to separate the chemical constituents into groups of different polarities (Nadir *et al.*, 1985). Many factors may affect the extractibility and hence the biological activity of the chemical constituents of plants. The pH of the extracting medium is one of these factors. For this reason it is important to use several extracting media to ensure recovery of all the active compounds (Nadir *et al.*, 1985). Two traditional extraction techniques are known, decoction and infusion: the first one, decoction, is prepared by placing the plant drug in cold water, bringing it to boil for 15 minutes or longer, and then allowing the mixture to stand for further 15 minutes, the extracts (aqueous or organic) are decanted or filtered and stored at –20°C until use. The second technique of extraction, infusion, is carried out by pouring boiling water or organic solvent on a specific quantity of plant material and allowing the mixture to stand for 10-15 minutes or more (Sofowora, 1982).

1.5 Laboratory identification of yeasts

The laboratory approch to the identification of yeasts and yeastlike organisms recovered from clinical speciemens has shifted from conventional tests (such as carbohydrate fermentation or assimilation, germ tube test, urease test and others) to the use of commercially available systems (Koneman *et al.*, 1985). The rapid urease test is useful for screening the urease producing yeasts. If the urease test is positive, other rapid conventional methods or commercially available systems may be used to identify *Cryptococcus neoformans.* The germ tube test, is helpful to screen for the presence of *Candida albicans.* If the test is

positive, the identification of *C. albicans* may be made and further testing is not required. Fermentation or assimilation test: A technique which is considered as one of the most important methods for identification of all yeasts except *C. albicans*.

It is based on the use of biochemical features including the carbohydrate utilisation profiles (Kwon–Chung *et al.,* 1992) for identification purposes. Commercial Systems: API 20C STRIP. It is widely used to identify different types of yeast. It includes 10 cupules resembling 12 different tests, all reagents are presented in their dehydrated form. Commercial systems are relatively expensive and are limited to use in laboratories with a suffeciently heavy work load to make their use cost effective (API20C Catalog, 2001).

1.6 Cytotoxicity of plant extracts against cell lines using MTT Assay

Cytotoxicity of plant extracts is usually evaluated based on their effects on both cell viability and proliferation. In case of any possible positive effect against yeast infections, it is necessary to further evaluate their cytotoxic effect on human cells. This is usually performed using human cell lines for the possible use and application of these extracts for treatment purposes.

1.6.1 MTT Assay

The most convenient modern assays for determination of cell viability and cell proliferation have been developed in a microplate form (96-well plates). The advantages of this miniaturization (Riss and Moravec, 1993):

1. It allows many samples to be analyzed rapidly and simultaneously.
2. The microplate form also reduces the amount of culture medium and cells required as well as the cost of plastic ware.
3. Calorimetric assays allow samples to be measured directly in the microplate reader.

Microplate assay has been developed based on different parameters associated with cell viability and cell proliferation. The most important parameters used are DNA synthesis like (3H)-TdR proliferation assay, and metabolic activity like MTT, XTT, and MTS assays (Riss and Moravec, 1993).

1.6.2 MTT Assay (background)

Background information: 3-(4.5-Dimethyl thiazole-2-yl)-2.5 diphenyl-tetrazolium bromide (MTT) reduction is one of the most frequently used methods for measuring cell proliferation and neural cytotoxicity. It is widely assumed that MTT is reduced by active mitochondria in living cells (Lui *et al.*, 1997; Riss and Moravec, 1993).

1.6.3 MTT assay application

1. MTT assay is designed to be used for the non-radioactive, spectrophotometric quantification of cell proliferation and viability in cell populations using 96-well plate format. It can be used for:
2. The measurement of cell proliferation in response to growth factors, cytokines, mitogens, and nutrients (Huang *et al.*, 1998).

3. The analysis of cytotoxic and cytostatic compounds like anti-cancer drugs and other pharmaceutical compounds (Gergel *et al.*, 1995; Wong and Goeddel, 1994).

4. The assessment of growth inhibitory antibodies and physiological mediators (Fanijul *et al.* 1996).

1.6.4 MTT assay principle

The assay is based on the cleavage of the tetrazolium salt MTT, in the presence of an electron coupling reagent, by active mitochondria. The water insoluble formazan salt produced has to be solubilized in an additional step. Cells, grown in a 96-well tissue culture plate, are incubated with the MTT solution for approximately 2 hours. After this incubation period, a water insoluble formazan dye is formed. After solublization, the formazan dye is quantitated using microplate reader (ELISA reader). The absorbance revealed directly correlates to the cell number (Roch, 1999). Advantages for MTT assay (Riss and Moravec, 1993) include:

1. Safe: no radioactive isotopes are used.
2. Accurate: the absorbance revealed strongly correlates to the cell number.
3. Sensitive: low cell number is detected.
4. Fast: the use of a multiwell plate reader allows a larger number of samples to be processed.
5. Easy: no washing steps and no additional reagents are required.

The disadvantages of this assay (Promega, 1996) include:

6. Requires volatile organic solvent to solubilise the formazan product.

7. Plate can not be read and returned to incubator for further color development.

1.7 Treatment of candidal infections

1.7.1 Treatment of abnormal vaginal discharge

Antibiotics are effective for the treatment of infections caused by bacteria and other medications are available to treat conditions such as Trichomonas and *Candida*. Because *Candida* infections are so common, there are now a variety of over-the-counter medications available in to treat *Candida*. The treatment choice of abnormal vaginal secretions or discharge depends on the cause.

Appendix C shows some of the antifungal drugs currently used in Nablus City (Q. Y. Aslan, Personal communication, October 20, 2003). These agents can be divided into six different groups: the antifungal agent alone, antifungal with antibacterial (to treat any bacterial infection), antifungal with antibacterial and cortisone (to treat the symptoms of infection as well as treatment of infection itself), antifungal with cortisone and at last fungistatic agent like salicilic acid.

1.8 Objectives of the current study

Until recently, information about the epidemiology of candidiasis among Palestinians is lacking. Therefore, the current study is aimed at:

1. Carrying out a comprehensive population-based epidemiological study of candidiasis among women attending a private gynecologist clinic, and among school children age 15-18 years in Nablus City.

2. Investigating the susceptibility of recovered *Candida* isolates to selected anticandidal drugs and to certain plant extracts.

3. Evaluating cytotoxic effects of certain plant extracts on selected human cell lines.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Subjects of the study

A total of 463 school children from 12 classes (tenth and first secondary grades) aged 15-18 years, comprising 251 males and 212 females, were examined for symptoms of cuataneous candidiasis in Nablus city. In addition, 119 women aged 17-55 years suspected for vaginal candidiasis, attending a gynecologist private clinic in Nablus city, were also surveyed for *Candida spp.* during the period of January - May 2002. Table 2.1 shows the distribution of school children among the various selected schools.

Table 2.1 Distribution of schools used for sample collection in Nablus city

School name	No. of examined students
Girls schools	
1. Kamal Jomblat	
Class 1*	**40**
Class 2	**23**
2. Jamal Abed Elnaser	
Class 1	**52**
Class 2	**37**
3. Salahyia	
Class 1	**22**
Class 2	**38**
Total Girls	**212**
Boys schools	
1. Malek Talal	
Class 1	**49**
Class 2	**41**
2. Qadry Tokan	
Class 1	**44**
Class 2	**43**
3. Zafer El-Masri	
Class 1	**34**
Class 2	**40**
Total Boys	**251**
Total (all schools)	**463**

*** Class 1, 1st secondary grade; Class 2, primary 10th grade.**

2.2 Collection of epidemiological data

Clinical examination for school children was carried out by the researcher under the supervision of a dermatologist, and by a gynecologist in the case of women attending private clinic. Detailed inspection of the foot was done by

searching for signs or symptoms of infection with *Candida spp.* All suspected cases were interviewed and data was recorded using specially designed questionnaires included demographic data on age, sex, use of any medication especially antibiotics and immunosuppressants, place of residence, hygienic behavior and other criteria (Appendix A).

2.3 Specimen collection and culture

For school children; collection of clinical samples was done by passing a sterile swab over infected area, after cleaning the foot thoroughly with ethyl alcohol. A sterile swab was used to obtain vaginal discharge samples for women population. Swabs were immediately placed in a sterile culture tubes filled with Sabouraud Dextrose Agar (SDA) liquid medium (Appendix B). Culture tubes were then incubated at 37°C for 24-48 hours to allow yeast growth.

When turbidity in the medium was recognized, yeast suspension was streaked out on a solid SDA plates in duplicates. The plates were then incubated for further 24-48 hours to obtain clear well separated colonies. Colonies were examined and used for the needed biochemical tests. Cultures were maintained on SDA slants at 4°C by periodically subculturing (Kown-Chung and Bennett; 1992).

2.4 Media

SDA medium ammended with two antibiotic-antifungal agents [Chloramphenicol (0.05mg/L) / Cycloheximide (0.5mg/L)] was used throughout this study.

2.5 Identification of cultures from primary isolates

Cultures of primary isolates were characterized based on their physiological and morphological characteristics.

2.5.1 Identification of *Candida albicans* by Germ Tube Test

Germ tube test was used in identifying *C. albicasns* (Kown-Chung and Bennett; 1992). The test was carried out follows:

1. Cells were picked up by hocking a pure colony lightly with a sterile loop.
2. Cells were then suspended in human serum 0.3-0.5 ml at room temperature and rub the loop a gainst the wall of the tube.
3. Serum cultures were then incubated at 37°C for 2.5 to 3 hours
4. After incubation; a drop of the serum culture was placed on a clean slide and examined under the microscope using low and high powers.

Formation of germ tubes in yeast cells was observed in a yeast positive isolate obtained from vaginal discharge (Figure 2.1).

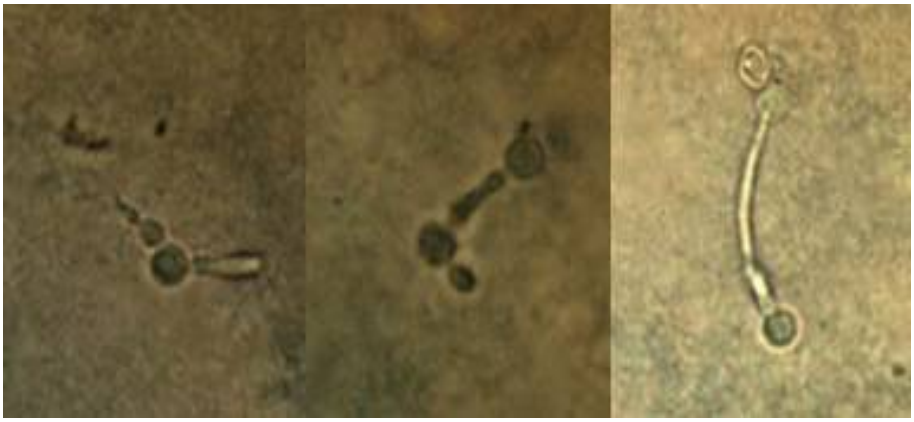


Figure 2.1 A micrograph showing clear formation of germ tubes in *Candida albicans* isolate grown in serum for 3h at 37°C.

2.5.2 Biochemical features

Clinical *Candida* strains were further tested using germ tube test in serum, urease production, fermention test classically or by using API 20C Kit (Konenan and Roberts, 1985).

2.5.2.1 Fermentation test

Fermentation test was carried out as follows:

1. For each isolate, 7 tubes were set up, each containing a 9.8 ml of one of the following solution: 1% Sugars: Glucose, Sucrose, Lactose, Maltose, Galactose, Trehalose and Dextrose.

2. To each of these tubes, 0.2ml of inoculate suspension was added (prepared equivalent to a McFarland standard no. 5 using SDA broth medium).

3. Tubes were then incubated at 37°C for 48 hours and a change in color was used as an indication for a positive reaction.

2.5.3 API20C kit

The API20C system is widely used compared to the Uni-Yeast-Tek system and that's why it was used in this work. API *Candida* is a standardized system for the identification in 18-24hs of yeasts most frequently encountered in clinical microbiology (API20C Catalog, 2001). Identification of yeast with API20C system was done by (Biomeriux API 20 C AUX, 1997) carrying out 20 tests using the API 20 C strips. Interpretation of the results was performed "manually", using the identification table provided by the manufacturers. The test was carried as follows:

1. Fresh isolated colonies were suspended in 0.85% NaCl medium
2. Suspention was loaded into API wells and then incubated at 37°C for 18-24 hours.
3. Identification of species was based on identification table provided by the manufacturer.

2.5.3.1 API yeast- identification

API yeast-identification was carried out as described by the manufactureres (API20C Catalog, 2001).

1. A yeast suspension equivalent to a McFarland standard 5 was prepared using a sterile Woodsen applicator stick. Yeast emulsification was carried out in 3ml sterile distilled water.
2. Yeast suspensions were used as quickly as possible (within 15 minutes) to inoculate the test strip.

3. Each microcupule was inoculated with 2 or 3 drops (to fill the well) of the yeast suspension.

4. Strips were placed within the humidity chamber supplied by the manufacturer and were incubated for (18-24) hours at 37°C.

5. A 7-digit number is generated and identification was made by using profile register supplied by the manufacturer.

2.5.4 Urease test

Urea agar base (Difco) was prepared in solid forms and then despensed in tubes. Urease test was carried out as follows:

1. Inoculation of the surface of urea media by a loopfull of pure yeast cultures which then were incubated for 4 days at 37°C.

2. Development of deep-red color throughout the medium indicates a positive reaction.

2.6 Susceptibility of Candida species to selected plant extracts

2.6.1 Collection of plant material

Selection of plant species used in this study was based on their use in Palestinian folkloric medicine in treatment of dermatomucosal infections (Table 2.2). Mature plants were collected from several areas in West Bank during the spring and summer seasons (2000-2001). Aerial parts were collected, dried in the shade, and growned into a powdered material using an appropriate seedmill. Powdered material was then stored in labeled and sealed plastic bags. All collected plants were authenticated by Prof. M. S-Ali Shtayeh (Department of Biological Sciences, An-Najah University, West Bank).

2.6.2 Extraction

Crude extracts of each plant were obtained using two different solvents: aqueous solvent (boiling distilled water, aqueous extract), and organic solvent (cold 95%. ethanol, ethanolic extract).

2.6.2.1 Aqueous extracts

Aqueous extracts were prepared using two techniques; a freeze dried water extract and a rotatory evaporated water extracts. Two aliquots (100g each) of powdered plant material were soaked in 500-1000ml of boiling distilled water for one week at room temperature. Mixtures were stirred daily. Extracts were then filtered using muslin or Whatman filter paper no.1. One of the aqueous filtrates was evaporated in vacuum (Kandil *et al.,* 1994) and dried using a rotatory evaporator. The second filtrate was dried using freeze dryer (Labconco, model 445/F, type J T/C). Dried materials were then stored in labeled sterile bottles and kept in the freezer at -20°C, till used.

2.6.2.2 Ethanolic extracts

A 100g powdered plant material was soaked in 300-800 ml of 95% ethanol for 5 days at room temperature (Kandil *et al.,* 1994). The mixture was stirred daily for regular infusion. Extracts were then filtered in muslin or Whatman filter paper no.1 and were then dried using a rotary evaporator at 60°C. Dried extracts were then stored in sterile glass bottles and stored at - 20°C till use.

2.6.3 Sterilization of plant extracts

Two grams of origenally dried extract were dissolved in 10ml of 10% dimethyl sulfoxide (DMSO) to obtain a final concentration of 200mg/ml.

Aqueons solutions were filtered using Whatman filter paper no.1. The filtrate was then passed through a millipore filter (0.45 Nm) using an autoclaved sterile glass filter holder. Sterile filtrates were stored in screw capped sterile tubes at 5°C until use.

2.6.4 Anticandidal activity screening methods of test microorganisms

2.6.4.1 Microorganisms testing

Clinical *Candida* isolates, previousley identified by the various tests, were tested for their susceptability to various plant extracts (Table 2.3). For this purpose the disk diffusion method was used:

Table 2.3 Selected yeast species tested for anticandidal effect of plant extracts.

Isolate No.	Source
1.	*Candida albicans*
2.	*C. guillermondii*
3.	*C. krusei*
4.	*C. parapsilosis*
5.	*Geotrichum sp.*
6.	*Tricosporon sp.*

2.6.4.2 Disk Diffusion Method: A 6mm diameter disks were prepared using Whatman filter paper no.1, placed in a glass petridish and were then autocalved for 15 minutes. Twenty-five microleters of plant extracts were applied to steriled disks and were then dried under laminar flow disk sterile bench. The final content of each disk was 5 mg of extract (Ali-Shtayeh *et al.*, 1997; Murray *et al.*, 1995).

2.6.4.3 Inoculum preparation by direct colony suspension method

Part of an isolated colony of different *Candida* species was inoculated into 5ml Saboroud Dextrose Agar Broth (Andrews, 2002), or equivalent or sterile distilled water tubes and incubated for 24-48 hours at 37°C. The growth turbidity in SDA broth was adjusted by further incubation or dilution with sterile physiological saline to achieve a 0.5 Mcfarland neplelometer tube no. 0.5 (10^8 CFU/ mL) using a spectrophotometer at 625nm (optical density 0.08-0.1).

2.6.4.4 Preparation of 0.5 Mcfarland

A 0.5 ml of 0.048M $BaCl_2$ was added to 99.5 ml of 0.18 M H_2So_4 (1% w/v) with constant stirring. The standard solution was then distributed into screw cap tubes and sealed tightly to prevent evaporation. Tubes were stored in dark at room temperature. Before use, the solution was agitated vigorously in a vortex mixer to have a homogeneous turbidity.

2.6.4.5 Susceptibility test

2.6.4.5.1 Disk diffusion method

Inoculation of agar plates was performed using the adjusted candidal suspension within 15 minutes as follows (Andrews, 2002 and Jawetz, *et al.*, 1995):

1. A sterile cotton applicator was dipped into suspension, excess was removed by turning the swab against the inner side of the container.
2. The inoculum was then streaked over the entire surface of agar plate evenly.

3. Plates were then dried at room temperature for 3-5 minutes.
4. Extract disks were then distributed on the surface of inoculated agar plates using a sterile foreceps, taking into consideration a constant spaces between them.
5. Appropriate reference antibiotic disks (10mg/ml) were used for each plate. This was considered as positive control for strain sensitivity and accuracy.
6. Negative controls were also used by applying sterile disks soaked with solvent.
7. Trials were carried out in triplicates.
8. SDA plates were incubated for 24-48 hours upside down at 37°C.
9. Inhibition zones were then measured using a transparent ruler taking the mean value for triplicates (Murray *et al.,* 1995).

Table 2.4 Reference antifungals used as anticandidal agents

No.	Name of antibiotic	Concentration used for each disk
1.	**Nyststin**	**5-10 mg\ml**
2.	**Econazole**	**5-10 mg\ml**

2.6.4.5.2 Broth dilution method

Media were placed in 8 tubes, each containing 9ml SDA broth and autoclaved. 0.6ml of candidal suspension was then added to each tube.

2.6.4.6 Preparation of the extract dilutions

Nine different dilutions of each stock solution were prepared as shown in (Table 2.5) (Rippon, 1988).

Table 2.5 Different dilutions for each stock solution of plant extracts.

Tube No.	Stock solution (200mg / ml)	Emulsifier (DMSO)	Final concentration (Mg/ml)
1	**0.35**	**-**	**70**
2	**0.30**	**0.05**	**60**
3	**0.25**	**0.10**	**50**
4	**0.20**	**0.15**	**40**
5	**0.15**	**0.20**	**30**
6	**0.10**	**0.25**	**20**
7	**0.05**	**0.30**	**10**
8	**0.03**	**0.33**	**5**

2.6.4.7 Minimum candicidal concentration (MCC)

After incubation of test tubes containing candidal suspension with different extracts, subcultures were made from the visually clear tubes, on SDA plates and the MCC was interpreted using tubes that showed no growth on agar plate (Irobi *et al.,* 1994).

2.7 Anti proliferation assay

2.7.1 Cell lines

The following human cell lines were used to study the cytotoxicity of three plant extracts: pulp fibroblasts from human third molars (Dr. I. ABOUT; Laboratoire IMEB; Faculte d'Odontologie; 27BD Jean Moulin; 13385 MARSIELLE cedex 5; France), Human carcinoma cells (HT29), Hepatic cells ($HepG_2$) and Human lung fibroblasts (L929) (European Collection of Cell Cultures, ECACC, Salibury, Wiltshire, UK).

2.7.2 Plants used to study the cytotoxicity on human cell lines

Three plant extracts, chosen on the base of availability, were used in this study (*Pareteria diffusa*, *Anagalis arvensis* and *Anthemis tinctoria* L.)

2.7.3 Cell culture

All cell lines were maintained at 37°C in a humidified incubator 5% CO_2 in the air (Eilon *et al.,* 2000; Halicka *et al.,* 1997; Kelner *et al.,*1998; Bahk *et al.,* 1998). Cells were seeded in plastic petri dishes with 10ml media for each.

2.7.4 Cells harvesting and counting

Cells were incubated for 2-7 days untill a confluent grwoth was achieved. Minimum Essential Media (MEM) (GIBCO, Invitrogen Corporation) was then aspirated and cells were harvested with a 3ml 0.05% Trypsin / 0.02% EDTA solution (Onozawa *et al.,* 1998; Castaneda and Kinne, 1999). Harvested cells were then placed in 10ml fresh media.

2.7.5 MTT assay

Preparation of MTT dye was performed by disolving 75mg of [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazoliumbromide 50ml of RPMT-1640 medium with the exclusion of L-glutamin and phenol red (Sigma). The dye was then kept in the dark in 50ml tubes at 2°C for further study. 1.5-1.8 million cells were transferred into 3 microwell-plates, each has 96 wells, for MTT assay. The final volume in each well was 100µl suspension. Each plate has 0.31 cm^2 growth area, flat bottom, gamma sterilized, tissue culture-treated and transmissible (TPP, Europe /Switzerland). The cells were allowed to adhear in a 5% Co_2 incubator at 37°C for 24-48 hours. After that, plates were washed with PBS for at least three times for each well. A 100 μl plant extract was then added to each well, except for the first column which was loaded with 200 μl to result

in the highest concentration. After 24 hours incubation, viable cells were quantitated as follows: **Quantitation of viable cells;** viable cells were quantitated by 3-[4.5-dimethy) thiazol-2-Yl]-2.5-diphenyl tetrazolium bromide (MTT) (MERCK, Germany). In brief 100 μl of MTT solution (3mg/ml) was added to each well and left for 3hours at 37°C, followed by a washing step under shaking using absolute DMSO solution to dissolve violet crystals (Srivastava *et al.*, 1998). Viability was quantitated by measuring A_{570}, using a 96 microwell plate-reader with a reference wave length of 650 nm. The percentage of cell survival defined as (mean A_{650} of treated wells / A_{650} untreated control wells) × 100%. (Srivastava *et al.*, 1998).

2.7 Statistical analysis

Data were analyzed using SPSS (Statistical Package for Social Sciences). Percentages and Chi-square tests were used for evaluating the degree of significance with 95% confidence ($p< 0.05$).

Relative anticandidal activity (RAA) was determined as follows: RAA = [(inhibition zone diameter mean of active plant)^2/(inhibition zone diameter mean of reference antibiotics)^2] × 100%

CHAPTER THREE
RESULTS

3.1 Incidence of fungal infections and associated symptoms among females complaining from vaginal discharge

Data on the incidence of fungal infections and associated symptoms in woman complaining from vaginal discharge are presented in figure 3.1 and table 3.1. Out of 119 studied cases, 63 (52.9%) were yeast positive. Symptoms of itching, dysparenia, burning and dysuria, urinary tract infection and other symptoms were encountered in the following percentages 22.5, 8.9, 8.9, 6.3, and 17.5, respectively. Differences on the occurrence of these symptoms were statistically significant (χ^2 =198.73, df=4, *P*= 0.000). Yeast negative cases were represented by 56 (47.1%). Symptoms of itching, dysparenia, burning and dysuria, urinary tract infection, other symptoms and bad odor were encountered in the following percentages 12.5, 4.8, 14.3, 0.0, 21.4 and 7.9 respectively (Table 3.1).

Table 3.1 Yeast infections and associated symptoms among females with complaint from vaginal discharge

Symptoms	Yeast Positive (No. %) 63 (52.9)	Yeast Negative (No. %) 56 (47.1)
Itching	**14 (22.5)**	**7 (12.5)**
Dysparenia	**5 (8.9)**	**3 (4.8)**
Burning and Dysuria	**5 (8.9)**	**8 (14.3)**
Urinary tract infection	**4 (6.3)**	**-**
Other symptoms	**11 (17.5)**	**12 (21.4)**
Bad odor	**5 (7.9)**	**-**

Out of 119 studied cases, 67 were city residents, 42 were village residents and 8 were refugee camp residents (Table 3.2). Yeast infection rates of 47.8, 61.9 and 38.5, were observed among city, village and refugee camp residents,

respectively. Differences on the occurrence of infection rates were statistically significant (χ^2 =18.17, df =2, *P*= 0.000) and in favor of village inhabitants. The highest rate was among village residents followed by city residence and the lowest was among refugee camp residents. With respect to the distribution of yeast infection among age groups, age group 21-30 showed the highest rates of infection among both city (62.5%) and village (69.2%) inhabitants, whereas age group 31-40 showed the highest rate of infection (60%) among camp inhabitants. Variations on the infection rates among the different age groups were statistically significant and in favor of age group 21-30 years (χ^2 =50.7, df =3, *P*= 0.000).

Table 3.2 Association between yeast infection, place of residence and age group among women studied cases.

Place of Residence	Age groups	Yeast Infection Status	
		No.% of +ve cases	No.% of – ve cases
City	≤20	3(9.4)	8(25)
	21-30	20(62.5)	15(46.9)
	31-40	8(25)	7(21.8)
	>40	1(3.1)	2(6.3)
	Total	**32 (47.8)**	**35 (52.2)**
Village	≤20	6(23.1)	6(37.4)
	21-30	18(69.2)	4(25)
	31-40	2(7.7)	3(18.8)
	>40	-	3(18.8)
	Total	**26 (61.9)**	**16 (38.1)**
Camp	≤20	1(20)	-
	21-30	1(20)	5(62.5)
	31-40	3(60)	3(37.5)
	>40	-	-
	Total	**5 (38.5)**	**8 (61.5)**

Table 3.3 Occurrence of yeast species among women and student population based on various identification methods.

Yeast Spp.	School Children		Women No. (%)
	Males No. (%)	Females No. (%)	
C. albicans	5 (5.5)	8 (19)	**42** (66.7)
C. guillermondii	57 (62.6)	11 (26.2)	**16** (25.3)
C. parapsilosis	5 (5.5)	2 (4.8)	-
C. krusei	11 (12.1)	6 (14.3)	-
C. famata	-	2 (4.8)	-
Saccharomyces cervisiae	3 (3.3)	-	2 (3.2)
Geotrichum spp.	5 (5.5)	4 (9.5)	-
Tricosporon spp.	5 (5.5)	9 (21.4)	3 (4.8)
Total of positive cases	91/100)	42/ (100)	63/ (100)

Candida albicans was found to be the most common yeast species among females suffering from fungal infections 42 (66.7%), followed *C. guillermondii* (25.3%) (Table 3.3). Other species, *Saccharomyces cervisiae* and *Tricosporon spp.* were represented by 3.2% and 4.8%, respectively.

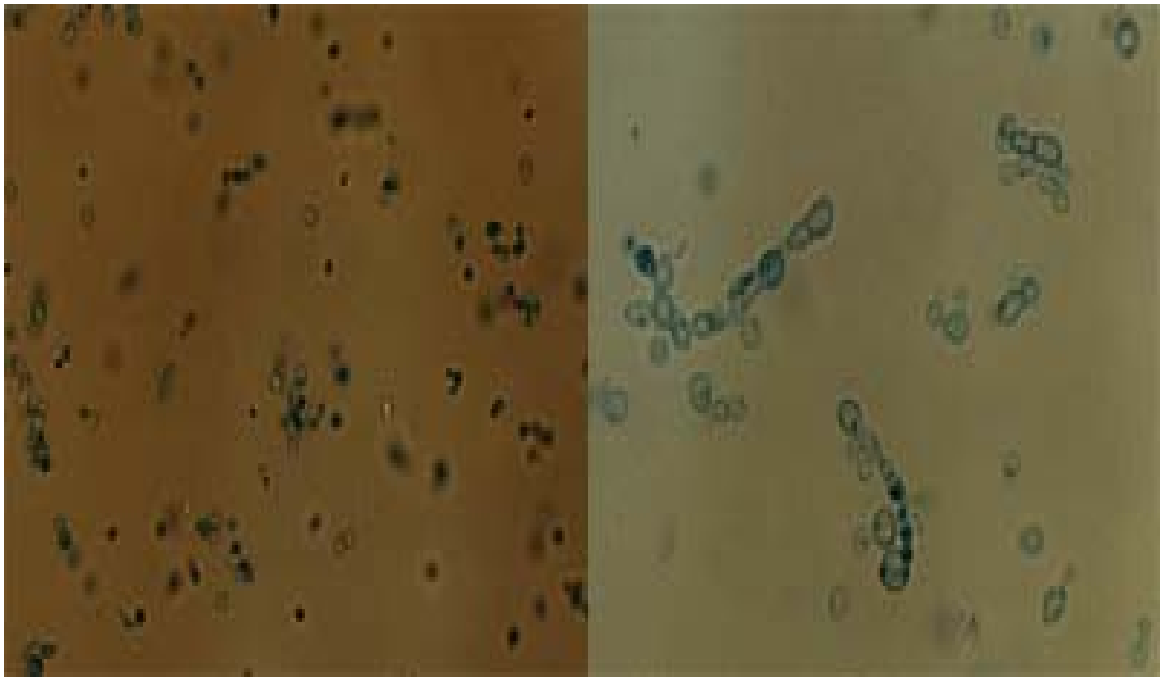
The most prevalent species observed in school children were; *C. guillermondii* (males; 62.6%, females; 26.2%), *Tricosporon spp*. (males; 5.5%, females; 21.4%) and *C. albicans* (males; 5.5%, females; 19%). Low prevalence rates were observed for *C. parapsilosis, C. krusei* and *Geotrichum spp.* among both genders. *Saccharomyces cervisiae* was observed only in males (3.3%), however, *C. famata* was observed only in female student population (4.8%). Positive growths and clear budding for some of these isolates are presented in Figures 3.1 and 3.2.

API20C Kit was used as a confirmatory method. Out of 40 selected tested isolates using this kit, 31 isolates were identified as yeast positive and the rest 9 isolates were unidentifiable (Table 3.4 and Figure 3.3). All these samples were

originally proven to be yeast positive isolates using classical identification methods.

A B

Figure 3.1 Slant gels (A) showing yeast growth on SDA media (TTSG28M2, TQSG11M39 were isolated from students; THVG3S3 is a vaginal isolate), (B) shows three cultured isolates (upper row SDA with cycloheximide, the lower SDA without cycloheximide- each column represents the same isolate).



A B

Figure 3.2 Micrographs showing yeast budding on SDA broth; (A) isolated from skin (B) a vaginal isolate.

Figure 3.3 Three API20C strips inoculated with three different isolates of *Candida*.

Table 3.4 Identification of representative yeast positive isolates using API20C Kit.

No. of isolates (No. %)	Identification by API20C Kit
9 (22.5)	Unidentified by the kit
4 (10)	***C. albicans***
11 (27.5)	***C. guillermondii***
2 (5)	***C. parapsilosis***
2 (5)	***C. krusei***
1 (2.5)	***C. famata***
1 (2.5)	***Geotrichum spp.***
1 (2.5)	***Tricosporon spp.***
9 (22.5)	***Saccharomyces cervisiae***
Total number of isolates 40	Total types of yeast 8

With respect to the infectious status, pregnant women showed the highest rate of infection. The infection rates of 58.1%, 52.8% and 43.8% were observed among pregnant, non-pregnant and post delivery women, respectively (Table 3.5). Disease symptoms were more frequent and conspicuous in pregnant women compared to the other two groups, especially non-pregnant women.

Itching was encountered in 27.8% and 21.1% of pregnant and non pregnant women, respectively. The rates of 5.6% and 2.6% were observed for dysparenia among pregnant and non pregnant women, respectively. The percentages of 11.1% and 5.3% were observed for burning and dysurea among pregnant and non pregnant women, respectively. However, the percentages of 11.1% and 2.6% were observed for UTI among pregnant and non pregnant women, respectively. Also the percentages of 10.5, 14.3 for bad odor were observed in non- pregnant and post delivery women, respectively. Differences in the occurrence of the various symptoms of infection rates among women in different pregnancy status were not statistically significant (χ^2 =26.411, df =24, *P*= 0.333).

Table 3.5 Association between pregnancy status, yeast infection and its associated symptoms.

Pregnancy status	Yeast infectious status No. %	Symptoms					
		Itching No. %	Dysparenia No. %	Dysurea And Burning No. %	UTI No. %	Other Symptom No. %	Bad odor No. %
Pregnant 31(26.1%)	+ve 18 (58.1)	5(27.8)	1 (5.6)	2(11.1)	2(11.1)	3(16.7)	-
	- ve 13 (41.9)	3(23.1)	1(7.7)	3(23.1)	-	3 (23.1)	-
Non-pregnant 72(60.5%)	+ve 38 (52.8)	8 (21.1)	1 (2.6)	2 (5.3)	1(2.6)	7(18.4)	4(10.5)
	-ve 34 (47.2)	4(11.8)	2 (5.9)	6(17.5)	-	8 (23.5)	
Post delivery 16(13.4%)	+ve 7 (43.8)	1(14.3)	1 (14.3)	-	1(14.3)	1(14.3)	1(14.3)
	-ve 9 (56.2)	9(100)	2 (22.2)	-		1 (11.1)	

+ Positive, - Negative

3.2 Occurrence of foot cutaneous candidiasis among school children

Out of 463 examined school children, 199 were suspected to have cutaneous fungal infection based on clinical characterization. Out of 251 studied male population, 91(36.3%) were found to be yeast positive based on the results of SDA culture media. With respect to females, out of 212 studied population, 42(19.8%) were found to be yeast positive. The prevalence rates of cutaneous candidiasis of 36.3% and 19.8% were found among males and females, respectively (Table 3.6).

Table 3.6 Occurrence of cutaneous candidiasis among school children.

Student Groups	No. of studied cases No. (%)	Suspected cases No. (%)	Yeast +ve No. (%)	Yeast -ve No. (%)
Male	251 (100)	130 (51.8)	91 (36.3)	39 (63.7)
Female	212 (100)	69 (32.5)	42 (19.8)	27 (80.2)
Total	463 (100)	199 (42.9)	133 (28.7)	66 (14.3)

Table 3.7 Cutaneous candidiasis and associated symptoms among suspected cases.

No. of studied cases	Infectious Status No. (%)	Symptoms				
		Erythema No. (%)	Scaling No. (%)	Mal odor No. (%)	Maceration No. (%)	Interdigital No. (%)
Males 130	+ve 91 (36.3)	29 (31.9)	19 (20.9)	24 (26.4)	7 (7.7)	84 (92.3)
	-ve 39 (63.7)	15 (38.5)	11 (28.2)	21 (53.8)	2 (5.1)	36 (92.3)
Females 69	+ve 42 (19.8)	23 (54.8)	31 (73.8)	16 (38.1)	3 (7.1)	24 (57.1)
	-ve 27 (80.2)	17 (62.9)	8 (29.6)	6 (22.2)	1 (3.7)	16 (59.3)

Erythema, scaling, mal odor, maceration and interdigital were found in male suspected cases with the following percentages 31.9%, 20.9%, 26.4%, 7.7% and 92.3%, respectively. The rates of 54.8%, 73.8%, 38.1%, 7.1% and 57.1% were found for erythema, scaling, mal odor, maceration and interdigital among female cases, respectively (Table 3.7). The occurrence of interdigital was of the most common prevalent symptoms. With the exception of interdigital, all observed symptoms were more prevalent among females compared to males.

Combined symptoms with various rates were observed, among these; interdigital and scaling 11.1%, interdigital, erythema and mal odor 10.1% and interdigital and mal odor 8% (Appendix D). Variations on the occurrence of combined symptoms among school children were statistically significant (χ^2 =268.53, df = 21, *P*= 0.000).

Table 3.8 shows occurrence of cutaneous candidiasis among suspected cases of school children according to place of residence. The highest rate of

infection was observed among males camp inhabitants (85%), followed by city residents (66.2%) and village (40%). All three females from villages had infected feet, whereas, those from city and camp showed the rates 68.9% and 38.1%, respectively (Table 3.8). Variations on the infection rates among female population were statistically significant and in favor of those reside in villages (χ^2 =63.6, df =2, *P*= 0.000).

Table 3.8 Occurrence of cutaneous candidiasis among suspected cases of school children according to place of residence.

Gender	Infectious Status No. (%)	Place of residence		
		City No. (%)	Village No. (%)	Camp No. (%)
Males 130	+ve 91 (36.3)	53(66.2)	4(40.0)	34 (85)
	-ve 39 (63.7)	27(33.8)	6(60.0)	6 (15)
Females 69	+ve 42 (19.8)	31(68.9)	3(100)	8 (38.1)
	-ve 27 (80.2)	14(31.1)	-	13(61.9)

Association between cutaneous candidiasis and other skin disorders among school children showed that acnes, eczema, allergy and other mycotic infections, rather than candidiasis, were predominant among males. The highest association rates were found for other mycotic infections (19.8% among males; 7.1% among females) (Table 3.9). Association between cutaneous candidiasis and other skin disorders were statistically significant (χ^2 =415.2, df =8, *P*= 0.000).

Table 3.9 Association between cutaneous candidiasis and other skin disorders among suspected cases of school children.

Gender	Infectious Status No. (%)	Associated skin disorders					
		Acnes No. (%)	Eczema No. (%)	Allergy of skin	Other mycosis No. (%)	Other infections No. (%)	None No. (%)
Males 130	+ve 91(36.3)	10(10.9)	2(2.2)	3(3.3)	18 (19.8)	4(4.4)	54(59.4)
	-ve 39(63.7)	4(10.3)	-	-	8 (20.5)	-	27(69.2)
Females 69	+ve42(19.8)	-	4(9.5)	2(4.8)	3 (7.1)	2(4.8)	31(73.8)
	-ve 27(80.2)	1(3.7)	2(7.4)	2(7.4)	1 (3.7)	-	21(77.8)

Out of 91 positive male cases, 57.1% reported that they dry their feet after washing, 26.4% reported that they use common facilities and 58.2% were with contact with domestic animals. On the other hand, 37.5% of female positive cases (42) reported that they dry their feet after washing, 30.9% reported that they use common facilities and 80.9% were with contact with domestic animals (Table 3.10). Variations on the infection rates with respect to the use common facilities and were in contact with domestic animals were statistically significant, (χ^2 =12.6, df = 1, *P*= 0.000) and (χ^2 = 54.3, df = 1, *P*= 0.000), respectively. Differences in the infection rates with respect to the drying of foot practice showed no statistical significance (χ^2 =0.068, df = 1, *P*= 0.795).

Table 3.10 Association between cutaneous candidiasis and other practices among school children

Students	Yeast infection No. (%)	Other practices					
		Drying of feet after washing No. (%)		Use of common facilities No. (%)		Presence of domestic animals No. (%)	
		Yes	No	Yes	No	Yes	No
Males 130	+ve 91(36.3)	52 (57.1)	39(42.9)	24(26.4)	67(73.6)	53(58.2)	38(41.8)
	-ve39(63.7)	28 (71.8)	11(28.2)	10(25.6)	29(74.4)	24(61.5)	15(38.5)
Females 69	+ve 42(19.8)	15 (35.7)	27 (64.3)	13 (30.9)	29 (69.1)	34 (80.9)	8 (19.1)
	-ve 27(80.2)	6 (22.2)	21 (77.8)	5 (18.5)	22 (81.5)	20 (74.1)	7 (25.9)

3.3 Anticandidal activity of selected plant extracts

Susceptibility of 4 yeast species was tested using 18 different plants and two different antifungal drugs (Nystatin and Econazole). Relative anticandidal activities of these extracts were evaluated. Almost all tested extracts showed anticandidal activities to various degrees based on estimates of inhibition zones. *Allium sativum* showed the highest (100%) relative anticandidal activity compared to reference antibiotics. Obvious inhibition zones were also observed for *Pistacia lentiscus*, S*alvia dominica* and *Petroselinum sativum* with relative anticandidal activities of 3.8 %, 2.25% and 2.25%, respectively. The least activity (0.35%) was observed for *Anagalis arvensis L., Gagea cloranth, Sonchus oleraceus L., Coridothymns capitatus* and *Inula viscosa L.* compared to reference antibiotic (Table 3.11). Figures 3.3 and 3.4 illustrate the percentages of relative anticandidal activities of 18 plant extracts against selected yeast spp. For better comparison *Allium sativum* was excluded (Figure 3.4).

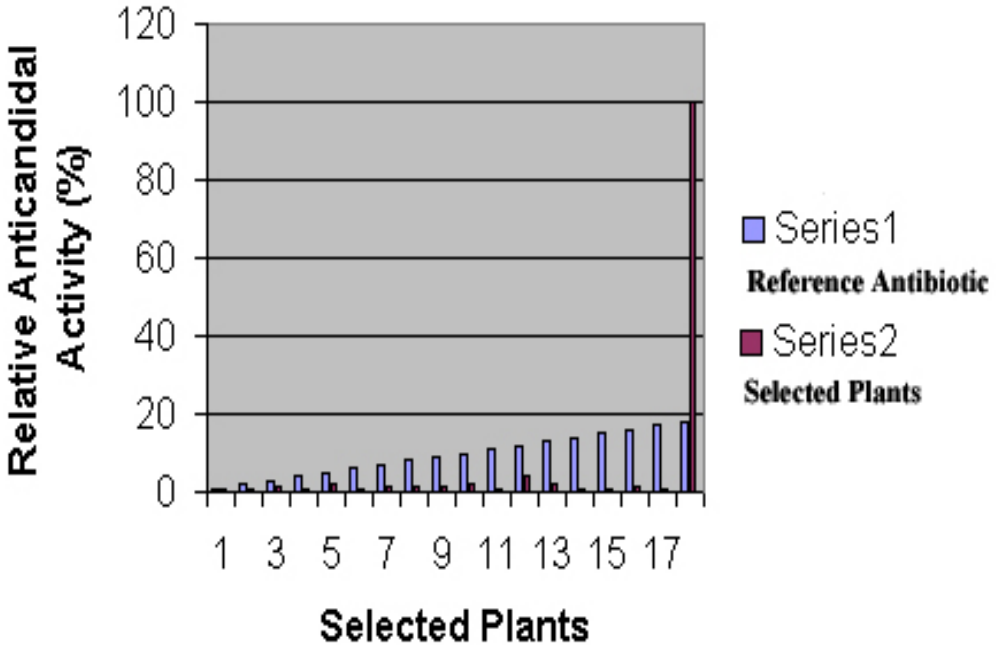


Figure 3.4 Diagram representation of relative anti-candidal activity for selected plants (1.*Lawsonia inermis* L; 2. *Juglans regia* L.; 3. *Calycotome villosa ;* 4. *Rosmarinus officinalis* L.; 5. *Anthemis tinctoria* L.; 6. *Inula viscosa* L.; 7. *Styrax officinalis* L.; 8. *Ziziphus spina-christi* L.; 9. *Campanula rapanculuis* L. ; 10. *Petrosilinium sativum;* 11. *Sonchus oleraceus* L.; 12. *Pistacia Lentiscust* L. ; 13. *Salvia dominica* L. ; 14. *Gagea cloranth* ; 15. *Anagalis arvensis* L.; 16. *Parieteria diffusa* ; 17. *Coridothymns capitatus* ; 18. *Allium sativum* L.).

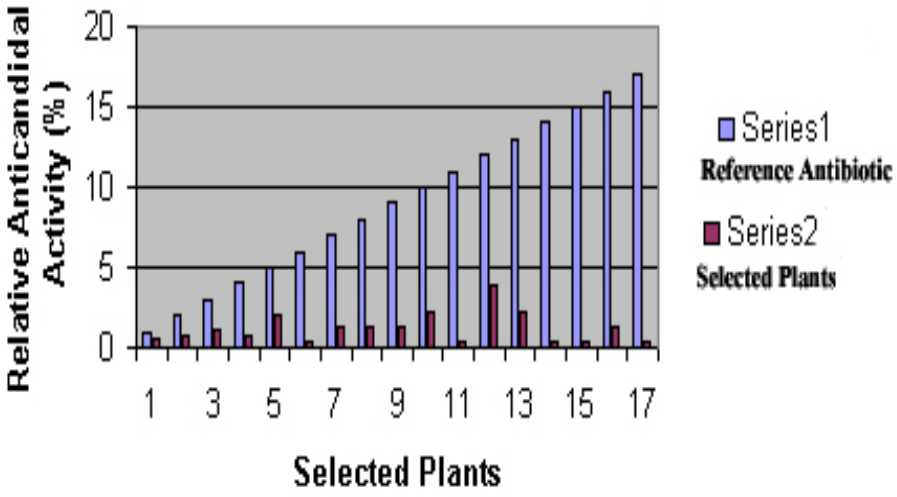


Figure 3.5 Diagram representation of relative anticandidal activity for selected plants excluding *Allium sativum.* (See figure 3.3 for used plants).

Table 3.11 Percentages of relative anticandidal activity values of selected plants used for anticandidal susceptibility testing.

Species / Family	Common Name	Arabic name	Tested Yests	*Relative activity
1.*Lawsonia inermis* L.	Henna	حناء	*C. albicans*	0.56
2. *Juglans regia* L.	Walnut	جوز	*C. albicans*	0.69
3. *Calycotome villosa*	Thomy broom	قنديل	*C.guillermondii* *C.neoformans*	1.6 0.63
4. *Rosmarinus officinalis* L.	Rosemary	حصلبان	*C. albicans* *C. neoformans*	0.74 0.69
5. *Anthemis tinctoria* L.	Yellow cammomile	بابونج أصفر	*C. albicans*	1.95
6. *Inula viscosa* L.	Inula	عرق طيون	*C. guillermondii*	0.35
7. *Styrax officinalis* L.	Snow bell	عبهر	*C. parapsilosis*	1.37
8. *Ziziphus spina-christi* L.	Syrian Christ thorn	دوم السدر	*C. parapsilosis*	1.37
9. *Campanula rapanculuis* L.	Bell flower	ورد الجرس	*C.guillermondii*	1.38
10. *Petrosilinium sativum*	Parsley	بقدونس	*C. guillermondii*	2.25
11. *Sonchus oleraceus* L.	Mustard	خردل	*C. guillermondii*	0.35
12. *Pistacia Lentiscust* L.	Mastic lentisk	سريس	*C. parapsilosis*	3.8
13. *Salvia dominica* L.	Sage	خواخة	*C. guillermondii*	2.25
14. *Gagea cloranth*	Gagea	زعيتمان	*C. guillermondii*	0.35
15. *Anagalis arvensis* L.	Red pimpernes	عين الجمل	*C. guillermondii*	0.35
16. *Parieteria diffusa*		عشبة الدم	*C. guillermondii*	1.38
17. *Coridothymns capitatus*	Thyme	زعيمة	*C. guillermondii*	0.35
18. *Allium sativum* L.	Garlic	الثوم	*C.albicans*	100

- Relative activity = (Inhibition zone diameter mean of active plant)^2 / (Inhibition zone diameter mean of reference antibiotic)^2× 100%

MIC values for the most effective plants tested against three isolated yeast spp. compared to two reference antibiotics were evaluated (Table 3.12).

Table 3.12 MIC values for most effective plants tested against three yeast species.

Most active plants	**Tested yeast species**		
	C. albicans	***C. krusei***	***Geotrichum spp.***
	Minimum Inhibitory Concentration values (MIC)		
Pistacia lentiscus	MIC ≥ 60	MIC ≥ 70	MIC ≥ 70
Allium sativum	MIC ≥ 30	MIC ≥ 10	MIC ≥ 10
* Nystatin	MIC ≥ 7.5	MIC ≥ 5	MIC ≥ 5
* Econazole	MIC ≥ 60	MIC ≥ 60	MIC ≥ 60

*** Reference antibiotics**

3.4 Cytotoxicity of selected plant extracts against human cell lines

Cytotoxic effects of three selected plants (*Anthemis tinctoria* L., *Parieteria diffusa* and *Anagalis arvensis)* were tested against known normal human cell lines. MTT assay was used and percentages of cell mortality were plotted against extract concentrations in micrograms. Figure 3.6 shows the effect of these plants against cell line HT29. All extracts showed cytotoxic effect at ≥ 500µg, however, more pronounced effects were observed for *Parieteria diffusa* and *Anagalis arvensis,* respectively.

Histograms used in cytotoxicity study were based on measured optical densities for cell mortality percentages at five different plant extracts concentrations. Cell mortality % = Mean of optical density of blank - mean of optical density of extract / mean optical density of blank X100% (Appendix D).

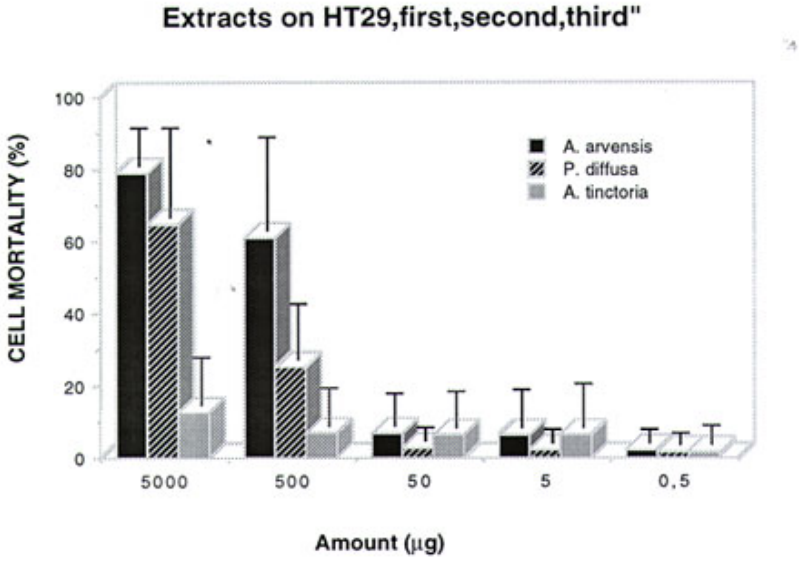


Figure 3.6 Cytotoxicity of three plant extracts against human cell line HT29

Data presented in Figure 3.7 shows the cytotoxic effects against L929cell line. Extracts from *Parieteria diffusa* and *Anagalis arvensis*, extracts showed cytotoxic effect at ≥ 500µg, however, more pronounced effect was observed for *Anthemis tinctoria* L. at ≥ 5000µg. The other two extracts showed almost the same effect using both concentrations.

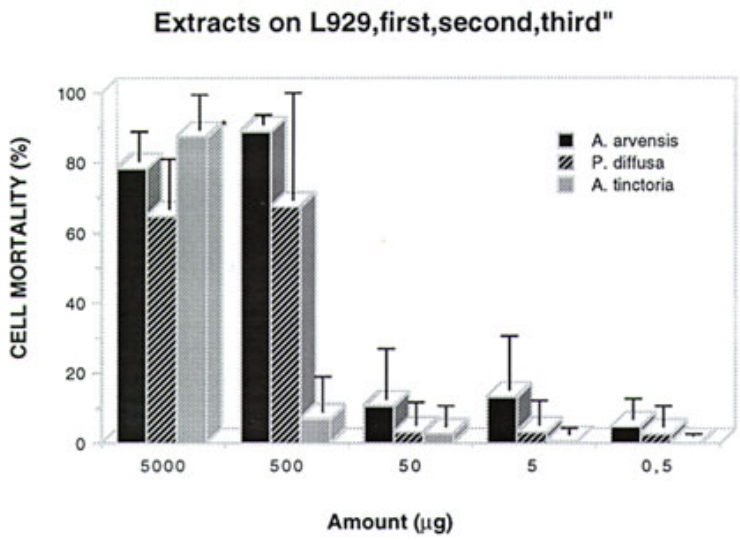


Figure 3.7 Cytotoxicity of three plant extracts against human cell line L929.

Data presented in Figure 3.8 shows the cytotoxic effects against HepG2 cell line. All extracts showed cytotoxic effect at ≥ 5000µg, however, *Anagalis arvensis* showed almost the same effect at ≥ 500µg.

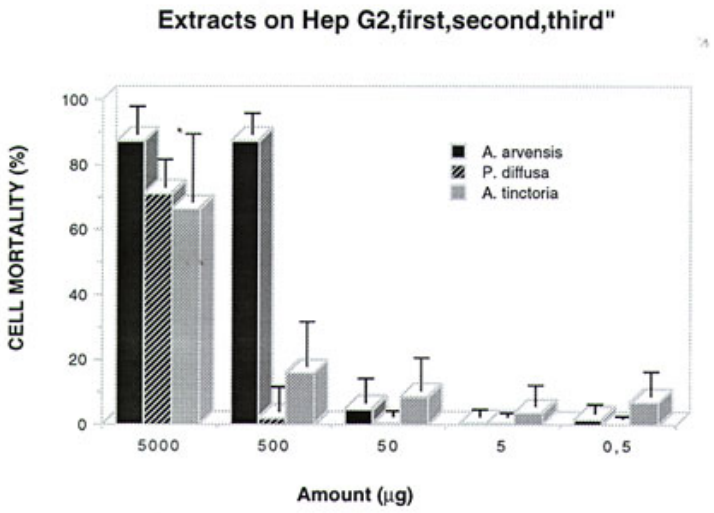


Figure 3.8 Cytotoxicity of three plant extracts against human cell line HepG2.

Data presented in Figure 3.9 shows the cytotoxic effects against Pulp Fibroblasts cell line. *Anthemis tinctoria* L. showed a pronounced cytotoxic effect at ≥ 5000µg, however, *Anagalis arvensis* and *Parieteria diffusa* showed reduced effect at this concentration in comparison with that at 500µg.

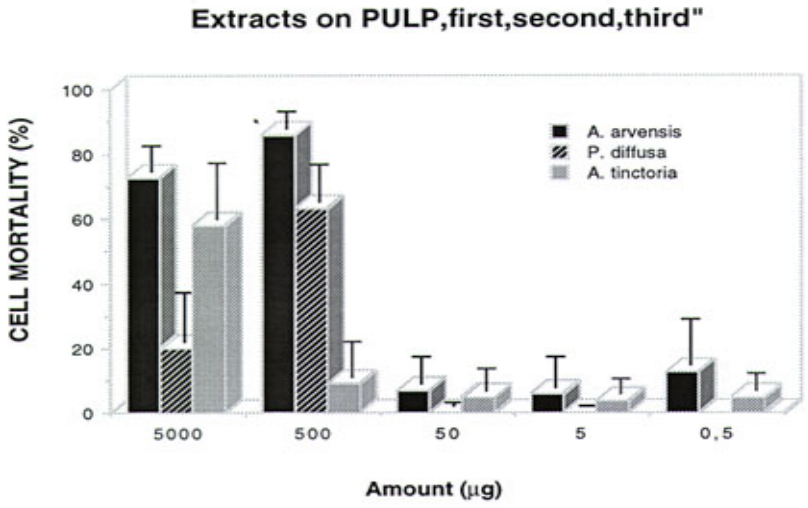


Figure 3.9 Cytotoxicity of three plant extracts against human Pulp fibroblast cell line.

CHAPTER FOUR
DISCUSSION

4.1 Incidence rates, etiological agents, predisposing factors and clinical presentation of vaginal candidiasis among women suffering from vaginal discharge

The fungus, *Candida albicans*, is the microorganism most frequently involved as an etiological agent in vaginitis (Hall, 2003; Garland *et al.*, 2002; Gulmezoglu, 2002). *Candida albicans* infections are clinically associated with vulvar irritation, dysuria, and a white (cheesy) discharge. Risk factors for vaginal candidiasis include pregnancy, use of oral contraceptives, estrogen therapy, end of menstrual cycle, diabetes, and antibiotic therapy (Reed *et al.*, 1993; Eckert *et al.*, 1998; Rex *et al.*, 2000; Sobel, 2002).

In our study population, out of 119 females suffering from vaginal discharge, the incidence rate of yeast infection was 52.9% (Table 3.1), of which 66.7% were due to the infectious agent *C. albicans* (Table 3.3). Itching and irritation were the most prevalent symptoms among yeast positive cases, each represented by 22.5%. These symptoms are well known for their association with yeast infections (Table 3.1). Variations on the occurrence of these symptoms were statistically significant (χ^2 = 198.7; df = 10; P =0.000). These findings were in agreement with several previous reports in this respect (Suhonen *et al.*, 1999).

Candida infections are usually due to impaired epithelial barrier functions and occur in all age groups, but are most common in the new born and the elderly (Murray *et al.*, 2000). Association between yeast infections and age in this study showed that the age group 21-30 years was represented

with the highest rate of infection. This was clear from the findings among both city (62.5%) and village inhabitants (69.2%), which constitute the majority of the studied population. This is consistent with several previous reports where this age group represents the most active sexual and reproductive age (Roberts, *et al.,* 1989). It is worth noting that age group 31-40 showed the highest rate of infection among the residents of refugee camps (Table 3.2). Recent antibiotic use, young age, and absence of current gonorrhea or bacterial vaginosis might be behind this observation which requires further investigation (Eckert *et al.,* 1998). Differences in the infection rates among the various age groups were statistically significant (χ^2= 50.7; df = 3; *P*=0.000).

Pregnant women were represented with the highest incidence of yeast infection (58.1%) compared to non-pregnant and post delivery groups (Table 3.5). This is an expected observation as pregnancy is considered as a predisposing factor for infection (Schmidt *et al.,* 1997).

Findings on incidence rates of infection among non-pregnant (52.8%) and post delivery (43.8%) were also expected as most women experience such infections in their life time especially at reproductive age as yeast infections seems to favor high estrogen levels which in turn stimulates glycogen secretion, which provides extra nutrition for yeast growth (Hillier and Lau, 1997).

Several symptoms were observed in association with yeast infection and the most common symptom was itching especially among pregnant women 27.8% (Table 3.5). Our findings in this respect are consistent with those reported by Abbott, (1995).

Village inhabitants showed the highest rate of yeast infection (61.9%), followed by the rates of 47.8% and 38.5% among city and refugee camp inhabitants, respectively (Table 3.2). The variations in the incidence rate of infection were statistically significant (χ^2 =18.17; df = 2; P = 0.00). This might be partially explained by the fact that a better health services are offered for both refugee camp and city inhabitants compared to that offered to village inhabitants. On the other hand the observed low rate of yeast infection among refugee camp inhabitants was not expected and might be explained by the fact that these women suffered from other infections, mainly of bacterial origin, which limits yeast colonization (Eckert *et al.*, 1998).

Due to the limited number of women using contraceptives, it was difficult to link any correlation between contraceptive use and yeast infections in the current study. It is also worth noting that the use of antibiotic and antifungal drugs was excluded in our study due to the fact that only four women reported to be under treatment (Appendix C).

4.2 Incidence rates, etiological agents, predisposing factors and clinical presentation of cutaneous candidiasis among suspected cases of school children

The incidence rate of cutaneous candidiasis among school children population was 28.7% (Table 3.6), with *C. guillermodii* as the predominant etiological agent among male infected cases (62.6%), whereas it was represented by 26.2% among female infected cases (Table 3.3). This is an expected observation as this species is well known for causing numerous human infections mostly of cutaneous origin and also from normal skin (Suhonen, *et al.,* 1999). Other etiological agents including *C. albicans, C. parapsilosis, Geotrichum spp, Saccharoomyces cervisia, C. krusei*, *Geotrichum spp.* and *Tricosporon spp*. were also observed with various prevalence rates (Table 3.3). All of these species are known for their colonization of human skin (Suhonen *et al.,* 1999).

Differences in the infection rates caused by *C. guillermodii* in both genders might be partially due to the fact that male's skin is composed of a thicker keratin layer which provides a favorable environment for yeast growth compared to female skin (Kwon-Chung and Bennett, 1992). Sport activities and the lack of wearing or misuse of sport shoes might also account for the higher incidence of infection among males as these conditions can generate moist, heat and friction which are preferable conditions for cutaneous yeast infections (Merlin *et al.,* 1999; Svejgaard *et al.,* 1983; Clayton and Hay, 1994; Perea *et al.,* 2000; Philpot and Shuttleworth, 1989).

Moisture, heat, friction and maceration of the skin are considered to be the major predisposing factors in normal patients for candidiasis. These factors in addition to acidity promote proliferation of yeast and explain the frequency of involvement of the large poriorificial skin folds (Brocks *et al.*, 1999). This may explain the presence of clinical signs of erythema and maceration especially among male population in the current study. Since males were represented with higher rate of yeast infection one should expect to see a higher rate for combined symptom of erythema and maceration among this group. The major clinical sign of cutaneous candidiasis was interdigital (Table 3.7). This is an expected observation in yeast infections of skin due to the humid environment (Al- Sougair, Moawad and Al-Humaidan, 1991).

Variations in the prevalence of infection rates based on place of residence were statistically significant ($\chi^{2 =}$ 63.6, df =2, *P=0.000).* The highest rate of infection was among male population of refugee camp residents. The fact that most of these school children were living under poor hygiene conditions and with inadequate footwear might explain this high incidence (Roig and Rodrigue, 1987; Kamihama *et al.*, 1997; Ajao and Akintunde, 1985). It is worth noting that the total number of female village residents was only three (Table 3.8) and were represented with the highest rate of infection among female population (100%). Cutaneous candidiasis and associated skin disorders were statistically significant ($\chi^{2=}$ 415.2, df = 8, *P*=0.000).

The most associated disorder among males was other mycotic infections in the feet, other than cutaneous candidiasis, and the majorities were diagnosed by a dermatologist. This is an expected consequence as candidiasis could be primary or secondary infection. Primary infection usually arises when the person's immune system is compromised which provides a preferable environment for yeast colonization, while secondary infection could be caused indirectly as a consequence of another infection that weakens the body defense mechanisms, thus feet mycoses could be convenient predisposing factors of cutaneous candidiasis. In females, eczema was the predominant associated disorder and signs were observed in both the arms and hands (Table 3.9). It is well known that under certain conditions that leave the immune system weakened, such as eczema, extreme stress, poor eating habits or exhaustion, candida will proliferate and virtually explode in the system (Hussein, 2000).

The use of common facilities such as towels seems to indicate that this practice did not affect the rate of cutaneous candidiasis as those who reported the nonuse of common facilities were represented with higher rates of infection. Differences in the rate of infection among those who practiced the use of common facilities and those who did not were statistically significant (χ^2 =12.6, df = 1, P=0.000). Such finding is expected as cutaneous candidiasis is not known to be contagious infection (Table 3.10).

Contact with domestic animals showed a positive effect on the rate of cutaneous candidiasis in both genders, reported a contact with animals, and

showed a much higher rate of infection compared to those who reported no contact (Table 3.10).

Data on the practice of drying of feet were contradictory among both males and females (Table 3.10). Females who reported that they did not practice feet drying were with higher rate of infection (64.3%) compared to those who did not (35.7%), while males who practiced drying their feet seems to show a higher rate of infection (57.1%) compared with those who did not (42.9%). Variations in the rate of infection with respect to this practice were of no statistically significant values (χ^2=0.068, df = 1, *P*=0.795). Our findings among females are expected as humid wet environment is known to be a predisposing factor for yeast infection and are in agreement with data reported by Leibovici *et al.,* (2002). However, the most possible explanation for the findings among male study population is most likely that they might pretend that they practiced the habit of feet drying while in fact they did not.

The occurrence of yeast species was confirmed using API20C Kit (Table 3.4). The finding of certain undifferentiated yeast species using this kit strongly indicates the lack of specificity of this method and hence the use of the modified API20CAUX kit may yield better differentiation.

4.3 Anticandidal activity of selected plant extracts

Eighteen different selected plants known for their antifungal and antibacterial activities were tested in our study in search for safe and effective anticandidal activities compared to reference antibiotics (Nystatin and

Econazole). Out of these plants, four were found to be with promising anticandidal activities; these include *Allium sativum, Pistacia lentiscus, Salvia dominica* and *Petroselinum sativum* (Table 3.11). *Allium sativum* (garlic) showed an activity exceeding that of both reference antibiotics. Garlic is a very important natural antifungal –antibiotic. It contains the volatile oil, allium, which converts to allicin when crushed or sliced. Exposed to air, allicin converts to diallydisulphide, which is a powerful bactericide and fungicide also effective against many viruses (Hussein, 2000). The other three plants were with activities similar to that shown by reference antibiotics.

4.4 Cytotoxicity of selected plant extracts against human cell lines

It is well known that plant extracts inhibit some enzymatic activities. Our aim was to evaluate the possible use of such plants for treatment purposes, thus it was essential to study the cytotoxic effect on human cells. The cytotoxic effect of three selected plant extracts (*Anthemis tunctoria* L., *Parieteria diffusa* and *Anagalis arvensis*) were tested against four normal human cell lines using MTT assay. All tested plants showed toxic effect, however, they seem to have variable effects at different concentrations depending on the used cell line. These results need further investigations in order to determine the value of such plants in treating yeast infections.

4.5 Concluding remarks and recommendations

1. To avoid possible disease consequences of vaginal infections, it is essential to accurately diagnose and identify causative agents involved in such infections as this will lead to a better treatment and control management. In women who fail to respond to treatment, it is important to reestablish the diagnosis to make sure that the cause of the symptoms remains the same.

2. Although yeast infection is not considered to be sexually transmitted, examination and treatment of the sexual partner may be recommended in cases of frequent recurrence.

3. The finding of this study shows a strong association between yeast infection and both itching and irritation, thus, it is essential to avoid scratching as this may further irritate the area. On the other hand, women should wear cotton underpants and avoid tight clothing as such practices will limit the generation of both moist and heat which are considered as predisposing factors for yeast growth. Practice good hygiene is also of great importance to limit yeast infections.

4. It is best to avoid sexual intercourse when symptoms are at their worst. If a sexual partner needs treatment, refrain from genital contact until both partners complete treatment. This is clear from our findings on infection associated with dysparenia in certain cases.

5. The findings on garlic antifungal activity strongly recommend its use in our diets. Diet is a very important contributing factor in vaginal candidiasis, thus it is recommended to use whole foods diet that includes: fresh vegetables

including garlic and fruits, fish and poultry, seeds and whole grains, essential fatty acids (such as olive oil and nuts) and plenty of water.

6. The findings of extremely high incidence of cutaneous candidiasis among school children draw the attention for more detailed investigations on yeasts and their reservoirs as yeast, in our study, seems to be the major causative agent involved in primary and secondary infections.

7. Finally, it is essential to increase the awareness of the public regarding yeast infections and this can be achieved through especially educational designed programs.

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Appendices

Appendix A

MSc. Project

Candidiasis in the city of Nablus: An Epidemiological Study
An-Najah University, Nablus

Number: **Date of collection:**

Sampling site: □ Fingers and nails □ Mouth □ Folds (□ Interdigital □ Inframammary □ Intertrigenous) □ Vagina □ Blood □ Other

Health institution and person in-charge:

Name of Patient: **Age:** **Gender: □ Male □ Female**
Occupation: **Residence:**
Marital Status: **□ Single □ Married**
Women :
Pregnant: **Use of contraceptives: □ Oral □ Artificial**
Post delivery:

Medical History:

Previous (before 1 year): Type: **Duration:**

Present: Disease: **Initiation date:**
Use of Drugs:
Symptoms:
Period of Treatment (duration):

Predisposing Factors:
□Diabetes
□Preceding Surgery
□Iatrogenic Immunosuppresion
□Intravenous Catheters
□Prolonged Administration of Antibiotics
□Cytoreductive Chemotherapy
□Neutropenia
□Hematologic Malignant Diseases
□Burns
□Diaper in Infants
□Tight Clothes
□Frequent Exposure of Hands to Water (fingers and nails(
□Low-Birth Weight Neonates

M.Sc. Project

Tinea pedis in Palestine: An Epidemiological Study in School Children in Nablus City

An-Najah University, Nablus

Questionnaire

Date:…….. Age:……

No.:…… Residence:……..

Name: Occupation:……..

1st. Medical History:

1. **General Medical Condition:**
2. **Previous occurrence:**
3. **Duration of condition:**
4. **Current treatment:**

□Topical □ Systemic

5. **Other skin disorder:**

□Yes □ No

6. **Trauma**
7. **Drug administration**

2nd. Environmental exposure:

□ Athletes □ Non Athletes

- **Type of sport: …….**
- **Use of common facilities such as:**

□ Shoes □ bath □Towels

3rd.Physical examination

- **Socio-economic condition:**

□ Good □ Bad

- **Personal hygiene:**

□ Good □ Bad

- **Family history of *Tinea pedis*……..**
- **No .of family members……**
- **Drying of foot after using water:**

□ Yes □ No

-
- **Location of the lesion:**

□ Hair scalpel □ Skin scraping □ Nail clipping

- **Type of *Tinea pedis:***

Intertriginous: □Scaling □Prurities □Macerated □Mal odor □Erythema
Planter: □Scaling □Hyperkeratosis □Acute exudative eruption
Vesiculo-bullous:

Dorsum:
Association:

- **Dealing with domestic animals……:**
- **Feet infected: □ Right □ Left □ Both**
- **Type of onychomycosis:**

Distal and lateral subungual (DLSO)
Proximal subungual (PSO)
Superficial white (WSO)
Total dystrophic (TDO)
Foot care:

□ Good □ Bad

- **Shoes:**

□ Tight □ good

- **Number of persons sleep at the same room:……..**
- **Associated findings:**

Infection involving hair follicle
Secondary bacterial infection
Persistent hyperpigmentation

Appendix B

Media, Stains and Reagents

Sabouraud Dextrose Agar-SDA; (Koneman, E. W., &Roberts, G. D.1985)

Dextrose	20gm
Peptone	10gm
Distilled water	1000ml
Agar	17gm
Cycloheximide	(0.05%)
Chloramphenicol	(0.005%)

Urease test medium, (Beneke, E. S. &Rogers, A. L. 1980)

Useful for identification of some yeast species especially *Cryptococcus neoformans* which hydrolyses urea and the medium become deep red.

Peptone	1gm
Agar	20gm
NaCl	5gm
KH_2PO_4	2gm
Glucose	5gm
Distilled water	1L

Dissolve by heating, 5ml of phenol- red solution (0.2%in 50% ethanol) was added. Autoclaved at 121ºC for 15 minutes, cooling, and 100ml of urea (20% aqueous solution, sterilized by filtration) was added. Tubes and slants were prepared. A small amount of yeast suspension was streaked out over the surface of media and incubated at 37ºC. Results were read in four days. A deep red color through the medium indicated a positive reaction.

Minimum Essential Media (MEM)

GIBCO
Invitrogen Corporation

Milieu Essential Medium[1] (MEM) (suite)

Réf.	21090	21430	11700	31095	41090
Composants	1X Liquide mg/L	10X Liquide mg/L	Poudre mg/L	1X Liquide mg/L	1X Liquide mg/L
SELS INORGANIQUES :					
$CaCl_2$ (anhyd.)	–	–	200,00	–	–
$CaCl_2 \cdot 2H_2O$	264,00	2640,00	–	264,00	264,00
KCl	400,00	4000,00	400,00	400,00	400,00
$MgSO_4$ (anhyd.)	–	–	97,67	–	–
$MgSO_4 \cdot 7H_2O$	200,00	2000,00	–	200,00	200,00
NaCl	6800,00	68000,00	6800,00	6800,00	6800,00
$NaHCO_3$	2200,00	–	–	2200,00	2200,00
$NaH_2PO_4 \cdot H_2O$*	–	–	140,00	–	–
$NaH_2PO_4 \cdot 2H_2O$	158,00	1580,00	–	158,00	158,00
AUTRES COMPOSANTS :					
D-Glucose	1000,00	10000,00	1000,00	1000,00	1000,00
Rouge de Phénol	10,00	100,00	6,00	10,00	10,00
Succinate de Sodium	–	–	100,00	–	–
Acide Succinique	–	–	75,00	–	–
ACIDES AMINES :					
L-Arginine • HCl	126,00	1260,00	126,00	126,00	126,00
L-Cystine	24,00	240,00	–	24,00	24,00
L-Cystine • 2HCl	–	–	31,00	–	–
L-Glutamine	–	–	–	292,00	–
L-Alanyl-L-Glutamine	–	–	–	–	406,00
L-Histidine HCl • H_2O	42,00	420,00	42,00	42,00	42,00
L-Isoleucine	52,00	520,00	52,00	52,00	52,00
L-Leucine	52,00	520,00	52,00	52,00	52,00
L-Lysine • HCl	73,00	730,00	72,50	73,00	73,00
L-Méthionine	15,00	150,00	15,00	15,00	15,00
L-Phénylalanine	32,00	320,00	32,00	32,00	32,00
L-Thréonine	48,00	480,00	48,00	48,00	48,00
L-Tryptophane	10,00	100,00	10,00	10,00	10,00
L-Tyrosine	36,00	360,00	36,00	36,00	36,00
L-Valine	46,00	460,00	46,00	46,00	46,00
VITAMINES :					
Pantothénate de Calcium D	1,00	10,00	1,00	1,00	1,00
Bitartrate de Choline	–	–	1,80	–	–
Chlorure de Choline	1,00	10,00	–	1,00	1,00
Acide Folique	1,00	10,00	1,00	1,00	1,00
i-Inositol	2,00	20,00	2,00	2,00	2,00
Nicotinamide	1,00	10,00	1,00	1,00	1,00
Pyridoxal HCl	1,00	10,00	1,00	1,00	1,00
Riboflavine	0,10	1,00	0,10	0,10	0,10
Thiamine HCl	1,00	10,00	1,00	1,00	1,00

1. Eagle, H. (1959) Science, 130, 432.

a. La composition originale indique le $NaH_2PO_4 \cdot 2H_2O$.

Appendix C

Antifungal drugs used in the city of Nablus

Drug groups	Common name	Chemical composition	Forms of drug	Manufacturer
1. Antifungal	Agistin	Clotrimazole 1%	Cream-vaginal cream-vaginal tablets -solution .	Agis
	Nestatin	Nystatin	Oral solution-cream-vaginal tablets-oral tablets	Taro
	Kandistan	Nystatin	Oral drops	Beir-zeit
	Candizone	Clotrimazole	Cream	Beir-zeit
	Itranox	Itraconazole	Capsules	Beir-zeit
	Daktazole	Miconazole	Oral gel-vaginal cream-cream	Al-quds
	Daktarine	Miconazole	Oral gel-vaginal cream-cream	Janseen
	Pitrex	Tolnaftate 1%	Cream-solution	Teva
	Bifonazole	Bifonazole	Cream	Teva
	Nesoral	Ketoconazole	Cream-tablets-shampoo	Janseen
	Lamisil	Terbinafine	Cream-spray-tablets	Novartis
	Fungazone	Miconazole	Cream	Dar al-shifa’
	Fungitrin	Miconazole	Cream-oral gel	Beir-zeit
	Sporanox	Itraconazole	Capsules	Janseen
	Mycoten	Miconazole	Cream-vaginal cream	Beit-jala
	Sporofulvin	Grisufulvin	Tablets-syrup	Beit-jala
	Grifolin	Grisufulvin	Tablets	Teva
	Orazole	Ketoconazole	Tablets	Beir-zeit
	Exodril	Naftifine	Cream	Novartis
	Gynofungitrin	Miconazole	Vaginal ovules	Beir-zeit
	Agispore	Bifonazole	Cream-gel-solution-shampoo	Agis

Table 1 b

2.Antifungal with cortisone	Fungicort	Miconazole with hydrocortizone	Cream	Beir-zeit
	Mycocort	Corticosteroid with miconazole nitrate with hydrocortizone	Cream	Al-quds
	Daktacort	Corticosteroid with miconazole nitrate with hydrocortizone	Cream	Janseen

Table 1 c

3.Antiantifungal with antibacterial with cortisone	Decomb	Nystatin-neomycin (aminoglycides)-gramicidin(cyclic antibiotic) with triamcinolone (hydrocortisone)	Cream	Beir-zeit
	Kenacomb	Nystatin-neomycin (aminoglycides)-gramicidin(cyclic antibiotic) with triamcinolone (hydrocortisone	Cream-ointment	Squib
	Dermacombin	Nystatin-neomycin (aminoglycides)-gramicidin(cyclic antibiotic) with triamcinolone (hydrocortisone	Cream-ointment	Taro
	Polycutan	Neomycin with clotrimazole with dexamethasone	Cream	Agis
	Medihist	Dexamethazone acetate with clotrimazole with neomycin	Cream	Beir-zeit
	Triderm	Gentamycin with betamethazone dipropionate with clotrimazole	Cream	Schering plough
	Tevacutan	Neomycin with clotrimazole with dexamethasone	Cream	Teva
	Multiderm	Steroid with diflucortolone with chlorquinaldol	Cream	Agis

Table 1.d

4.Antifungal with fungistatic	**Phytoderm compositom**	**Tolonftate 1% with salicylic acid with zinc oxide**	**Cream**	**Teva**

Table 1.e

5. .Fungistatic with cortisone	**Diprosalic**	**Salicylic acid with steroid with betamethasone with dipropionate**	**Ointment - lotion**	**Schering plough**

Table 1.f

6. Fungistatic	**Fungimon**	**Undicylinic acid with zinc undicylinate with aluminium chlohydrate**	**Powder**	**Trima**
	Selsun	**Selinium sulfide 2.5% (detergent of dandruff and for treatment of (*Tinea versicolor*)**	**Shampoo**	**Janseen**
	Sebocel	**Selinium sulfide 2.5% (detergent of dandruff and for treatment of *Tinea versicolor*)**	**Shampoo**	**Taro**
	Whitefield's	**Salicylic acid with benzoic acid**	**Ointment**	**Gama**
	Whitefield's	**Salicylic acid with benzoic acid**	**Emulsion**	**Sammon**

Appendix D

Measured optical density values used in evaluating % of cell mortality for three different plant extracts at 5 different concentrations, using the equation:

% cell mortality= Mean of optical density of blanc- Mean of optical density of plant / Mean of optical density of blanc X 100%.

BLANC	5000	500	50	5	0,5
2,125	89,72	84,66	22,66	29,81	0
2,028	89,27	77,28	20,09	30	0
2,125	92,44	91,49	19,77	29,04	9,86
2,11	92,71	90,76	19,77	32,62	12,71
2,095	93,12	92,76	7,64	27,87	0
2,081	91,22	92,98	9,86	26,33	0
2,095	90,9	76,38	0	20,67	8,77
2,125	90,95	93,57	22,94	25,88	7,64
2,426	92,85	89,04	13,61	9,86	0,00
2,368	92,62	78,46	16,38	11,35	0,00
2,25	92,85	91,26	20,99	5,83	0
2,293	93,8	89,59	23,98	20,4	0
2,368	91,04	88,5	23,21	3,12	0
2,396	92,08	90,72	28,28	5,83	0
2,293	93,43	65,11	23,98	1,62	0
2,23	92,17	87,05	16,38	0	0
2,21	75,96	25,50	0,00	0,00	6,92
0,13	75,96	33,57	0,00	0,00	0,00
	74,95	49,17	2,11	0	0
	75,68	37,43	0	0	3,93
	74,58	43,3	0	0	1,54
	75,41	40,55	0	0	0
	75,68	19,81	0	0	0
	75,87	16,97	0	0	0
	81,83	16,05	0	0	2,14
	78,8	15,5	0	0	3,90
	79,63	40,18	0	0	
	79,54	45,59	0	0	
	79,26	27,33	0	0	
	78,25	33,02	0	0	
	77,15	0	0	0	
	74,49	6,69	0	0	
	71,28	70,17	0	0	
	70,17	68,63	0	6,92	
	64,95	69,05	6,85	2,99	
	69,91	69,4	2,22	2,64	
	75,55	66,06	2,39	0,76	
	72,13	71,28	0,34	0,68	
	65,21	68,37	2,9	2,47	
	66,15	61,7	1,28	2,73	
	60,94	67,6	0	0	
	73,24	70	0	0	
	71,11	67,52	0	5,47	
	71,7	69,4	0	1,88	
	57,35	62,3	4,44	2,39	
	63,24	69,74	0,68	3,41	
	61,96	72,13	3,41	1,62	
	60,76	64,87	2,19	0,76	
	78,75	60,80	6,63	6,56	
	10,80	26,28	9,26	10,37	

Diagrammatic representation of combined symptoms among suspected cases of school children

Reported cases on use of both contraceptives and drugs among women suffering from vaginal discharge

Table 2.2 Selected Plants used for Anticandidal Susceptibility Testing

Species / Family / Voucher No.	Common Name	Arabic name	Parts used	Popular usage	Ref. For folk popular uses
1. *Allium sativum* L. (Liliaceae)TA1	Garlic	الثوم	[6] LF	Skin and circulatory system (heart and blood vesseles) antihelmintics	4, 13
2. *Calycotome villosa* (Poir) (Papilionaceae) TA2	Thomy broom	قنديل	[2] AP		
3. *Campanula rapanculuis* L. (Campanulaceae) TA3	Bell flower	ورد الجرس	[2] AP		
4. *Coridothymus capitatus* Reichb (Labiatae) TA4	Thyme	زعيمة	[2] AP	Anti-inflammatory and antimicrobial activity for eye infection, headache, inflammation disphoretic, stomachache, carminative, whoopin cough, antihelmintic, antispasmodic, emmengonue and vermifiuge abdominal pain, heart disorders, dropsy, paralysis, blindness, respiratory.	22,4, 14, 6
5. *Gagea cloranth* (Bieb). Schult. Fill (Liliaceae) TA5	Gagea	زعيتمان			
6. *Inula viscosa* L. Ait (Compositae) TA6	Inula	عرق طيون	[2] AP	Treatment of diabetes, antihelmentic, expectorant, diuretic, for lung and bronchial disorders, anti-inflammatory, reconstiotuent, Hemmorrhoids, eye infections, muscle spasms, general tonic, local paralysis, mucus in the respiratory tract, rheumatism, toothache, skin diseases.	4, 22, 8, 17, 27, 25, 1, 9, 13, 18, 6, 20
7. *Juglans regia* L. (Juglandaceae) TA7	Walnut	جوز	[6] LF, [3] FR	Treat aczema, nervous problems, as food, for syphilis, antihelmentic, astringent, stomatheache, nerve tonic, treats scrofula, riclcetts, gastro-enteritis, vermifuge, as a hypoglycemic agent, antidote poison, tonic, dental hygiene, depurative, galactofuge, rubefacient, antisorophulons, antiseptic, skin diseases, antiparasitics, repellents.	4,8,9,12,17 8,25, 29, 6, 13
8.*Lawsonia inermis* L. (Lythraceae) TA8	Henna	حناء	[6] LF	Enlargment of liver and spleen, incalculosis, injaundice, inleprosy, skin disease, burns colds, anti-inflammatory activity, cytotoxic activity, hair and scalp problems, treat hair dandruff and split ends,remedy for split nails, for birthcontrol, fever, local anaesthetic, mouth ulcers, antifungal, used in dermatology in leprosy and skin disorders, anti-piuretic, analgesic.	3,12,17,13, 21, 2
9. *Parieteria diffusa* (Mert & kock) (Urticaceae) TA9		عشبة الدم	[2] AP	To stop bleeding from fresh skin wounds, antitrussive, hemorrohid lentitive, resolvent for skin sillammation Vulnerary, diuretic and departive, vermifuge, sedetive incases of intestinal colic.	5,11, 22

Species / Family	Common English name	Arabic name	Parts used	Popular uses	Ref. For popular uses
10. *Petrosilinium sativum* Moffm (Umbiliferae) TA10	Parsely	بقدونس	[4] WP	Gastronomic use, digestive, hypotensive, urination, intermittence and prostate Cancer. Renal lethiasis, Carminative diuretic, emmanogogue.	4,7, 6
11. *Pistacia lentiscus* L. (Anacardaceae) TA11	Mastic lentisk	سريس	[6] LF	For stomach pains, analysis, perspective covering for wounds, skin infections, cardiac stimulation, anti-inffammatory, migrane, seedative in gastralgia facilitate child birth, for frver breeth freshner, treat chest pain, expectorant, hair care for diarrhea in chlidren, could be masticated to sweeten breath, diueretic, swelling for gastrointestinal disorders, aid to minstruation, astringent, kideny stores, muscle paralysis, sore throat tract, Eczema.	12, 9, 4, 22, 27, 24, 19, 15,
12. *Anagalis arvensis* L. (Primulaceae) TA12	Red pimpernes	عين الجمل			
13. *Anthemis tinctoria* L. (compositae) tunctoria TA13	Yellow cammomile	بابونج أصفر	[1] FL, [6] LF, [5] RT		
14. Rosmarinus officinalis L. (Labiatae) TA14	Rosemary	حصلبان	[2] AP	For common cold, and purgative, dieuretic and cough, antiseptic for the circumcision wound, relaxation, gastronomic, antispasmodic and spice, urinary system.	19, 7, 6, 4
15. *Salvia dominica* L. (Salvia doraceae) TA15	Sage	خواخة	[1] FL		
16. *Sonchus oleraceus* L. (Solanaceae) TA16	Mustard	خردل	[4] WP		
17. *Styrax officinalis* L. (Styracaceae) TA17	Snow bell	عبهر	[6] YB		
18. *Ziziphus spina-christi* L. (Rhamnaceae) TA18	Syrian Christ thorn	دوم السدر	[6] LF	Treat blisters, bruises, chest pains, dandruff, fractures, headache, mouth and gum problems, laxative, pectoral, nutritive to cure toothache, astringent, anti-diarrheutic, fermifuges, anti-inflammatory (eye wash), analgesic, anti-rheumatic, purgative, stomach pain antihelmentic, backache, arthritis, gums, joints, skin disorders, abdominal pains, constipation, intestinal parasites, rhrumation, open wounds, boldness.	

[1] FL, flowers; [2] AP, aerial parts ; [3] FR; fruit ; [4] WP, whole plants; [5] RT, roots; [6] LF, leaves; [7] YB, young branches.

1Abu Zarga et al., 1998; 2.Ali –Shtayeh et al., 2000; 3 Ali-Shtayeh et al., 1997 ; 4Ali-Shtayeh et al., 1998 ; 5Al-Said et al., 1988 ; 6 Amico & Sorce, 1997 ; 7 Barel & Yashphe, 1991 ; 8 Benayache et al., 1991 ; 9Caceres et al., 1990 ; 10 Dafni & Yanive, 1994 ; 11 Dutta & Nath, 1998 ; 12 El-Kamali & Khalid, 1998 ; 13Gribanovski sassu et al., 1969 ; 14 Guarrera, 1999; 15 Haykel & Omar, 1988 ; 16 Karim & qurasan, 1986; 17 Karting et al., 1991; 18 Kinghorn & Balandrin, 1993; 19 Merzouki & Ed-Derfoufi, 1997; 20 Mostaqul Huq et al., 1999; 21 Ong & Norzalina, 1999; 22 Palevitch et al., 1984; 23 Palevitch & Yaniv, 1991; 24Sanez et al., 1997; 25 Shah et al., 1991; 26 Sulieman et al., 1988; 27 Yaniv et al., 1987; 28 Yesilada et al., 1993; 29Yoshida et al., 1995.

جامعة النجاح الوطنية
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قدمت هذه الأطروحة استكمالا لمتطلبات درجة الماجستير في العلوم الحياتية بكلية الدراسات العليا في جامعة النجاح الوطنية في نابلس، فلسطين

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الملخص

هدفت الدراسة الحالية لتقييم مدى وبائية الخمائر الفطرية في مجموعتين شاملتين ضمت النساء اللاتي يعانين من إفرازات مهبلية والتي قد تكون دليلا على وجود المرض، وكذلك وجود هذه الخمائر في أقدام أطفال المدارس من الفئة العمرية 15 ـ 18 سنة والذين يعانون من ظهور أعراض كالتهيج الجلدي. كذلك هدفت الدراسة إلى تقييم تأثيرات بعض النباتات التي تستخدم في الطب الشعبي على نمو هذه الخمائر. كما هدفت أيضا إلى دراسة تأثير ثلاث نباتات لمعرفة مدى سميتها على خلايا الإنسان لتقدير التراكيز الممكن استخدامها للقضاء على الخمائر و الحول دون تسمم خلايا الإنسان.

تم جمع عينات الدراسة الطبية في الفترة مابين كانون الاول ـ أيار عام 2002م، حيث شملت 119 عينة جمعت من نساء يرتدن عيادة طبية خاصة في مدينة نابلس، بالإضافة إلى 463 عينة جمعت من مدارس ثانوية مختارة (3 مدارس بنين و 3 مدارس بنات). أما النباتات المستخدمة في الطب الشعبي كان عددها 18 نبات بالإضافة لدراسة تأثير سمية 3 منها على أربعة أنواع من خلايا الإنسان.

بينت النتائج ان 9.52% من النساء يعانين من الاصابة الفطرية كما لوحظ وجود اعراض عدة تتطابق مع وجود هذه الخمائر وكان أكثرها الشعور بالتحسس و الحكة (5.22 %)، متبوعة بأعراض أخرى كالألم أثناء الجماع (9.8%)، الحرقة والألم أثناء التبول(8,9%)، التهابات في الجهاز البولي (3.6%)، الرائحة السيئة للإفرازات (9.7%) وأعراض أخرى (5.17%). ظهر المرض بأعلى نسبة في الفئة العمرية 21 ـ 30 سنة، حيث كانت نسبة الاصابة لدى هذه الفئة (2.69%) ولوحظ كذلك ان فئة النساء الحوامل كانت الأكثر عرضة للاصابة بالمرض حيث كانت نسبة الإصابة لدى هذه الفئة (1.58%).

أما بالنسبة للإصابه بالخمائر الفطرية في الأقدام عند طلبة المدارس فكانت (36.3%) عند الذكور و (19.8%) لدى الإناث. وكان وجود التشققات واحمرار في الجلد بين الأصابع من اكثر الاعراض انتشارا حيث لوحظ لدى (92.3%) من الذكور و (57.1%) من الإناث. سجلت أعلى نسبه إصابة فطرية لدى الطلاب الذكور والذين يعيشون في المخيمات حيث وصلت إلى (85%) في حين كانت اعلى الاصابات عند الإناث سكان القرى حيث وصلت إلى (100%). فيما يتعلق بالأمراض الجلدية والمصاحبة للخمائر تبين ظهور علاقة واضحة حيث كانت الفطريات الاخرى هي الأكثر انتشارا (19.8%) لدى الذكور بينما سجلت الاكزيما أعلى نسبه مصاحبه للخمائر لدى الإناث (9.5%) . لم يلاحظ اي علاقة واضحة ما بين استخدام المناشف المشترك والإصابة بالخمائر أو انتشارها فيما اظهر التعامل مع الحيوانات الأليفه علاقة واضحة مع الاصابة الفطرية وظهرت بنسبة (58.2%) عند الذكور وبنسبة (80.9%) عند الإناث. أما تجفيف الأقدام فلم يوضح علاقة منطقية وبالتحديد لدى الذكور حيث ادعى (57.1%) منهم عادة تجفيف الأقدام في حين كانوا مصابين بالخمائر الفطرية. أما العلاقة فكانت واضحة ومنطقية لدى الإناث حيث سجلت نسبه إصابة اكبر عند الإناث اللواتي سجلن عدم تجفيف الأقدام.

أما بالنسبة لتأثير النباتات الطبية على هذه الخمائر فقد لوحظ ان كافة المستخلصات المستخدمة اظهرت تأثيرات وبنسب متفاوتة كان أعلاها لنبات الثوم حيث وصلت فعاليته إلى (100%) مقارنه مع العقارات المستخدمة لهذا الغرض ، في حين أظهر كل من نبات السريس (3.8%)، المريمية (2.25%) والبقدونس (2.25%) فعالية عالية. أما أقل النباتات فاعلية فكانت عين الجمل، زعيتمان، خردل والزعيمة حيث سجلت جميعها فعالية بنسبه (0.35%) فقط.

فيما يتعلق بتقييم سميه بعض هذه النباتات (عين الجمل، بابونج اصفر وعشبه الدم) على خلايا الإنسان (خلايا اب السن ، خلايا رئوية ، خلايا الكبد و خلايا سرطانية) فاظهرت النتيجة أن المستخلصات الثلاثة كان لها تأثيرا سميا واضحا عند تركيز ≤ 500 ميكروغرام ضد الخلايا السرطانية بينما تأثيرا اكبر كان ملاحظا لكل من عشبه الدم وعين الجمل. واظهر المستخلص النباتي للبابونج الاصفر أعلى نسبة للسمية على الخلايا الرئوية بينما اظهر النباتين عشبة الدم وعين الجمل تأثيرا واضحا عند التركيز ≤ 500 ميكروغرام فقط. المستخلصات الثلاثة أظهرت سميتها على خلايا الكبد على تركيز ≤ 5000 ميكروغرام مع ملاحظة أن نبات عين الجمل كان له نفس التأثير تقريبا على تركيز ≤ 500 ميكروغرام. اظهر نبات البابونج الاصفر أعلى نسبة سمية عند تركيز ≤ 5000 ميكروغرام على خلايا اب السن، بينما أظهر كل من نبات عين الجمل وعشبة الدم تأثيرا سميا مخفضا عند هذا التركيز مقارنة مع تركيز ≤ 500 ميكروغرام.