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Effect of cold aqueous plant extract (Citrus aurantium and Solanum melongena) against the Giardia lamblia parasite and Entamoeba histolytica in vitro and measuring the LD50 for extracts in Al-Muthanna province.

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ABSTRACT

The current study was conducted in the laboratory of the postgraduate college of Sciences / University of Muthanna. To investigate the parasite *Giardia lamblia* parasite and *Entamoeba histolytica* among the reviewers for each of Rumaitha General Hospital, Children's Hospital and childbirth, and during this period the collection of 100 stool specimens of patients with diarrhea. The samples were visually and microscopically examined using a direct swab, and results showed the presence of the parasite *Giardia lamblia* by 10.8%. As *Entamoeba histolytica* increased by 16.66%, and the study included parasite culture *Giardia lamblia* in the TYI-S-33adapting medium and proven successful, as well as the cultivation of a parasitic *Entamoeba histolytica* condition in beef liver infusion medium , and after the culture has been experimenting with use of cold aqueous for each Peel of *citrus aurantium*, peel *solanuomme longena* with three concentrations(100,75,50%)and it turned out that these extracts are effective in eliminating both parasites, and showed a concentration of 75% and a clear impact in the elimination of both parasites, It also found significant differences between different concentrations under the moral level($P \leq 0.01$)and($P \leq 0.05$). and showed 100% concentration effect by the largest in the elimination of both parasites. The median lethal dose account of plant extracts water aqueous for each of the, bitter orange peel *Citrus aurantium*, eggplant *Solanuomme longena* if the dose is lethal to extract bitter orange (7.75) g / kg body weight median lethal dose to extract eggplant (7.25) g / kg body weight.

Keywords: Citrus aurantium, Solanum melongena, Giardia lamblia, Entamoeba histolytica

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INTRODUCTION

Given the global trend in the present time to focus on the use of herbs and medicinal plants as a remedy; for the free components of the side effects that accompany the chemically manufactured drugs that negative symptoms caused on the body and health that may lead to the emergence of other diseases such as cancer and diseases of genetic mutations or illnesses cause birth defects as well as the negative impact on the body's immune resistance to disease in other (1); So the researchers to extract the active ingredients in natural plant work to become an important source for the manufacture of drugs (2).

Constitute the parasites reasoned mainly diarrhea is the most important intestinal parasites nurse two parasitic *Entamoeba histolytica* and parasite *Giardia lamblia* if these known parasites of protozoan global parasitic proliferation infect infants and children in developing and developed countries and in spite of that for they are among the causes of intestinal diseases most common in World (3).

There are a number of pathological effects arising from infected by microbiological intestinal such as diarrhea, weight loss, loss of appetite and abdominal distension and fullness gases with pain, nausea, fever, vomiting and also cause atrophy of intestinal villi leading to impaired intestinal absorption of proteins, carbohydrates and vitamins such as vitamin B and vitamins is dissolved (4**).**

Entamoeba histolytica

General Description of the parasite

This parasite is going through four distinct forms in its life cycle is the Trophozite stage Precyst stage, Cyst stage, and Metacyst stage (5).

Pathogenicity and Symptoms

Pathological effects of *E. histolytica* depends on: the severity of infection, host resistance, the case of the gut, as well as on the nature of Nutrition (6). We have between Gonin and Trudle(2003) ,(7) to *E. Histolytica* infected man be in two forms either pathogen to certain individuals or unpathogen for other individuals; types of diseases that invade tissue called *E. histolytica* name and species unpathogen called *E. dispar* name often injuries in temperate regions is not pathogen, because most people are carriers of the disease without the appearance of any clinical symptoms, on the contrary of them in tropical and subtropical areas, where most of the infections are in this unpathogen areas and be a parasite *E. histolytica* is responsible for millions of cases of *Entamoeba histolytic*a abscess liver amoebic where be a parasite endemic in these areas, divide the symptoms of infection parasite *E. histolytica* into two types: intestinal, and outside the Extraintestinal, and rely symptoms on two main factors: the first is a parasite site in the host, and the other occurrence of penetration or tissue invasion (8).

Giardia lamblia

General Description of the parasite

The flagella protozoan parasite *Giardia* who intrudes in the upper part of the small intestine of humans and vertebrate animals, *G. lamblia* parasite has two forms are developed trophozoitstage (9). And cyst stage (10).

Pathogenicity and Symptoms

The parasitic pathogen effects depend in severity for reasons related to either the host or the causative with respect virulence (11) if it affects the upper part of the channel intestinal of Duodenum and jejunum and causes presence symptoms and phenomena accompany the state of diarrhea (12).

And that the difference in virulence between the strain *G. lamblia* as well as the immune status of the body are the ones who determine the progression of the disease. We have been clinically described the symptoms associated with cases of diarrhea caused by the parasite *G.lamblia* by the researcher Zeibigs(1997)

,(8) that can be seen in general on a patient with a noticeable symptoms: diarrhea is often a water with a foul smell with diarrhea greasy Steatorrhea, bulging with stomach cramps Abdominal cramp, loss of appetite Anorexia, weight loss Weight loss, vomiting and sometimes accompanied by fever (13). NoteDoglioni*etal*. (1992),(14) By studying the histological examination of the channel gastrointestinal tract of patients suffering from *Giardia* disease by Sight deviceand Biopsy atrophy of the villi twelve decimal, it did not change in fasting and increased in the ileum, while epithelial cells may take up clearly (14).

Solanuomme longena

Active substances to plant eggplant *Solanuomme longena*

Eggplant is a source of iron, calcium, phosphorus, potassium, vitamin B. and his familiar fresh weight of the wet material ratio of 92.7, 1.4 protein, fiber ratio of 1.3, 0.3 fat, 0.3 percentage metallic materials. The remaining percentage of 4 consist of carbohydrates and vitamins A and C(15,16)

Citrus aurantium

Active substances in plant bitter orange peel *C. aurantium*

study Carvalho - freitas& costa (2002),(17) that the volatile oils extracted from the fruits of *C. aurantium* green plant using steam distillation method containing the oil d - limonene by (90.4%) .referred Fisher & Scott (1997),(18) to Contain genus Citrus on High percentageoil d - limonene .This is a monounsaturated oil from turbines ringed is found in most plants while refer achammaa (1989), (19) to Contain fruitsof plant *C. aurantium* on oil d - limonene by (90%) and it same orange oil except ifsmell better and taste bitter and contain many other compounds taste bitter :

aurantimarin by 2.5 - 1.5%, aurantimaric acid by 2 - 0.1%
naringin also called aurantun, Isohesperidin by 3% - 0.4

Add to contain peels on fixed oil , a resinous material and Hesperidin a Scared tasteless and there is a rate ranging between % 8-3.

MATERIALS AND METHODS

Culture of *G .lamblia* and *E .histolytica*In vitro

Add one gram of faeces containing thetrophozoit stage and cyst stage ofthe parasite *giardia lamblia*to theculturmediumreaper and itTY1-S-33 adaptingand distilled water and by five replications of the medium and has note emergency parasite changes in the hours and days. As well as taking the One gram of feces containing the trophozoit stage and cyst stage of*E .histolytica* parasite to the culture medium and it beef liver infusion and distilled water and by five replications of the medium and has emergency Note the parasite changes in the hours and days.

Estimate vital parasite

Use water Eocene 1% solution as the Eocene pigmentation pigment content, all except the living protoplasm. (20).

Test the effectiveness of cold aqueous extracts of plants, *citrus aurantium,solanuomme longena* against each of the parasite *Giardia lamblia* and *E. histolytica* outside the body of the organism

To make the test for each of the aqueous extracts create the concentrations required for each extract was first a (100%, 75%, 50%), and by making stocks solution for each extract by taking 1 g of dried powder in 10 ml of distilled water, and adjust the population density and vitality of the parasite grown outside the body organism, was used counting blood cells slice and dye Eocene concentration (1%), if you put a drop of the culture medium parasite *Giardia lamblia*, or from culture medium of *e. histolytica*, on a clean glass slide and then added a drop of dye Eocene 1% and after mixing withdrawn 10 Microliter by minutes and place on

absorbent counting blood cells slide and examined under a microscope 40X magnification strongly if extracted the total number of parasites(21) by applying the following equation:

The total number of parasites = number of parasites in 4 large boxes × 2500 × 2

To calculate the percentage of the number of live parasites used the equation depending on the non-permeable Eocene dye into the cell parasitic living, namely:

The percentage of parasite living = number of parasites is unpigmented By Eocene / total number of parasites × 100

I repeated the count to three times at each experiment and adopted the ofArithmetic meanthe replicates

Conducted the test and that the treatment of the tubes planted parasite concentrations extracts where treated the first group concentration of 50%, and treated the second group concentration of 75%, and treated the third group concentration of 100%, while the fourth group Filled with distilled water as a control group, gently requested the pipes for the distribution of Abstract equally evenly inside the culture medium of the parasite and it left the incubator 37 degree C for 24 hours, and then took the pipe and the estimated percentages of killing parasites using counting blood cells slice and dye Eocene (1%) if extracted percentages killing parasites from the following law:

The percentage of parasites slain = number of parasites pigmented By Eocene / total number of parasites × 100

The appointment median lethal dose (LD50) of cold aqueousplant extracts for each plant *citrus aurantium* peel and *solanuomme longena*

It was given one of the groups and both sexes 1 ml of Normal saline by Stomach Tube through the Oral administration and represented this group control (C), while in same waygiven graded doses of aqueous extract cold of the peel of each plant *citrus aurantium* and *solanuomme longena* extract separately (10,7.5,5,2.5) g / kg for animals other aggregates (T1, T2, T3, T4) respectively, and both sexes, the animal monitored for 24 hours and recorded data about virtual changes in behavior and mortality quotient, (22).

Calculations

Identified Themedian lethal dose LD_{50} according to the equation Behrens and Karber (1953),(23) which:

$$\frac{\sum axb}{n}$$ highest dose- =LD50

Where:
a: the difference between the two doses in straight sets
b: the average number of dead animals in straight sets
n: number of animals in each group

Statistical analysis:

The results of a statistical analysis of the data using statistical program ready SPSS (2008)were used Chi-square(X2) by Dunkin 'polynomial to find a moral differences.

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RESULTS

Culture of *G. lamblia* In Vitro

Culture Medium	Days stage	1	2	3	4	5	6	7	8	9	10	11	12
Adapting TYI-S-33	trophozoit	+	+	+	+	+	+	+	+	+	+	-	-
	cyst	+	+	+	+	+	+	+	-	-	-	-	-
Distilled water	trophozoit	+	-	-	-	-	-	-	-	-	-	-	-
	cyst	+	+	+	+	+	-	-	-	-	-	-	-

Seen from the table {1} results of laboratory development of the parasite ***G. lamblia*** in TYI-S-33 modified medium, if the Note growth trophozite stage where up to the tenth day of the culture either cyst stage remains for the seventh day as the photo(1). As the parasite when put in distilled water (control) was the duration of the trophozoit stage is been survival one day, either cyst stage was staying in distilled water in a five-day period.

Table(1)showed*Giardia lamblia* in the culture medium.

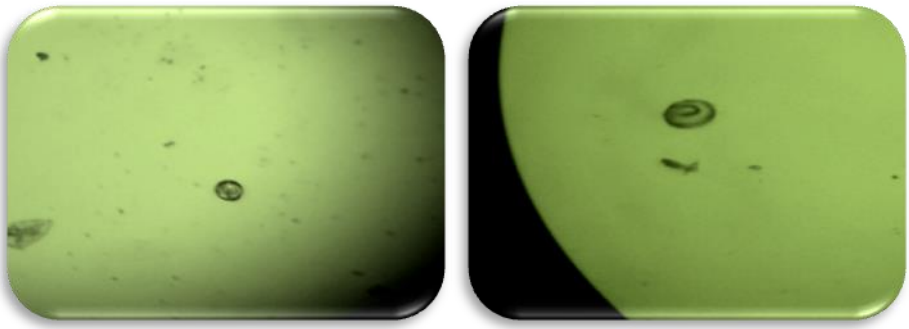

Image (1) describes the cyst stage of*Giardialamblia* in the culture medium (X40).

Culture of *E. histolytica*In Vitro

Seen from the table {2}results of laboratory development of the parasitic*E.histolytica* in beef liver infusion medium if the Note growth trophozoit stage where up to the eighth day of thecultur, either cyst stage remains for day in the twelfth as in photo(2) either when e.*histolytica* parasite cultivation in distilled water (control) trophozoit stage is been survival one day, either cyst stage was staying in distilled water is the seven-day period.

Table (2) *Entamoeba histolytica* parasite development in Beef liver infusion

Culture Medium	Days Stage	1	2	3	4	5	6	7	8	9	10	11	12
Beef liver infusion extract	trophozoit	+	+	+	+	+	+	+	+	-	-	-	-
	Cyst	+	+	+	+	+	+	+	+	+	+	+	+
Distilled water	trophozoit	+	-	-	-	-	-	-	-	-	-	-	-
	Cyst	+	+	+	+	+	+	+	-	-	-	-	-

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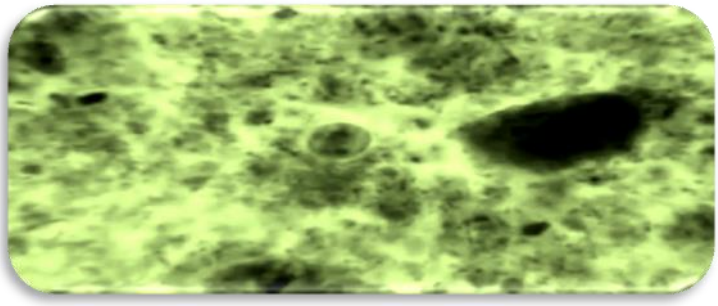

Image (2) cyst stage visualizations of dysentery beef liver infusion medium X40.

Effectiveness of cold aqueous extracts of peel for each plant *citrus aurantium and solanuomme longena* Against parasitic *Entamoeba histolytica* outside vivo

Test results showed the effectiveness of the water aqueous extracts in the killing *Entamoeba histolytica*, as in the form (1) and table {3} If the population density of the parasitic *Entamoeba histolytica* (29000) and the percentage of the number of living parasite (% 86.2), either prepare parasites murdered and percentages for the murder of aqueous extracts of *citrus aurantium* have been observed extract effective in killing *Entamoeba histolytica*and percentages were killed and within the time the experiment It was noted by the chi square test that each of the concentration (%100.75% 0.50%) significant effect on the parasitic amoeba dysentery and also noted the existence of significant differences between the concentrations of the extract of bitter orange on the prospect($P \leq 0.05$)by Dunkin 'polynomial.

when the concretion 100% (51.7) and the concentration of 75% (34.48) and the concentration of 50% (34.48). The aqueous extract of the eggplant has shown effective at killing the parasite and reached the proportion of killing at a concentration of 100% (68.9) and the concentration of 75% (51.7) and decreased the ratio of the concentration of 50% (34.48)It was noted that there were significant differences between the concentrations of the extract on the possibility of eggplant($P \leq 0.01$)by Dunkin 'polynomial. Did not death occur remember for the group of negative control, which has not added to the culture medium where any antagonist of the parasite.

Table (3) percentages of *Entamoeba histolytica* aqueous extracts from each plant(bitter orange, eggplant)

Connection	*S. melongena*	*C. aurantium*
50%	34.48 **c**%	%34.48 **b**
75%	51.7 **b**%	%34.5 **b**
100%	68.9 **a**%	51.7 **a**%
x^2	**11.858**	**5.07**
P	**0.01**	**0.05**
Df	2	2

X^2 =The value of Chi-square
P =Level of significance
df =degrees of freedom
(A, b, c)the different letters indicate the presence of significant differences between the concentrations of a possible(**P≤0.01**) by the Dunkin' polynomial.

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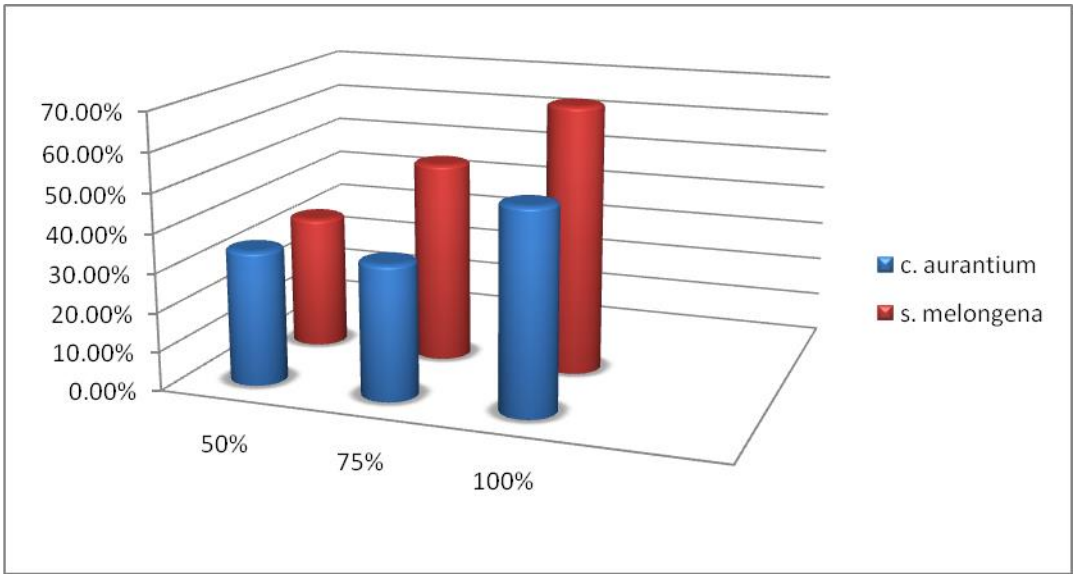

Form(1)percentages of *Entamoeba histolytica* aqueous extracts from each plant(bitter orange, eggplant).

Against the parasite *Giardiato lamblia* outside vivo

Test results showed the effectiveness of the aqueous plant extracts to kill the parasite *Giardialamblia* as in the table {4}and form(2). If was the population density of the parasite (32,500) and the percentage of the number of living (84.61%), while the preparation of parasites killed and percentages for killing The aqueous extract of *citrus aurantium* have shown effective at killing the parasite and reached the proportion of killer at a concentration of 100% (61.53) and the concentration of 75% (61.53) and decreased the ratio of the concentration of 50% (46.2)And it was also observed by the chi square test that each of theconcentration(%100.75% 0.50%) significant effect on the parasite *Giardia lamblia* as well as the presence of significant differences between the concentrations of the extract of orange on the prospect(P≤0.05)by Dunk in 'polynomial.

As for the eggplant extract water it has been observed in the effectiveness of the extract to kill *Giardia lamblia* and percentages were killed and experience within the time, when connection 100% (61.5) and the concentration of 75% (46.2) and the concentration of 50% (46.15)And it was also observed by the chi square test that each of the concentration(%100.75% 0.50%) significant effect on the parasite *Giardia lamblia* as well as the presence of significant differences between the concentrations of the extract of orange on the prospect(P≤0.05) by Dunkin 'polynomial. death did not remember to the negative control group that were not added to the culture medium where any antagonist of the parasite.

Table (4) percentages of Giardia lamblia aqueous extracts water to (,bitter orange, eggplant).

Connection	***S.melongena***	***C. aurantium***
%50	%46.15 **b**	46.2 **b**%
%75	46.2 **a**%	61.5 **a**%
%100	%61.5 **a**	61.53 **a**%
X^2	**4.9**	**4.86**
P	**0.05**	**0.05**
Df	2	2

X^2 =The value of Chi-square
P =Level of significance
df =degrees of freedom
(A, b, c)the different letters indicate the presence of significant differences between the concentrations of a possible(**P≤0.05**) by the Dunkin' polynomial.

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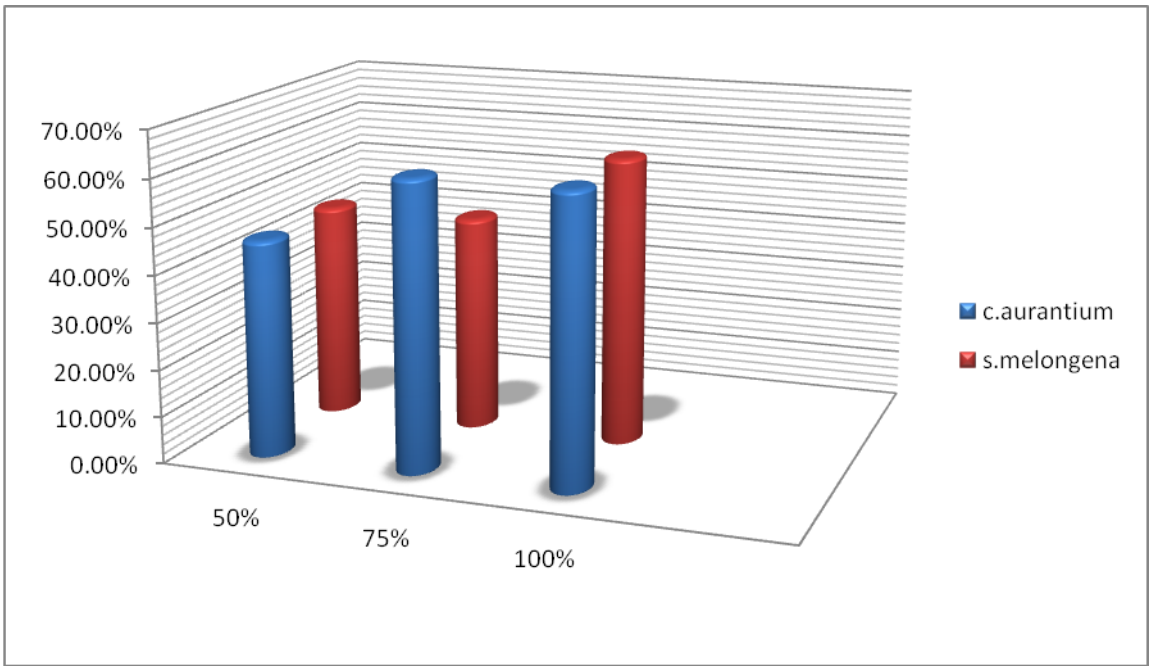

Form(2) percentage of *Giardia lamblia* aqueous extracts from each plant(bitter orange, eggplant).

Results determine the medium lethal dose of aqueous extracts of the plant cold *citrus aurantium* , *solanuommelongena*
The phenomenon of changes in the behavior of mice and calculate the value of the LD50

The results of the current study table (5) and Table (6) to determine the lethal dose for half the preparation of rat LD50 due to the toxic effects of aqueous extracts of the peel of *citrus aurantium* and peel *solanuommelongena*and observed visually on the behavior of mice if represented a lack of appetite Anorexia and lack of activity Hypo activity and weakness Kinetic Motor Weakness increases these symptoms unit with increasing dose while this was a few changes were identified in the early hours of the dosage oral delivery of low-lying, if you reach a maximum at 10 dose)) g / kg of body weight, which led to the deaths of some animals, which amounted to (1.2, 5) respectively during the next twenty-four hours to extract dosage was preparing eggplant and dead animals (1,1,5) of *citrus aurantium* extract.

Table (5) half way lethal dose of aqueous extracts of bitter orange coldcalculation

concretion	doses	N	a	B	Now+next/2b	ab
control	-	5	-	-	-	-
T1 %25	2.5	5	-	-	-	-
T2 % 50	5	5	2.5	1	0.5	1.25
75%T3	7.5	5	2.5	1	1	2. 5
100 %T4	10	5	2.5	5	3	7.5
					Total	**11.2 5**

$$\frac{\sum axb}{n}$$ highest dose- =LD50
= 10 - 11.25 / 5= 7.75 g / kg of body weight.

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Table (6): halfway lethal dose of aqueous extract sof*solanuomme longena* cold calculation

concretion	doses	N	A	b	Now+ next/2b	ab
control	-	5	-	-	-	-
T1 %25	2.5	5	-	-	-	-
T2 % 50	5	5	2.5	1	0.5	1.25
75%T3	7.5	5	2.5	2	1.5	3.75
100 %T4	10	5	2.5	5	3.5	8.75
					Total	**13.7 5**

$$\frac{\sum axb}{n}$$

highestdose-=LD_{50}
= 10 - 13.7 5 / 5= 7.2 5 g / kg of body weight.

DISCUSSION

Culture of *G. lamblia* In Vitro

The table shows (5-4) the results of the implant in the TYI-S-33 adapting medium growth trophozite stage it for up to tenth day and cyst stage remains for the seventh day of the implant, an outcome approach to the findings of the Kubaisi (2007) ,(24) and reached Ghanimi, (2013) ,(25) who were able development of the parasite for a period of (11) days and this is not consistent with what his record Clark & Diamond (2002) ,(26) where Benoit that it is possible to keep the parasite alive for an indefinite period, provided supply center-nutrient continuously . And explains this use of sterilization technology by filtration Milipore filter, and that does not lead to damage the center components if the method was used Autoclave some of the material contained within the culture medium components it is possible that it nutrients may damage the high temperature autoclaves (121 m and 15 Joe) leading to a lack of continued optimal growth as well as the containment of the center on (L- cysteine), which helps the parasite in the adhesion process and provides Oxidative Lowland, addition it increases the effectiveness and vitality phase activist during growth (27). as well as the secondary transfer of isolates and to depletion of nutrients and the accumulation of metabolic products of secondary and that may lead to influence The parasite growth (28).

When you put the parasite in distilled water, the duration of survival and one day to the process of vegetative and five days for the process cyst stage and that the lack of nutrients necessary for the growth of the parasite and the accumulation of secondary materials metabolic which is toxic and then fading due to the depletion of the stocks of foodstuffs cyst stage(26).

Culture of *E. histolytica*In Vitro

The results of the transplant showed in the beef liver infusion extract medium appearance of the first growth of the parasite after the third day of the transplant and the increasing growth significantly when transferring cultured to his second Sub culture planting, and proved that the center of success in the parasite cultivation indicating that a good compromise for the attributes of the elements food serving parasite growth, but trophozoit stage it does not last more than eight days and cyst stage remain for a period of 15 a day, a result comparable to the findings of the (29), which was able to parasite development for a period of 10 days using the calf fetal serum in the preparation of the culture medium (Semi solid medium for parasitic Amoebae) , As well as close to what is the result reached by the(24), which used plasma horse in the preparation of the same center-user, while in the current study used serumMan is the result of converging the findings of (30) .also shows(31) the possibility of parasite development amoebic dysentery for ten days at this center. This is not consistent with what his record (26) and (32),where They pointed to the possibility of keeping the parasite alive for an indefinite period, provided nutrient supply culture medium on an ongoing basis. The reason not to stay alive for a long time may be due to the sensitivity of the parasite to some of the surrounding as light environmental conditions, and the continued lack of necessary for growth and reproduction such as, metals,

starch as a result consumed by a parasite key components, as well as the metabolic products of the parasite affects the pH of the medium and change becomes inappropriate for the child's and stay alive longer in the middle That was its development(29).It can als obe affected by the parasite during his transfer from the middle initial to the secondary center being exposed to oxygen and dieorcystion, or the presence of reviving microscopic secondary resistance to antibiotics, which can contribute too vercome the parasite; given by the effects of the outputs of metabolic toxicity of the central leading to alack of sustaining the center for long periods(28).

effectiveness of cold aqueous extracts of peel for each plant *citrus aurantium*, *solanuomme longena* against *Giardia lamblia* and *E. histolytica* outside vivo

Results showed cold aqueous extracts of both plant(bitter orange, eggplant) effective in killing all of the parasite *Giardia lamblia* and parasitic *E. histolytica* where he showed the concentration of100%betterin the killer of each of the parasitic *E. histolytica* increased by86.2% to extract orange, 51.7% of the extract of bitter orange, 68.9% of eggplant extract. As a parasite *Giardia lamblia ,* show 100% concentrationby61.5% to orange extract, 61.53% of the bitter orange extract, 61.5% of the extract eggplant .And conform to this result with what exists (33). The mechanism, which is due being responsible for the toxicity of phenols of microorganisms include inhibition of enzymes by oxidizing compounds (Oxidizing compound) possibly by interacting with specific groups or by random interaction with proteins (34). The flavonoids, the work includes the ability to overlap with protein membrane and overlap with the walls of the bacterial cell as it is most familiar of fat has been working to tear up the cell membranes of microorganisms (35).According to Chi-square test concentrations for each moral differences may be due to a difference insolubility of the extract and the different microbiology resistance and the results of this research and found that the water plant extracts used her effective in eliminating the parasite *Giardia lamblia* and *E. histolytica*, From the results of this research and found The aqueous extracts effective in eliminating the parasite *Giardia lamblia* parasite and *E. histolytica*.

Results determine the medium lethal doseLD50 of aqueous extracts of the plant cold *citrus aurantium , solanuomme longena*

It has been studied fatal half the dose for the purpose of knowledge of toxic effects by measuring the LD50 It is known that some plants compounds with toxicity such as plant bitter orange, eggplant effects have been observed to influence the toxic after dosage mice orally and has animal control for 24 hours, where it was noted the deaths of animals at dose (7.25 g / kg) to extract eggplant .at dose (7.75 g / kg) of bitter orange extract. And of which it is clear that the impact of extracts toxic effect on the white mice used in the experiment of bitter orange peel vegetarian eggplant (36) as these extracts affect the analysis of red blood cells resulting in the death of the animals.

Conclusions

- parasites development efficiency outside the body of the organism , where the effectiveness of the (beef Liver infusion Agar) in the growth of the parasite *Entamoeba histolytica* and effectiveness of TY1-S-33 adapting medium in the development of parasite *Giardia lamblia* .
- showed cold water extracts from each plant peel (bitter orange , eggplant) effective in the elimination of all of the parasite *Giardia lamblia* and *Entamoeba histolytica* outside the body of the organism**.**

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