transmit the infection (Moldwin *et al*, 1992). Also, no culture systems are widely available to clinicians.

To improve the acceptability of diagnosis by culture, a plastic envelope method was devised, which permits both immediate examination and culture in one self-contained system (Beal *et al,* 1992).

Culture have Disadvantages of culture method include testing time and availability , because Swab is put in broth and incubated an aerobically at 37°C. Growth is usually detected within 48 hours, and samples without growth after 7 days are considered negative for trichomoniasis (Patel *et al*, 2000).

Culture is especially important for diagnosing trichomoniasis in men since the wet preparation findings are usually negative. Urethral swab, urine, and semen cultures are used to maximize sensitivity (Workowski *et al*, 2006).

1.2.8.5 Papanicolaou (Pap) smears:

Trichomonads may be viewed on Papanicolaou (Pap) smear, but this test yields low sensitivity and should not be relied on for diagnosis (50%). False-positive results are also common with this technique.

Because cultivation methods are relatively slow and wet-mount preparation lacks sensitivity, the staining of parasites in fixed and unfixed smears was introduced. Staining techniques such as acridine orange (Fripp *et al*, 1975), Leishman (Levett *et al,* 1980), periodic acid-Schiff (Rodriguez *et al,* 1973), and Fontana (Nagesha, *et al*, 1970) have been introduced to improve the

sensitivity of direct microscopy.

Papanicolaou (Pap) staining holds considerable appeal in the diagnosis of trichomoniasis because it is routinely used in gynecologic screening for cytologic abnormalities, particularly in populations with a high prevalence of STD (Berggren *et al,* 1969; Levett *et al*, 1980; Spence *et al,* 1980; Perl *et al*, 1972), however, (Perl *et al*, 1972) reported a 48.4% error in diagnosis due to false-positive and false-negative findings when Pap smears were used as the sole criterion for diagnosis and treatment of T. vaginalis infection.

Staining techniques have their limitations since T. vaginalis does not always appear in its typical pear-shaped form with flagella. It often appears as rounded forms resembling polymorphonuclear leukocytes, and occasionally the typical morphologic characteristics are lost during fixation and staining, making the etiologic identification difficult (Perl, 1972).

1.2.8.6 Antibody-Based Techniques:

There are an estimated eight serotypes observed in *T. vaginalis* (Ackers *et al*, 1990). However, by immunoblot analysis, a wide variety of antigenic markers are seen (Garber *et al*, 1986).

Various techniques including agglutination, complement fixation, indirect hemagglutination, gel diffusion, fluorescent antibody, and enzyme-linked immunosorbent assay have been used to demonstrate the presence of antitrichomonal antibodies (Jaakmees *et al,* 1966; Mason *et al,* 1979; Mathews *et al*, 1983; Sibau *et al,* 1987; Teras *et al*, 1966). However, the

serum or local antibody response to a pathogen depends on several factors, such as the nature of the antigen or pathogen, its live or inactivated form, its local concentration, and the frequency and length of immune system stimulation. It has several inherent disadvantages.

In some instances, an antibody response is not observed either because the system is too insensitive to detect low levels of specific antibodies or because the serum humoral response has not yet been elicited. Since trichomonal antibodies may persist for a long time after treatment, current and past infections cannot be distinguished.

Direct detection of *T. vaginalis* antigens in clinical specimens by using monoclonal antibodies holds promise as a rapid method in the diagnosis of trichomoniasis. (Krieger *et al*, 1985) obtained two broadly reactive monoclonal antibodies which identified all 88 strains of *T. vaginalis* obtained from diverse geographic areas. Monoclonal antibodies to immunogens such as the 62- and 65-kDa proteins for the detection of T. vaginalis from clinical specimens gave similar results to those of wet-mount preparations (Lisi *et al,* 1988). Furthermore, the use of monoclonal antibodies to proteins such as cell detaching factor (CDF; 200 kDa) and cysteine protease (60 kDa), which immunogens are observed in all the isolates of *T. vaginalis*, could provide an alternative method for the detection of trichomoniasis.

Trichomonas Direct Enzyme Immunoassay and Fluorescent Direct Immunoassay, which use peroxidase and fluorochrome-labeled cocktails of monoclonal antibodies to various *T. vaginalis* structures, were as sensitive and specific as culture techniques (Thomason *et al*, 1989). Also, the results can be obtained in one hour, allowing both diagnosis and administration of treatment in a single patient visit.

1.2.8.7 DNA Techniques:

Recombinant DNA techniques have been increasingly used in clinical laboratories to improve the specificity and sensitivity of T. vaginalis diagnosis.

The use of PCR methods helps detect nonviable organisms and also has the ability to detect cells and target sequences in clinical samples that have undergone fixation or partial degradation (Underhill *et al*, 1974). The commercially available Affirm VP system (MicroProbe Corp, Bothwell, Wash.) uses synthetic oligonucleotide probes for the detection of both Gardnerella vaginalis and T. vaginalis from a single vaginal swab (Briselden *et al*, 1994).

The technique was found to be superior to wet-mount preparation. However, false-negative results were encountered when this technique was compared with culture technique (80% sensitive compared with culture-positive samples) (Briselden *et al*, 1994).

The dot-blot hybridization technique, which uses a 2.3-kb *T. vaginalis* DNA fragment as a probe, can detect *T. vaginalis* DNA from vaginal exudate. However, because of the instability of the probe and the special care needed in the handling and disposal of the radioactive material, the technique has significant drawbacks (Rubino *et al*, 1991). These limitations could be overcome by using the 2.3-kb fluorescence-labeled DNA probe for identification of *T. vaginalis* by the DNA in situ hybridization technique. The usefulness of these techniques for identifying asymptomatic carriers needs to be evaluated.

Polymerase chain reaction (PCR) methods yield a high sensitivity (84%) and specificity (94%). Although not yet widely available, PCR has great diagnostic potential (Sobel, 2005).

1.2.9.1 Trichomoniasis therapy:

Until 1959, topical vaginal preparations available against trichomoniasis provided some symptomatic relief but were ineffective as cures (Lossick, *et al*, 1991). These local treatments did not penetrate the vaginal epithelium, urethra, Skene's glands, and Bartholin's glands, which harbor the organism (Lossick *et al*, 1991).

1.2.9.2.1 Metronidazole:

Today, the standard treatment for trichomoniasis is 500 or 250 mg metronidazole (Flagyl given orally, three times a day for 7 days, or in a single 2-g dose. Both the infected patient and sexual partner, whether

symptomatic or asymptomatic, should be treated to prevent reinfection. Both regimens are equally effective (Workowski *et al*, 2006; Hager, 1980; Underhill *et al*, 1974).

The success rate ranges from 82 to 88% and is almost 95% when the sexual partner is treated simultaneously (Heine *et al*, 1993). The single 2-g dose is preferred, since less total drug is required, patient compliance is better, and there are fewer side effects (Hager *et al*, 1980; Lossick, 1982).

1.2.10.1 Deterrence and Prevention:

All sexually transmitted diseases can be prevented by using adequate protection during sexual intercourse. Effective forms of protection include male and female condoms.

Other preventive measures are similar to those for other forms of vaginitis, including wearing loose cotton clothing and not using douches, vaginal deodorants, or sprays (Schwebke and Hook, 2003).

Also Abstinence from sexual intercourse prevents trichomoniasis, limiting the number of sexual partners decreases the risk of trichomoniasis and Spermicides that contain nonoxynol-9 are not recommended for the prevention of sexually transmitted diseases.

Frequent use is associated with disruption of the genital epithelium, which may be associated with an increased risk of HIV infection and other sexually transmissible agents. (Workowski and Berman, 2006)

The government of the medical and social aspects of sexually transmitted disease is none the responsibility of the dendrologist alone. Close collaboration between the public health administrators at government and local level is vital importance. Their responsibility may be as follows:

1.2.10.2 Governmental responsibility:-

According to (Omer, 1987):

1- Financial: the government had to provide adequate services for all people.

2- Legislative: the government need to make available laws to authorize an official service which may include: the certification of specialists and establishment of the others low that combat prostitution.

2- International co-operation: the government has the responsibility for making and signing ratifying international agreements of the availability of treatment for foreigners.

4- Health education: the government should not inform the public effectively and all education materials must be presented in an understandable and acceptable manner.

5- Epidemiological: the government needs determine the incidence and tends of trichomniasis in each area.

6-: Measures should be taken to abolish promiscuity and improve the society morals.

Clinician s Responsibilities:-

The venerolgist got a role to play in control of trichomoniasis. His major duties may be as follows:

1- Administration of sexual transmitted disease (S.T.D) clinics to deliver a good service.

2- Collection of reliable statistics of his work.

3- To take a part in training of junior staff.

4- Participation in health education program by continuous advice and suggestion to those who are preparing material for the purpose.

5- To provide good diagnosis, treatment and follow-up tricomoniasis.

6-Medical education is one of the responsibilities of the medical people.

1.2.11 Plant understudy:

1.2.11.1 Identification:

Genus **lawsonia** species **inermis** *varity cultivar common* **name henna mignonette tree egyption privt** family **lythraceae** specimen number **s10343** data source **color nat p17;Dyepl p55;medpl p225;Frgnt p211**

1.2.11.2 PALANT DESCRIPTION:

Growing Henna somewhere in your garden will give you ample conversation material for garden club tours and the like. It will also fill the air with the delicious fragrance of its blooms. Mentioned in the Song of Soloman (as Camphire), Henna has been used in perfumes since around 1500 BC and is currently grown commercially in several African Countries, India, Pakistan and Iran. Henna is also well-known for the dye its leaves produce, used for coloring hair, darkening fingernails and temporary body art which can stay

in the skin for a month or more. Please note that Henna dye is not USDA approved for direct skin contact and such practices are actually illegal. It is approved for use as a hair dye. This desert oasis plant

1.2.11.3 Chemical Constituents and Components:

Main chemical components are lawsone, esculetin, fraxetin, isoplumbagin, scopoletin, betulin, betulinic acid, hennadiol, lupeol, lacoumarin, quinone and napthaquinone.

1.2.11.4Action:

Tannins:

1. It inhibits the growth of gram-positive and gram-negative bacteria.
2. It exhibits significant healing response against excision and incision wounds.
3. It is absorbed into the stratum corneum (skin) immediately and protects skin from ultraviolet radiations.
4. It is a weak direct acting haemolytic agent

1.2.11.5 Curing Diseases:

1. The flowers are intellect promoting and useful in cephalagia, burning sensation, insomnia and fever.
2. Henna has active elements that help protect against many surface fungi and bacteria and has been used for its cooling and astringent properties.
3. In hot climates, henna is used as protection against sun, to soothe sunburn and to reduce body temperature.

1.2.11.6Application:

We at Apex International are one of the prominent cultivators & exporters of Herbs and Oils from India.

CHAPTER TWO

MATERIAL

&

METHODS

2.1. MATERIALS AND METHODS

2.1.1 Materials:

2.1.1.1 Chemicals and Reagents:

Analytical grades of the following chemicals were used

Amyl Alcohol	the British Drug House, Engl
Chloroform	AR SD Fine- Chem. Ltd
Ethanol	Avondale Laboratories, Ltd
Methanol	Loba Chemie PVT Ltd
Sodium chloride	British Drug House, England.
	Gibco, Grand island, N.Y., USA Trepan blue
Dimethyl solphoxedase DMSO	Merck Chemicals, Darmstadt, Germany

2.1.2 Chemotherapeutic agents:

In all experimental we used metronidazole (flagyl®) powder. Patch No. 070501. Manufactured in May, 2007. Expired in May, 20011. Company; Wujiang Li town pharmaceutical.

2.2.2. Plant materials:

The selected plant species collected between January 2008 and April 2008. The plants material used for study were barks and leaf of authenticated by henna mignonette tree egyption privt the taxonomists of Medicinal and Aromatic plants Research Institute (MAPRI).All plant parts will air -dried, coarsely powdered mechanically and then used for extracts preparation.

2.2.2.1-prepration of Crude extracts:

Extraction was carried out according to the method described in (Harbone, 1984). To prepare extracts for screening of antitrichomonal activity, briefly ; parks and fruits were separated from the other parts of the plant, the parks and fruits were then dried under the shadow with good ventilation and then ground finely in a mill, (50 g) were macerated in 250 ml of Chloroform for 3 h at room temperature with shaking and for 24 h at room temperature, the supernatant was decanted and clarity field by filtration through a filter paper (0.45μ), after filtration, the solvent was then removed by rotary evaporator at 55 ° C. Each residue was weighed and the yield percentage was determined (% of dry weigh) and stored at-20 ° C for further analysis in tightly sealed glass vial.

The residue of plant was macerated in 250 ml of methanol for 3 h at room temperature with shaking and for 24 h at room temperature, the supernatant was decanted and clarity field by filtration through a filter paper (0.45μ), and evaporated under reduced pressure. Extract was re-dissolved or suspended in methanol and the final volume was adjusted to give the appropriate concentration and will be kept in refrigerator until the time of their use.

2.2. 3 Parasite used:

Trichomonas vaginalis was used in all experiments and these parasites take from patient with positive trichomoniasis.

2.2.4 Culture medium:

To transport the sample from hospital to institute used nutrient broth medium as transport media. Trophozoites of Trichomonas vaginalis maintained in SPLM medium. Sub culturing of the parasite was performed at 37 ± 1°C in RPMI medium containing 5% bovine serum. The trophozoites were maintained for the assays and were employed in the log phase of growth.

2.2.5 Patients and diagnostic methods

In our study *Trichomonas vaginalis* was searched in women who are applied to Umbada and Ibrahim Malik Hospitals with PID or vaginal discharge complaints. Urine sample were obtained in sterile container. All positive samples trichomoniasis were examined by two methods; wet mount preparations and CPLM cultivation methods.

2.2.5.1 Wet mount preparations

A vaginal discharge sample was taken for *Trichomonas vaginalis* diagnosis using wet smear as fallows briefly, each subject was given a wide mouth, leak-proof universal specimen container and was instructed on how to collect her own urine sample. About 20mls of urine sample was obtained from each participant. For the purpose of the research, no personal identifies (names, ID number, address, etc) were used on the urine sample of the participant. Instead bar-coded numbers were used to ensure obscurity of subjects, to facilitate laboratory procedures and minimize the chances of errors during

the handling of the urine specimens. The samples were placed into a tube and centrifuge deposit of urine (Ackers et al, 1978). A drop was put on a slide and cover slip applied and the deposit examined under high power field 40X of light microscope for motile flagellates.

2.2.5.2 CPLM cultivation method

The culture technique previously described (Oyerinde, 1999) was followed but with horse serum substituted with bovine serum. Mid stream urine samples were collected from indoor hospital patients, the urine samples were centrifuged and take the deposit and were placed in CPLM medium. The cultivated materials were incubated at 36 °C in anaerobic condition and their microscopic examination was made after 24 and 48 hours. This was done by taking a drop from the bottom of the culture using sterile Pasteur pipette, transferring to a slide and examined under the high power objective .

2.2.5.3 Inoculums:

Trichomonas vaginalis was inoculated in the RPMI 1640 medium and incubated at 37 °C for 48 hours. Parasites were counted under the microscope in haemocytometer.

2.2.6 In vitro susceptibility assays:

In vitro susceptibility assays used the sub- culture method of Cedilla Rivera et al 2002. This is highly stringent and sensitive method for assessing the anti-protozoal effects (gold standard) particularly in *Entamoba histolytica*,

Gairdia intestinalis and *Trichomonas vaginalis* (Gillin and Diamond, 1981; Arguello-Garcia etal., 2004).

The extracts were dissolved in 0.005 µl of dimethyl sulfoxzide (DMSO) and were added to abondorve tube containing 0.950 µl D.W in order to reach concentration of 5 mg/ml. The concentrates were stored at -20 °C for further analysis.

The 96-well microtiter plates was divided to 12 part to take different each plant extract, positive control and negative control.

Twenty microliters of CRPMI medium were placed in the wells-except the first three of wellsC-1- (40 µl of an extract solution of 5 mg/ml were added in the first three wells which the final concentrations were 100%) and 20 µl in the following (C-2 were 2250 µg/ml and C-3 were 1250µg/ml) to reach concentration 50% and 25% obviously; 80 µl of culture medium was complemented with parasite. The final volume in the wells was 100 µl.

Each test included metronidazole pure compound [(1-(2-hydroxyethl)-2-methyl-5nitroimidazole], a trichomonocide, was used as positive control in concentration 1250µg /ml, whereas untreated cells used as a negative controls (culture medium plus trophozoites). Samples were take for counting for 0, 24, 48, 72, 96, 120, 144, 168, 192, 219 and 240 hours. Samples were take and mixed with trepan blue in equal volume.

The final number of parasites was determined with haemocytometer and Microsoft excel program was used for the calculation of IC50 from linear regression equations. Three series of determinations were run for each plant extract.

The mortality of parasite % for each extracts activity was carried out according to the following formula:

$$\text{Mortality of parasite \%} = \frac{(\text{Control negative} - \text{tested sample with extract})}{\text{Control negative}} \times 100\%$$

Only 100% inhibition of the parasite (namely, when there no motile parasites observed) was taken as a positive (+) result.

2.2.7 Cell counting:

Cell counts were done using the improved Neubauer chamber. The cover slip and chamber were cleaned with detergent, cleaned carefully with distilled water and swapped with 70% ethanol, then dried. The liquid of cell suspension was mixed with equal volume of 0.4% trypan blue in a small tube. The chamber was completed with cell suspension. After cells had settled, the chamber was placed under light microscope. Using 40 X objective, cells in the 4 large corner squares (each containing 16 small squares) were counted.

2.2.8 Statistical analysis:

All data are presented as means. Statistical analysis for all the assays results were done using ANOVA test. Significance was attributed to probability values $P \leq 0.05$ or $P \geq 0.005$ in some cases.