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**In Vitro Antimicrobial Activities of Flaxseed Extract Against
Gastrointestinal Tract Infection**

**A dissertation submitted for partial fulfillment requirement of
MSc degree in Medical Laboratory Sciences
(Microbiology)**

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

الآية



قال تعالى:

بسم الله الرحمن الرحيم

﴿ وَاللَّهُ أَنْبَتَكُمْ مِنَ الْأَرْضِ بِأَنَّا ﴾

صدق الله العظيم

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الاهداء

إلى معلم البشرية ومنبع العلم نبينا محمد (صلي الله عليه وسلم)
إلى روح والدي الهادي عبد المنان اللهم ارحم ابي واذقه حلاوه الجنه وطيب مسكنه ولا تحرمه من
نعيمها واجعل لقائنا به في الجنه يارب العالمين.

الي روح والدتي سعيدة بشير
منذ أن رحلتي والبيت مظلم لم تنطفا به المصابيح بل انطفأت به القلوب.
إلى أزهار النرجس التي تفيض حباً وطفولة ونقاء وعتراً. الغاليات اللاتي مازلت يجبن على
إدراج العمر الاولي أخواتي عسجد والاء.
إلى من أخذ بيدي ورسم الأمل كل خطوه مشيتها أخواني عاصم وعزت.
أصدقائي الذين تسكن صورهم وأصواتهم أجمل اللحظات والأيام التي عشتها. هبه، ناهد ، زبيدة ،
خالدة.

إلى كل من ساعدني في انجاز هذا البحث شكري الجزيل وأمتناني إلى كافة الأهل والأصدقاء.
يامن رزقني الله بك يا أجمل واروع نعمه اعطاها الله لي يابسة رسمت على شفاهي يا نور عيني
ومصباح طريقي يا من شمعة اضاءت مصباح حياتي يا من يملأ قلبي بحبك وتملاً حياتي بفرحي
معك لن اجد أي كلمة أعبر بها عن عشقي لك أنت من ملكت عمري وحياتي وجعلتني أشعر بأني
ملكة وسط كل النساء يا من إذا طلبت منه النجمه من السماء أتى بها وقدمها لي وساده من الحرير
يا رمز نادر ايجادة يا كل شئ جميل بحياتي ادعو ربي ان يريح قلبك ويفسح صدرك ويزررك بخير
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Abstract

Background : Gastrointestinal tract Infections with a variety of infectious agents can occur in any part of the tract. Infections can range in severity from self Limited to life threatening.

Objectives: This study was aim to evaluate the antibacterial effect of extract of flax seed in bacterial gastrointestinal infections such as *E.coli*, *Salmonella typhi*, *B.cerus*, *Yersinia enterocolitica*.

Methods : This was descriptive study, carried out in medical and aromatic plant center institute, national center for research in Khartoum, from Feb to Aug 2018. The crude aqueous methanolic extract of flax seed was studied using the in vitro antimicrobial effect against gastrointestinal bacterial infection mechanistic basis was further elucidated by testing the inhibitory effect.

Result : the study showed *E. coli*, *yerasinia enterocolitica*, *salmonella typhi*, and *B.cerusto* were sensitive to flax seed extract in methanol.

The antibacterial effect of extract tested in different concentration such as 100%,50%,25% and 12.5% for *yerasiniaenterocolitica* ,*E. coli salmonella typhi*, and *B. cerusto* showed maxium zone of inhibition encounter in 100% concentration then 50% and least was 12.5% concentration of Flax seed extraction.

Conculosion : The study conducted that the flax seeds extract was effective as antibacterial against some bacteria with different concentrations and this helps to be used in the treatment of some diseases caused by the bacteria mentioned above.

المستخلص

الخلفية: اجريت هذه الدراسة في المركز القومي للبحوث الخرطوم في الفتره من فبراير إلى أغسطس 2018 بغرض اختبار فعاليات بذرة الكتان علي بعض البكتريا.

الأهداف:هدفت هذه الدراسة إلى تقييم فعاليه مستخلص بذور الكتان للبكتريا الممرضه للجهاز الهضمي (السالمونيلا التيفية، العصوية الشمعية، اليرسنية الملهبه للمعدة والقولون، الاشريكيه القولونيه).

المنهجية: شملت هذه الدراسة أربعة أنواع من البكتريا كمنترول ماخوذه من إدارة المعامل، زرعت البكتريا واختبر مدي تاثيرها بالمضادات الحيوية.

الاستخلاص الكحولي لبذرة الكتان اثبت فعاليته ضد البكتريا المختاره (السالمونيلا التيفية، العصوية الشمعية، اليرسنية الملهبه للمعدة والقولون، الاشريكيه القولونيه).

النتيجة: أظهرت الدراسة أن بكتريا (السالمونيلا التيفية، العصوية الشمعية، اليرسنية الملهبه للمعدة والقولون، الاشريكيه القولونيه) كانت حساسة لمستخلص بذور الكتان في الميثانول. تم اختبار التأثير المضاد للجراثيم في المستخلص بتركيز مختلفة 100%، 50%، 25%، 12.5% إلى بكتريا السالمونيلا التيفية، العصوية الشمعية، اليرسنيه الملهبه للمعدة والقولون، الاشريكيه القولونيه أظهرت الحد الأقصى لمنطقة التنشيط في تركيز 100% ثم 50% واقل كان 12.5% من استخراج بذور الكتان.

الخلاصة: أستنتجت الدراسة بأن بذرة الكتان لها نشاط مضاد لبعض أنواع البكتريا بتركيز مختلفه وهذا يساعد على استخدامها في معالجه بعض الامراض التي تسببها البكتريا المذكوره أعلاه.

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List of abbreviations

Abbreviation	Meaning
ATCC	American type culture collection
B.cereus	Bacillus cereus
C. difficile	Clostridium difficile
dl	Deciliter
E.coli	Escherichia coli
GI	Gastrointestinal tract
HUS	Hemolytic uremic syndrome
Kg	Kilo gram
Mg	Milligram
MHA	Mueller Hinton agar
MIC	Minimum inhibition concentration
ml	Milliliter
Mm	Millimeter
MRSA	Methicillin Resistance staphylococcus aureus
MSSA	Methicillin sensitive staphylococcus aureus
N. gonorrhoeae	Nisseriagonorrhoeae
NCCLS	National Committee for Clinical Laboratory Standards
S.typhi	Salmonella typhi
Y.enterocolitica	Yersinia enterocolitica
HWO	World health organization

H.inflenze	Heamophulisinfluenze
M. catarrhalis	Moraxella catarrhalis
Aug	August
Feb	Febuary
SPSS	Statistical package for the social sciences
C	Centigrade
E.test	Epsilo meter test
C.F.U\ML	Colony forming Unit\ milliliter
MBC	Minimum Bacterial Concentration
MIC	Minimum Inhibitory Conceneration
MOL\L	Mold per liter
MMHg	Millimeters of mercury
V\V	Volume per volume

Chapter one
Introduction, Rationale and Objectives

1.1 Introduction:-

Gastrointestinal tract Infections with a variety of agents can occur in any part of the gastrointestinal tract from the mouth to Anal Canal. infections can range in severity from self. Limited to life threatening, particularly if infection spreads from the gut to other part of the body. Infection are typically caused by the ingestion of exogenous pathogens in sufficient quantities to evade host defenses and then cause disease by multiplication, toxin production, or invasion through the gastrointestinal mucosa to reach the blood stream and other tissue in other cases, members of the normal of the normal flora of gastrointestinal tract can cause disease (warren levinson, 2014).

Gastrointestinal infections are viral, bacterial or parasitic infections that cause gastroenteritis, an inflammation of the gastrointestinal tract involving both the stomach and the small intestine. Symptoms include diarrhea, vomiting, and abdominal pain. Dehydration is the main danger of gastrointestinal infections, so rehydration is important, but most gastrointestinal infections are self-limited and resolve within a few days. However, in a healthcare setting and in specific populations (newborns/infants, immune compromised patients or elderly populations), they are potentially serious. Rapid diagnosis, appropriate treatment and infection control measures are therefore particularly important in these contexts. Gastrointestinal infections can be caused by a large number of microorganisms. (Robert V.Tauxe and Nathan M. Thielman ,2001)

What Are Gastrointestinal Infections?

Diarrhea, with its frequent and watery bowel movements, often is caused by gastrointestinal infections (although other illnesses and dietary changes also can be culprits). Germs such as parasites, viruses, or bacteria all can cause gastrointestinal (GI) infections. Which germs are responsible for diarrhea depends on the geographic area a person lives in and its level of sanitation, economic development, and hygiene standards. For example, countries that have poor sanitation or use human waste as fertilizer tend to have outbreaks of diarrhea when intestinal bacteria or parasites contaminate crops or drinking water. In developed countries like the United States, outbreaks of diarrhea are most often due to what we call food poisoning. Food poisoning happens when toxins made by bacteria in food that is not handled, stored, or cooked properly make a person sick. The viruses that cause diarrheal illness, also known as viral gastroenteritis, can pass through a household (or a college dorm or other place where lots of people live together) quickly because they're highly infectious. Luckily, the diarrhea usually goes away on its own in a few days. For healthy teens and adults, viral gastroenteritis is a common but minor inconvenience. But for little kids and people with chronic illnesses, it can lead to dehydration that requires medical attention. (Robert V. Tauxe and Nathan M. Thielman, 2001).

The flax seeds is anti-inflammatory flax possesses anti-inflammatory properties and it is a gluten-free seed. you can also use this seed cooking because of its anti-inflammatory properties. Those who possess gluten sensitivity can use this seed as an option. Flax seeds are

helpful in preventing your body against infection. Flax seed is quite safe but sometimes its overconsumption may lead to many side-effects. These are basically gastrointestinal side-effects which can lead to increase in the number of bowel movement, gastric trouble, nausea, abdominal pain, diarrhea, and constipation. Flax seed should be consumed in little quantities and you should drink plenty of water to avoid any side effects. In some cases, it can block your intestine. This type of laxative effect can happen due to the formation of large lignin chemicals in your body. you should discontinue consumption of flax seed as soon as notice any kind of side effects. sometimes, flax seed can be poisonous to you if you take it raw or unripe. (Charless Bollmann, 2015)

1.2 Rationale:

Traditional medicine is now very important in treatment of some diseases and is used by human. Its use is preferred because of low cost availability and minimal side effect of chemical substances.

For that this study aimed to determine the activity and effect of flax seed extract on the growth properties as antibacterial agent against pathogen isolated from patient with GIT infection.

With increasing of bacterial resistance against available antibiotics now become essential to look for traditional medicine.

1.3 Objectives:

General objective:

To assess in vitro antimicrobial activities of flax seed extract against bacteria causing gastro intestinal such as *E.Coli*, *B. cereus*, *Salmonella typhi* and *yeresinia entercolitica*.

Specific objectives :

- To detect flax seed antimicrobial activity against *E.coli*, *B.cereus*, *Salmonella typhi* and *yeresinia entercolitica*
- To associate the concentration of crude extract with bacterial inhibition zone
- To compare between sensitivity of antimicrobial durgs with flax seeds extract.

Chapter two
Literature Review

2. Literature review:

2.1 Definition:

The small seed of flax (especially *Linum usitatissimum*) used especially as a source of oil, as a demulcent and emollient, and as a dietary supplement. (Lisa Drayer, 2018)

2.2 Component :

Flaxseed contains all sorts of healthy components, it owes its primary healthy reputation to three of them:

- Omega-3 essential fatty acids, “good” fats that have been shown to have heart-healthy effects. Each tablespoon of ground flaxseed contains about 1.8 grams of plant omega-3s.

Lignin’s, which have both plant estrogen and antioxidant qualities.

Flaxseed contains 75 to 800 times more Lignin’s than other plant foods.

Fiber. Flaxseed contains both the soluble and insoluble types. (Elaine Magee, 2009)

2.3 Physical properties

Physical properties of flaxseeds have been evaluated as a function of seed moisture content, varying from 6.09% to 16.81% (db). In the moisture range, seed length, width, thickness, arithmetic mean diameter, and geometric mean diameter increased linearly from 4.27 to 4.64 mm, 2.22 to 2.38 mm, 0.85 to 0.88 mm, 2.45 to 2.63 mm and 2.00 to 2.12 mm respectively with increase in moisture content. Flax seed extract were tested against four type of the gram positive

and negative bacterial *Bacillus Cereus*, *E. coil*. (YalcinCoskuner and Ersan Karababa ,et al 2007).

2.4 Antimicrobial agents

Antimicrobial agents include naturally occurring antibiotics, synthetic derivatives of naturally occurring antibiotics (semi-synthetic antibiotics) and chemical antimicrobial compounds (chemotherapeutic agents). Generally, however, the term ‘antibiotic’ is used to describe antimicrobial agents (usually antibacterial)that can be used to treat infection.(Monica, 2006)

2.5Antibacterial and Anti biofilm Activity of Flaxseed:

The present study aimed to explore the antibacterial and anti-biofilm activity of flaxseed oil on some locally isolated bacterial pathogens. No inhibitory effect was noticed against.

Escherichia coli or *Enterococcus faecalis*. However, variable effects were developed against

Methicillin resistant *Staphylococcus aureus* (*MRSA*), methicillin sensitive *Staphylococcus aureus* (*MSSA*), Normal flora of small intestine:

Duodenum is adjacent to stomach and hence it is slightly acidic in nature. Therefore microorganisms in duodenum is similar to that of stomach. Mainly *Lactobacillus* and *Enterococcus* are found in deudenum.

From duodenum ileum, intestine become less acidic and hence number of microorganism increases.

In jejunum Enterococci, lactobacillus, Diphtheroid and *Candida albicans* are found.

In Ileum microorganism begins to resemble to that of large intestine.

Mainly obligate anaerobes such as *Clostridium perfringens*, *Bacteroides* and anaerobic *E. coli* are found. (Harith Jabbar and Ahmed saad ,et al, 2016)

2.6 Measure of antibacterial activity &resistance :

2.6.1 Susceptibility testing techniques

Laboratory antimicrobial susceptibility testing can be performed using:

A dilution technique.

A disc diffusion technique.

Dilution susceptibility tests:

Manual or semi-automated dilution susceptibility tests are performed in Microbiology Reference Laboratories for epidemiological purposes or when a patient does not respond to treatment thought to be adequate, relapses while being treated, or when there is immunosuppression. Dilution techniques measure the minimum inhibitory concentration (MIC). They can also be used to measure the minimum bactericidal concentration (MBC) which is the lowest concentration of antimicrobial required to kill bacteria. A dilution test is carried out by adding dilutions of an antimicrobial to a broth or agar medium. A standardized inoculum of the test organism is then added. After overnight incubation, the MIC is reported as the lowest concentration of antimicrobial required to prevent visible growth. By

comparing the MIC value with known concentrations of the drug obtainable in serum or other body fluids, the likely clinical response can be assessed.(Monica, 2006)

2.6.2 Disc diffusion susceptibility tests:

Disc diffusion techniques are used by most laboratories to test routinely for antimicrobial susceptibility. A disc of blotting paper is impregnated with a known volume and appropriate concentration of an antimicrobial, and this is placed on a plate of susceptibility testing agar uniformly inoculated with the test organism. The antimicrobial diffuses from the disc into the medium and the growth of the test organism is inhibited at a distance from the disc that is related (among other factors) to the susceptibility of the organism. Strains susceptible to the antimicrobial are inhibited at a distance from the disc whereas resistant strains have smaller zones of inhibition or grow up to edge of the disc. For clinical and surveillance purposes and to promote reproducibility and comparability of results between laboratories, WHO recommends the (NCCLS) modified Kirby-Bauer disc diffusion technique. National Committee for Clinical Laboratory Standards. (Monica, 2006)

Kirby-Bauer NCCLS modified disc diffusion technique : The validity of this carefully standardized technique depends on, for each defined species, using discs of correct antimicrobial content, an inoculum which gives confluent growth, and a reliable Mueller Hinton agar. The test method After incubation at 35 C for 16–18 hours, zone sizes are measured and interpreted using NCCLS standards. These are derived

from the correlation which exists between zone sizes and MICs. An approximately linear relationship exists between log MIC as measured by the dilution test, and the inhibition zone diameter in the diffusion test. A regression line expressing this relationship can be obtained by testing a large number of strains by both techniques. This has been done and enables zone diameter sizes to be correlated to MIC values in the NCCLS modified Kirby-Bauer technique. The NCCLS Kirby-Bauer technique should only be used for well-evaluated bacterial species. It is not suitable for bacteria that are slow-growing, need special nutrients, or require CO₂ or anaerobic incubation.(Monica, 2006)

2.6.3 Stokes disc diffusion technique:

In this disc technique both the test and control organisms are inoculated on the same plate. The zone sizes of the test organism are compared directly with that of the control. This method is not as highly standardized as the Kirby-Bauer technique and is used in laboratories particularly when the exact amount of antimicrobial in a disc cannot be guaranteed due to difficulties in obtaining discs and storing them correctly or when the other conditions required for the Kirby Bauer technique cannot be met .One way laboratories in developing countries.

performing the Stokes technique could change to a technique comparable to the WHO recommended Kirby-Bauer technique is to use highly stable Rosco Diagnostica antibiotic tablets (*Neo-Sensitabs*) instead of less stable paper discs. (Monica, 2006)

2.6.4 Antimicrobial discs:

The choice of antimicrobials to be included in difficulties in obtaining discs and storing them correctly or when the other conditions required for the Kirby-Bauer technique cannot be met. One way laboratories in developing countries performing the Stokes technique could change to a technique comparable to the WHO recommended Kirby-Bauer technique is to use highly stable RoscoDiagnostica antibiotic tablets (Neo-Sensitabs) instead of less stable paper discs. Rosco Neo-Sensitabs susceptibility testing Neo-Sensitabs antimicrobial tablets are standardized according to the 2004 MIC-breakpoints recommended by the NCCLS. The tablets are 9 mm in diameter and colour -coded. The formulae used to produce the tablets gives them a shelf life of about 4 years and many Neo-Sensitabs can be stored at room temperature. The same principles and quality control as used in the modified Kirby-Bauer method apply when using Neo- Sensitabs. An excellent 2004 booklet User's Guide- Neo-Sensitabs Susceptibility Testing is available from RoscoDiagnostica. This describes the principles and how to perform susceptibility testing and exactly how to measure and interpret zone sizes. The cost and local availability of Neo- Sensitabs can be obtained from Rosco Diagnostica. Modified Kirby-Bauer susceptibility testing technique Control each new batch of agar by testing it with a control strain of *E. faecalis* (ATCC 29212 or 33186) and co-trimoxazole disc. The zone of inhibition should be 20 mm or more in diameter. Store the plates at 2–8 C in sealed plastic bags. They can be kept for up to 2 weeks. For use, dry the plates with their

lids slightly raised in a 35–37 C incubator for about 30 minutes. Fastidious organisms: Unmodified Mueller Hinton agar is not suitable for susceptibility testing *H. influenzae*, *S. pneumoniae*, *N. gonorrhoeae*. Isolates of these organisms should be sent to a specialist microbiology laboratory for testing. Alternatively, the addition of lysed blood will enable such organisms to be tested. (Monica, 2006)

2.6.5 Turbidity standard:

Equivalent to McFarland 0.5 This is a barium sulphate standard against which the turbidity of the test and control inocula can be compared. When matched with the standard, the inocula should give confluent or almost confluent growth. Shake the standard immediately before use. Resistant: A pathogen reported as ‘resistant’ implies that the infection it has caused will not respond to treatment with the drug to which it is resistant irrespective of dose or site of infection. Intermediate: A pathogen reported as intermediately susceptible suggests that the infection it has caused is likely to respond to treatment when the drug is used in larger doses than normal or when the drug is concentrated at the site of infection, e.g. in the urinary tract. Consideration should be given to using other drugs that may provide more optimal therapy. Susceptible: A pathogen reported as susceptible suggests that the infection it has caused is likely to respond to treatment when the drug to which it is susceptible is used in normal recommended doses and administered by an appropriate route. (Monica, 2006)

2.6.6 Etest :

Confirmation of unusual resistance profiles can be performed using the Etest method. Etest is a quantitative technique for the determination of minimum inhibitory concentration (MIC) of antimicrobial agents against microorganisms and detection of resistance mechanisms. It comprises a predefined gradient of antibiotic concentration for a specific antibiotic on a plastic strip. When the Etest strip is applied onto an inoculated agar surface, eg Mueller Hinton, the gradient of antimicrobial agent is transferred immediately to the medium. After overnight incubation or longer, a symmetrical inhibition ellipse (elliptical shaped zone) centred along the strip is formed. The MIC is read directly from the scale in micrograms per millilitre ($\mu\text{g/ml}$) at the point where the inhibition ellipse edge intersects the strip.(Monica, 2006)

2.6.7 Nitrocefintest:

To detect beta-lactamase enzymes The nitrocefin biochemical test is a sensitive technique for detecting beta-lactamase producing strains of *N. gonorrhoeae*, *H. influenzae* and *M. catarrhalis*. Nitrocefin is a chromogenic cephalosporin which changes from yellow to red when its beta-lactam ring is hydrolyzed by betalactamase. Although the test can be performed using a nitrocefin solution, nitrocefin is very expensive, light-sensitive, and not easily obtained. Most laboratories find it more convenient to use a commercially available nitrocefin test such as the Oxoid betalactamase (nitrocefin) Touch Stick, code number BR0066A. Each pack contains 100 sticks. When stored frozen

(below -10°C) the sticks have a shelflife of about 1 year. The test is performed by touching a colony of the test organism with a stick and when the organism is beta-lactamase producing, the end of the stick turns pink-red within 15 minutes. Acidimetric test to detect beta-lactamase producing strains of *N. gonorrhoeae* and *H. influenzae*. When it is not possible to obtain the products to perform a nitrocefin test, a simple. (Monica, 2006).

2.6.8 Acidimetric test:

Can be used to detect beta-lactamase producing strains of *N. gonorrhoeae* and *H. influenzae*. The test is not sufficiently sensitive to detect beta-lactamases producing strains of *M. catarrhalis*. The acidimetric test is based on detecting a change in colour of the indicator bromocresol purple from purple to yellow due to penicilloic acid produced from the breakdown of penicillin (used in the test) by beta-lactamase. (Monica, 2006)

2.7 Common GI Infection

Here are a few types of GI infections that you may have heard about: *Salmonella* bacteria lead to between 1 and 5 million cases of diarrheal illness in the United States each year. These bacteria, a major cause of food poisoning, are frequently found in raw chicken or eggs.

Shigella bacteria are highly contagious and spread easily from person to person. They attack the intestinal wall and may cause ulcers that bleed. *Shigella* infections account for more than 160 million cases of diarrhea around the world each year. (J. Fernando DelRosario, 2015)

E. coli bacteria are found in the bowel movements of people and animals. Some strains of the bacteria secrete a toxin that can be life-threatening for little kids and older people. Others can cause traveler's diarrhea, a milder infection. *E. coli* infections spread through direct person-to-person contact or contaminated water or food, such as undercooked beef or unwashed fruit that came in to contact with animal manure.(J. Fernando Del Rosario,2015)

The *Giardia* parasite, which spreads easily through contaminated water and human contact, is another common cause of diarrheal infections in the United States. This parasite can spread in water parks and pools because it is resistant to chlorine treatment. Bathing in and drinking water from contaminated streams or lakes can lead to an infection and chronic diarrhea. Infants in childcare settings can become infected with *Giardia* and bring the parasite home, causing diarrhea in family members.(J. Fernando Del Rosario, 2015)

Another parasite, *Cryptosporidium*, is a common culprit behind diarrhea epidemics in childcare centers and other public places. *Cryptosporidium* often causes watery diarrhea that can last for 2 weeks or more. (J.Fernando Del Rosario, 2015)

2.8 Signs and Symptom:

Usually GI infections cause abdominal cramping followed by diarrhea. A person might also have:

A fever.

Loss of appetite.

Nausea.

Vomiting.

Weight loss

Dehydration.

Mucus or blood in the stool.

These symptoms typically last for a few days or longer. Symptoms that last for more than 2 weeks, however, are a sign of chronic diarrhea. Call your doctor if you think that you have chronic diarrhea or if you see blood in your stool.(J. Fernando DelRosario,2015)

2.9 Will Symptoms Appear:

The incubation period for a gastrointestinal infection depends on the particular germ causing it. For example, the *Shigella* incubation period is usually 2 to 4 days, but the period for viral infections ranges from 4 to 48 hours.

Parasitic infections generally have longer incubation periods; for instance, in a *Giardia* infection, symptoms can take from 1 to 4 weeks to appear. Then, depending on the type of germ and the person's overall health, a diarrheal infection can last for a few days or a few weeks. (J. Fernando Del Rosario, 2015)

2.10 GI Infections Contagious:

Diarrheal infections are highly contagious. They can spread from person to person via dirty hands, contaminated food or water, and some pets. Most cases are contagious for as long as a person has diarrhea, but some infections can be contagious for even longer.(J. Fernando Del Rosario, 2015)

Escherichia coli, often called *E. coli*, is the leading cause of travelers' diarrhea and a major cause of diarrheal disease in the developing world, especially among children. People usually contract *E. coli* through ingestion of water contaminated with human or animal feces.

Escherichia coli O157:H7

Escherichia coli O157:H7 is a Shiga toxin-producing form of *E. coli* bacteria, which causes gastrointestinal infections with symptoms including bloody diarrhea and vomiting. Though it usually resolves after a few days, it can sometimes (5-10% of infections) lead to hemolytic uremic syndrome (HUS), which can result in kidney failure if untreated. (Robert V. Tauxe and Nathan M. Thielman, 2001)

Adenovirus can cause diarrhea, fever, conjunctivitis, bladder infections and rashes, but the most common symptom is respiratory illness. After rotavirus, it is the most common cause of pediatric diarrhea. (Robert V. Tauxe and Nathan M. Thielman, 2001).

Rotavirus is the most frequent cause of diarrhea in young children and infants and it is responsible for the most severe cases. There is a vaccine for rotavirus, but globally it causes more than 1/2 million deaths per year in children less than five years old. Most of these are in emerging countries. (Robert V. Tauxe and Nathan M. Thielman, 2001).

Salmonella and *Shigella* are food-borne GI illnesses. *Salmonella* is common and is found in raw meats, poultry, seafood and eggs, as well as milk and dairy products. Acute symptoms include nausea, vomiting, abdominal cramps, diarrhea, fever, and headache. *Shigella* is

frequently found in water polluted with human feces. Symptoms of shigellosis (bacillary dysentery) include abdominal pain, cramps, diarrhea, fever, vomiting, and blood, pus, or mucus in stool.(Robert V. Tauxe and Nathan M.Thielman, 2001).

Staphylococcus aureus the most common cause of food intoxication, characterized by abrupt/violent onset, severe nausea, cramps, vomiting, and diarrhea using lasting 1-2 days. This opportunistic pathogen can be found on humans (skin, infected cuts, noses and throats) and has been associated with a wide range of foods including meat and meat products, poultry and egg products, salads, bakery products, and dairy products.(Robert V. Tauxe and Nathan M. Thiel man, 2001)

Yersinia enterocolitica, called *Y.enterocolitica*, is a relatively infrequent cause of diarrhea and abdominal pain. Infection is most often acquired by eating contaminated food, especially raw or undercooked pork products, as well as ice-cream and milk. Common symptoms are fever, abdominal pain, and diarrhea, which is often bloody.(Robert V. Tauxe and Nathan M. Thielman,2001).

Bacillus cereus. Commonly found in soil, is a gram-positive endospore forming bacterium that can sometimes cause foodborne illness. *B.cereus* endospores can survive cooking and produce enterotoxins in food after it has been heated; illnesses often occur after eating rice and other prepared foods left at room temperature for too long. The signs and symptoms appear within a few hours of ingestion and include nausea, pain, and abdominal cramps. *B. cereus* produces

two toxins: one causing diarrhea, and the other causing vomiting. More severe signs and symptoms can some times develop. Diagnosis can be accomplished by isolating bacteria from stool samples or vomitus and uneaten infected food. Treatment involves rehydration and supportive therapy. Antibiotics are not typically needed, as the illness is usually relatively mild and is due to toxin activity.(Robert V. Tauxe and Nathan M. Thielman, 2001).

2.11 Diagnosis:

When symptoms point to a possible gastrointestinal infection, diagnosis can be confirmed through laboratory tests used for culture or antigen detection from stool specimens. In certain cases (e.g. for *E. coli*, *Salmonella*, *C. difficile...*), antibiotic susceptibility testing is used to determine microbial resistance to antibiotic therapy, if appropriate. Particularly in hospital settings, rapid diagnosis provides important information for implementing infection control measures. To diagnose the cause of a diarrhea, it is helpful to consider where the context is a food-borne outbreak or “travelers’ diarrhea”.(Robert V.Tauxe and Nathan M. Thielman, 2001)

2.12 Normal flora of small intestine:

Duodenum is adjacent to stomach and hence it is slightly acidic in nature. Therefore microorganisms in duodenum is similar to that of stomach. Mainly Lactobacillus and Enterococcus are found in duodenum.

From duodenum ileum, intestine become less acidic and hence number of microorganism increases.

In jejunum Enterococci, lactobacillus, Diphtheroid and Candida albicans are found.

In Ileum microorganism begins to resemble to that of large intestine.

Mainly obligate anaerobes such as *Clostridium perfringens*, *Bacteroides* and anaerobic *E. coli* are found. (GaurabKarki ,2018).

2.12.1 Normal flora of large intestine:

Large intestine is anaerobic in nature. It contains obligate anaerobes and facultative anaerobes.

Clostridium perfringens, *Bifidobacterium*, *Bacteroides*, *Streptococcus fecalis*, *E. coil*. (GaurabKarki, 2018).

Chapter Three
Material and Methods

3. Material and Method:

3.1 Study design

This was prospective and descriptive study.

3.2 Study Area

The study was conducted in medical and aromatic plant institute, national center for research in Khartoum

3.3 Study duration:

The study was carried out from Feb to Aug 2018

3.4 Study population

Bacterial American Type Culture Collection ATCC of *E. coli*, *B. cerus*, *S. typhi*, *Y. enterocolitica*.

3.5 Sample size

The study included *E. coli*, *B. cerus*, *S. typhi*, *Y. enterocolitica* bacterial American Type Culture Collection ATCC from gastrointestinal tract infection.

3.6 Sample collection:

3.6.1 Collection of flax seeds

The flax seeds were purchased from market. they were identified and authenticated by medical and aromatic plants institute, national center for research in Khartoum.

3.6.2 Preparation of the crude extract:

Approximately 1 kg ground seeds were soaked in the aqueous-methanol(30:70 v/v) at room temperature for 3 days with occasional shaking. It was filtered through a double layered muslin cloth and subsequently through a filter paper. The residue was re-soaked in the

fresh solvent and the process was repeated twice to get maximum yield of crude extract from the plant material. The combined filtrate was concentrated in a rotary evaporator at 40 °C under reduced pressure (760 mmHg) to a thick, semi-solid mass, labeled as the crude extract of Flaxseed, with approximated yield of 8% and was stored at 20 °C until used (Ghayur and Gilani, 2005).

3.6.3 Bacterial microorganisms:

Bacillus Cerues ATCC 14579 (Gram + ve bacteria).

Escherichia coli ATCC 25922 (Gram –ve bacteria).

Salmonella typhi ATCC (Gram –ve bacteria).

Yersinia Entercolitica ATCC (Gram –ve bacteria).

3.6.4 Preparation of bacterial suspensions:

One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37° C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 10^8 - 10^9 C.F.U/ ml. The suspension was stored in the refrigerator at 4°C till used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique. Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37 °C for 24 hours. After incubation, the number of

developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension. Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

3.6.5 Testing of antibacterial susceptibility

3.6.5.1 Disc diffusion method:

The paper disc diffusion method was used to screen the antibacterial activity of plant extracts and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 1999). Bacterial suspension was diluted with sterile physiological solution to 10^8 cfu/ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No. 2 mm in diameter) were placed on the surface of the MHA and soaked with 20 μ l of a solution of each plant extract. The inoculated plates were incubated at 37 °C for 24h in the inverted position. The diameters (mm) of the inhibition zones were measured. The antibacterial activity results were expressed in terms of the diameter of zone of inhibition and <9mm zone was considered as inactive; 9-12mm as partially

active; while 13-18mm as active and 18mm as very active. (Sana Mukhtar and Ifra Ghori, 2012)

The results were expressed in terms of the diameter of the inhibition zone:

< 9 mm, inactive; 9-12 mm, partially active; 13-18 mm, active;

>18 mm, very active. (Tania Mariade Almeida Alves, Andréia Fonseca, 2000)

3.7 Data analysis:

Data were analyzed using computerized program SPSS version 20, by chi - square T test.

Chapter Four

Result

4.Results

The study was conducted during the period from Feb to Aug 2018.the present work studied the antibacterial activities of flax seeds extract against four stander bacteria from management laboratory.

On the standard organisms (*E. coli*, *salmonella typhi*, *yerasinia enterocolitica* and *B. cerus*) shown the Affectivity of Flaxseed begin From 12.5% concentration on all spp. And this effectiveness increase when the concentration of Flaxseed increase. **Table (4.1)**

Result of *E. coli*, *yerasiniaen terocolitica*, *salmonella typhi*, *B.cerusto* sensitivity of flax seeds extract by methanol show high in concentration 100% inhibition zone decrease gradually with concentration, result insignificant with p.value. **Table (4.1)**

Result of *E. coli*, *yerasinia enterocolitica*, *salmonella typhi*, *B.cerus* to sensitivity of flax seeds extract by methanol show high in concentration 50% inhibition zone decrease gradually with concentration, result insignificant with p.value. **Table (4.1)**

Result of *E.coli*, *Y.enterocolitica*, *salmonella typhoon*, *B.Cerus* to sensitivity of flax seeds extract by methanol show high in concentration 25% inhibition zone decrease gradually with concentration ,result insignificant with p. value. **Table (4.1)**

Result of *E. coli*, *yerasinia enterocolitica*, *salmonella typhi*, *B. cerusto* sensitivity of flax seeds extract by methanol show high in concentration 12.5% inhibition zone decrease gradually with concentration, result insignificant with p. value. **Table (4.1)**

Comparison of the antimicrobial activity between flax seeds extract and reference antibiotics against stander bacteria:

Flax seeds extract, when compared with reference drugs against bacteria stander ,showed better antibacterial activity with Ampicillin(20mm) and Nalidxic acid (19mm) against *E.coli*, the chloramphenicol (12mm)the less effect against *E.coli* compare the flax seeds extract, result see insignificant with p. value 0.2. **Table(4.2)**

Flax seeds extract, when compared with reference drugs against bacteria stander ,showed better antibacterial activity with Nalidxic acid (20mm) against *yerasinia enterocolitica*, the Ampicillin(16mm) and chloramphenicol (18mm) less effect against *yerasinia enterocolitica* compare the flax seeds extract, result see insignificant with p.value 0.2 **Table(4.2)**

Flax seeds extract, when compared with reference drugs against bacteria stander ,showed better antibacterial activity with Ampicillin(15mm), chloramphenicol(21mm) and Nalidxic acid (18mm) against salmonella typhi, result see insignificant with p. value 0.2.**Table(4.2)**

flax seeds extract, when compared with reference drugs against bacteria stander ,showed better antibacterial activity with Ampicillin (18mm), chloramphenicol(16mm) and Nalidxic acid(18mm) against *B.cereus*, result see insignificant with p. value 0.2 **Table(4.2)**

Table (4.1): inhibition zone (mm) of organism with extraction

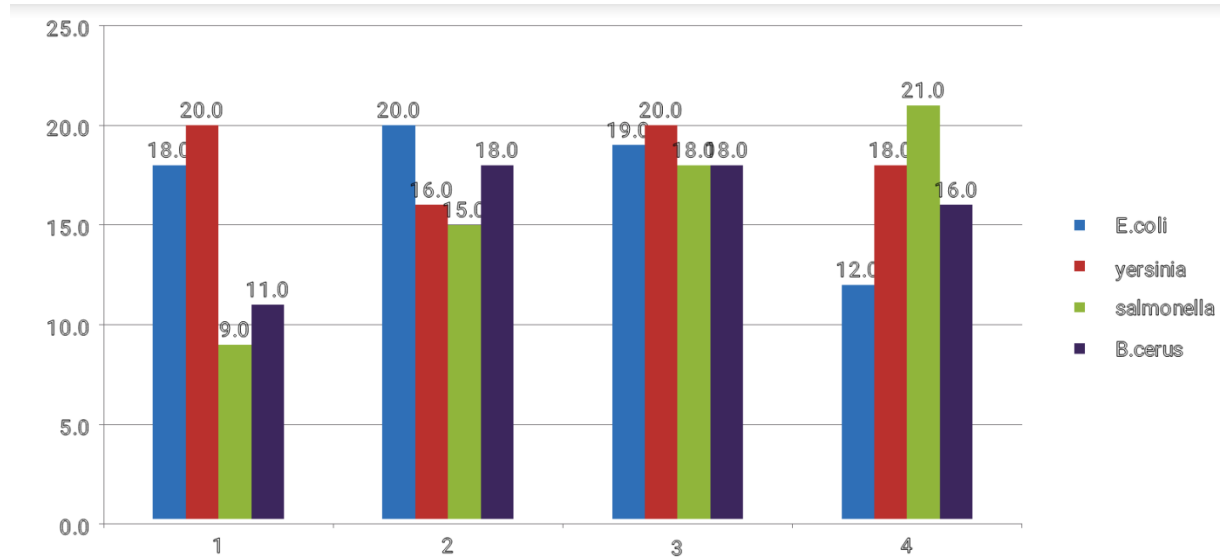
E.coli	100	50	25	12.5
Mean	17.2	8.3	6.7	5.8
P. value	0.001	0.003	0.002	0.006
B.cereus	100	50	25	12.5
Mean	10.7	8.5	7.3	6.5
P. value	0.001	0.001	0.002	0.002
Y.enterocolitica	100	50	25	12.5
Mean	19.0	16.7	7.5	6.7
P.value	0.001	0.000	0.001	0.002
S.typhi	100	50	25	12.5
Mean	8.5	8.0	8.0	6.8
P.value	0.001	0.005	0.001	0.001

Table (4.2): Comparison of sensitivity of the flax seed extraction and antibiotic against the organisms

Bacteria strain	Inhibition zone (mm)			
	Extraction	Antibiotic		
		Ampicillin	Nalidixic acid	Chloramphenicol
<i>E.coli</i>	18 mm	20 mm	19 mm	12 mm
<i>y.enterocolitica</i>	20 mm	16 mm	20 mm	18 mm
<i>S.typhi</i>	9 mm	15 mm	18 mm	21 mm
<i>B.cerus</i>	11 mm	18 mm	18 mm	16 mm
P. value	0.2	0.2	0.2	0.2

The P.value is insignificance less than 0.005

Figure (4.1):relationship between standerd bacteria and inhibition zone of extraction and some antibiotic.



Chapter Five

Discussion, Conclusion and Recommendation

5.1 Discussion

The present study determined the antimicrobial activity of extracted flax seeds using disc diffusion method. The result showed that antimicrobial activities against all of bacteria including *E. coli*, *Yersinia enterocolitica*, *Salmonella typhi*, *B. cereus* was highly effective on the bacteria with 100% inhibition at all concentrations, but was variation in the degree of antimicrobial activity on the bacteria species increased when the concentration increased.

Result of *E. coli*, *Yersinia enterocolitica*, *Salmonella typhi*, *B. cereus* to sensitivity of flax seeds extract by methanol show high in concentration 100% inhibition zone decrease gradually with concentration.

The result showed the antimicrobial activity against the *Y. enterocolitica* (20mm), *E. coli*(18mm), *S. typhi*(9mm), *B. cereus* (11mm), was highly effective on the bacteria with 100% inhibition at all concentration of flax seeds extract.

The antimicrobial activity of flax seeds against the *Y. enterocolitica* (17mm), *E. coli*(9mm), *S. typhi* (9mm), *B. cereus* (9mm) was different effective in the inhibition zone on the some concentration 50% ,the result see insignificant in methanol extraction with p.value

The antimicrobial activity of flax seeds against the *Y. enterocolitica*(8mm), *E. coli*(7mm), *S. typhi*(8mm), *B. cereus*(8mm) was different effective in the inhibition zone on the some concentration 25%, the result see insignificant in methanol extraction with p. value

The antimicrobial activity of flax seeds ,began from minimum concentration 12.5% Low inhibit in some bacteria (*Y.enterocolitica* (7mm), *E.coli* (6mm), *S.typhi* (7mm), *B.cerus* (6mm) was different effective in the inhibition zone on the concentration 12.5%, but the variation in the degree of antimicrobial activity on the bacteria organism which the inhibition zone of flax seeds against bacterial organism increased when the concentration increased.

Flax seeds extract, when compared with reference drugs against bacteria stander ,showed better antibacterial activity with Ampicillin (20mm) and Nalidxic acid(19mm) against *E.coli*, the chloramphenicol(12mm) the less effect against *E.coli* compare the flax seeds extraction, the result see insignificant in methanol extraction with p.value 0.2.

Flax seeds extract, when compared with reference drugs against bacteria stander ,showed better antibacterial activity with Nalidxic acid(20mm) against *yerasinia enterocolitica*, the Ampicillin (16mm) and chloramphenicol(18mm) less effect against *yerasinia enterocolitica* compare the flax seeds extract, the result see insignificant in methanol extraction with p. value 0.2.

Flax seeds extract, when compared with reference drugs against bacteria stander ,showed better antibacterial activity with Ampicillin (15mm), chloramphenicol(21mm) and Nalidxic acid(18mm) against *salmonella typhi*, the result see insignificant in methanol extraction with p. value 0.2.

Flax seeds extract, when compared with reference drugs against bacteria stander ,showed better antibacterial activity with Ampicillin(18mm), chloramphenicol(16mm) and Nalidxic acid (18mm) against *B.cereus*, , the result see insignificant in methanol extraction with p. value 0.2.

5.2 Conclusion

Flax seed had antibacterial activity, but with varying degree of effectiveness against bacterial *E. coli*, *B.cereus*, *Y. enterocolitica* and *S.typhi*.

Depth studies of active ingredient of flax seed to determine the active compound response to antibacterial activity

5.3 Recommendation

The is good source of environmentally and ecologically safe antibacterial and could be used for commercialization with appropriate dosage.

The resistance strain of bacteria should be put in more investigation and the resistance gene should be detected.

This work should be put in to more investigation for future practice in the field of pharmacology, phytochemistry, botany and other biological field for drugs discovery.

Chapter Six
References and Appendix

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