

**IN VITRO STUDY OF ENTAMOEBA HISTOLYTICA CAUSATIVE AGENT OF AMOEBIASIS WITH LEMON JUICE AT DIFFERENT CONCENTRATION SHOWED ANTIAMOEBIC PROPERTIES**Shrivastava Bhanu<sup>1\*</sup>, Shrivastava Vandana<sup>2</sup>, Shrivastava Archana<sup>3</sup><sup>1</sup>Deptt. of Microbiology, IASCA ITM University Gwalior M.P, India<sup>2</sup>Faculty of life Sciences in microbial biotech Research and Training centre Gwalior, India<sup>3</sup>Head of Department of Engineering chemistry, Gwalior Institute of Information Technology (GIIT) Gwalior M.P, India

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**ABSTRACT**

A species of parasite protozoa *Entamoeba histolytica* causing amoebiasis and amoebic dysentery characteristic include a single nucleus containing a small central karyosome and peripheral chromatin that is finely and regularly beaded. This is a single celled parasitic animal, that infects predominantly humans and other primates. Amoebic infection was first described by Fedor Losch in 1875 in St. Petersburg. In 1890, Sir William Osler reported the first North American case of amoebiasis when he observed amoebae in stool in abscess fluid from physician who previously resided in Panama. So we used the Lemon juice (Citrus) at different concentration against *Entamoeba histolytica* to treat the amoebiasis disease. *Entamoeba histolytica* shows the variety of growth due to the effect of Lemon juice (Citrus). Lemon juice is ingredient and it has anti amoebic properties against *Entamoeba histolytica* a causative agent of amoebiasis.

**Keywords:** *Entamoeba histolytica*, Lemon juice, amoebiasis, Citrus

**INTRODUCTION**

The species name *Entamoeba histolytica* was first coined by Firtz Schaudin in 1903. In 1913, in the Philippines, Walker and Sellards documented the cysts as the infective form of *Entamoeba histolytica*. The life cycle was then established Dobell in 1925.

Although most cases of amoebiasis are asymptomatic, dysentery and invasive extraintestinal disease can occur. Amoebic liver abscess is the most common manifestation of invasive amoebiasis, but other organ can also be involved, including pleuropulmonary, cardiac, renal, genitourinary and cutaneous sites. In developed countries, amoebiasis primarily affects migrants from and travelers to endemic regions, men who have sex with men and immunosuppressed or institutionalized individuals. Amoebiasis is second only to malaria in terms protozoa associated mortality. The combined prevalence of a amoebiasis liver abscess is estimated at 40-50 million cases annually worldwide resulting in 40,000-100,000 deaths<sup>7</sup>. Asymptomatic intestinal amoebiasis occurs in 90% of infected individuals. However, only 4-10% of individuals with asymptomatic amoebiasis who were monitored for one year eventually developed colitis or extraintestinal diseases<sup>18</sup>. In Japan and Taiwan, HIV seropositivity is a risk factor for invasive extraintestinal amoebiasis. This has not been observed elsewhere<sup>19</sup>. *Entamoeba histolytica* is transmitted via ingestion of the cystic form of the protozoa. Viable in the environment for weeks to months, cysts can be found in fecally contaminated soil, fertilizers or water or on the contaminated hands of food handlers. Fecal oral transmission can also occur in the setting of anal sexual practices or direct renal inoculation through colonic irrigation devices. The trophozoites can penetrate and include the colonic mucosal barrier, leading to tissue destruction and secretory bloody diarrhea and colitis resembling inflammatory bowel disease. In addition, the trophozoites can spread hematogenously via the portal circulation to the liver or even to more distant organs.

Metronidazole is widely used for treating amoebiasis but it has some side effects. This study based on butter milk used as a anti amoebic agent because it shows the physico chemical properties against amoebic infection. Buttermilk is a dairy product. Buttermilk is sweet in taste and their compositional and functional properties were determined by the different pH level<sup>21</sup>. Amoebiasis leads the major health problem in India and worldwide. About 15% of indian population are suffering from amoebiasis reported through India. In semi urban community in Delhi, the diagnostic method for amoebiasis was studied. 198 samples was infected by various

parasites in which preliminary samples<sup>24</sup>. *Entamoeba histolytica* associated with high morbidity and mortality to a major health problem throughout the world.

**MATERIALS AND METHODS****MATERIALS**

Direct microscopy for intestinal amoebiasis (from stool sample)

1. Stool sample
2. Centrifuge
3. Formal saline
4. Ether
5. Iodine
6. Distilled water

**METHODS**

The stool sample was taken and mixed thoroughly take 2 ml stool and dilutes it in 10 ml distilled water centrifuge and mix for 5 minutes at 300 rpm. Discarded the supernatant and take the pellet. Apart of pellet was use for acid fast staining in remaining pellet acid 5 ml 10% formal solution in pellet followed by 3ml of ether. Centrifuge at 300 rpm for 5 minute discarded supernatant and take the pellet and mix and make a slide and see it under microscope.

Cultivation of *E. histolytica* or culture method or NIH methods use the NIH media and ringer's solution as the material for (NIH media).

Fresh egg fluid 270 ml.

Ringer's solution 70 ml mix thoroughly, distribute 5-6 amount coagulate ringer's solution.

1. sodium chloride (NaCl) 8g/l
2. Calcium chloride (CaCl<sub>2</sub>) 2g/l
3. Potassium chloride (KCl) 0.2 g/ml
4. Distilled water 1000 ml

**Method**

The egg brake aseptically and collect the fluid in sterile 500ml flask containing glass beads. Bead the fluid mix yolk, albumin, filter through gauze and measure add the required amount for ringer solution and mix again now distribute 5 to 7 ml amount in screw cap bottle, inspissate in 850 g and coagulate in slanting position. Cool and overlay the silent with lock solution and then autoclave at 15 lbs. presser for 15 min all the work must be done with aseptic condition.

**Lock's solution formula**

Sodium chloride (NaCl) 8.00g  
Calcium chloride (CaCl<sub>2</sub>) 0.2g  
Potassium chloride (KCl) 0.2 g

Disodium hydrogen phosphate 2.0 g  
Magnesium chloride (MgCl<sub>2</sub>) 0.01 g  
Sodium bicarbonate (NaCO<sub>3</sub>) 0.4g  
Potassium dihydrogen phosphate 0.3g  
Distilled water 1000 ml  
pH range 7.1

Dissolve, autoclave at 15 lbs for 15 minutes than wile using adjust the reaction at pH 7.1 with N/10 HCl

#### Culture

About owe the inoculums from a rich culture showing 40-50 amoebae low pressure filled of microscope is put in the fresh medium bottles. A loopful sterile rich starch is also put in addition to penicillin (1000 per unit) of ever and addition of antiseptic culture bottle is incubated at 37<sup>0</sup>C and observed 24 hour subculture is done after an hrs inoculation.

#### OBSERVATION

Our worked by the observation tables

Present study showed following results by the observation table:

Table 1 showed the different growth of *Entamoeba histolytica* at 0.01 ml concentration of Lemon juice (Citrus) at different time on 24 hrs., 48 hrs., 72 hrs., after 24 hrs.,48hrs and 72 hrs. Showed heavy growth of culture. Figure.1

Table 2 showed the different growth of *Entamoeba histolytica* at 0.02 ml concentration of Lemon juice (Citrus) at different time on 24 hrs., 48 hrs., 72 hrs., after 24 hrs.,48hrs. Showed heavy growth of culture. While in 72 hrs showed growth. Figure.2

Table 3 showed the different growth of *Entamoeba histolytica* at 0.03 ml concentration of Lemon juice (Citrus) at different time on 24 hrs. showed growth while no growth observe at 48 hrs. 72 hrs. in culture.

Table 4 showed the different growth of *Entamoeba histolytica* at 0.04 ml concentration of Lemon juice (Citrus) at different time 24 hrs. showed low growth while no growth observe at 48 hrs. 72 hrs. in culture.

Table 5,6& 7 showed, no growth of culture observed at 0.05 ml up to 1ml concentration of Lemon juice (Citrus) at different time. Figure.3

#### RESULT AND DISCUSSION

Our worked on the Lemon Juice (Citrus) against the *Entamoeba histolytica* and they found Lemon Juice (Citrus) showed antiamoebic properties at due to its physico chemical properties. They found positive result against the amoebiasis, worked on the new effective agent metronidazole thiosemicarbazone against the *Entamoeba histolytica*. They found sensitivity of *Entamoeba histolytica* was evaluated<sup>1,20</sup> used some traditional plant for cure of amoebiasis. This study provides various between 1982 and 1999, on plant with antiamoebic activities and those which possess both antiamoebic & antiplasmodial activity. The results suggest that it is advisable to increase efforts towards the conservation of plants, in order to retain their economic and therapeutic significance.<sup>12</sup> Study based on a crude drug formulation. They used five medicinal herbs, like Boerhavia diffusa, Berberis aristata, Tinospora cordifolia, Terminalla chebula & Zingiber officinale. They used the plant extract. Their work on the MIC value of plant extract. They found that MIC is effective 1000 micro organism/ml compared with 10 organism/ ml for metronidazole.

So we used the Lemon juice (Citrus) at different concentration against *Entamoeba histolytica* to treat the amoebiasis disease. *Entamoeba histolytica* shows the variety of growth due to the effect of Lemon juice (Citrus). Lemon juice is ingredient and it has antiamoebic properties against *Entamoeba histolytica* a causative agent of amoebiasis.

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Table 1 Lemon Juice( Citrus ) used in 0.01 ml for *Entamoeba histolytica*

Time (Hours)	Growth of <i>Entamoeba histolytica</i>	
24hrs.	+++	Heavy Growth
48hrs.	+++	Heavy Growth
72hrs.	++	Growth

Table 2 Lemon Juice( Citrus ) used in 0.02 ml for *Entamoeba histolytica*

Time (Hours)	Growth of <i>Entamoeba histolytica</i>	
24hrs.	+++	Heavy Growth
48hrs.	+++	Heavy Growth
72hrs.	++	Growth

**Table 3** Lemon Juice( Citrus ) used in 0.03 ml for *Entamoeba histolytica*

Time (Hours)	Growth of <i>Entamoeba histolytica</i>	
24hrs.	++	Growth
48hrs.	-	No Growth
72hrs.	-	No Growth

**Table 4** Lemon Juice( Citrus ) used in 0.04 ml for *Entamoeba histolytica*

Time (Hours)	Growth of <i>Entamoeba histolytica</i>	
24hrs.	+	Low Growth
48hrs.	-	No Growth
72hrs.	-	No Growth

**Table 5** Lemon Juice (Citrus ) used in 0.05 ml for *Entamoeba histolytica*

Time (Hours)	Growth of <i>Entamoeba histolytica</i>	
24hrs.	-	No Growth
48hrs.	-	No Growth
72hrs.	-	No Growth

**Table 6** Lemon Juice (Citrus ) used in 0.06 ml upto 0.9ml for *Entamoeba histolytica*

Time (Hours)	Growth of <i>Entamoeba histolytica</i>	
24hrs.	-	No Growth
48hrs.	-	No Growth
72hrs.	-	No Growth

**Table 7** Lemon Juice (Citrus ) used in 1ml for *Entamoeba histolytica*

Time (Hours)	Growth of <i>Entamoeba histolytica</i>	
24hrs.	-	No Growth
48hrs.	-	No Growth
72hrs.	-	No Growth

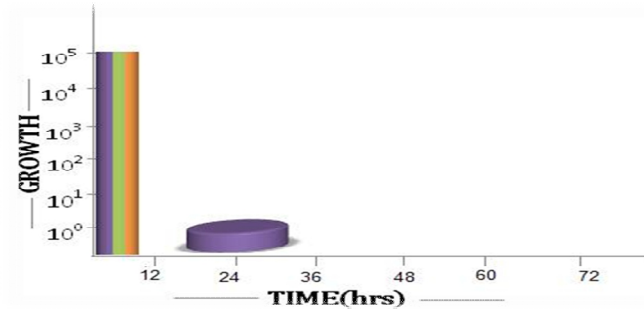


Figure 1 Show table 1 results ( Graph plotted between Cell growth and Time )

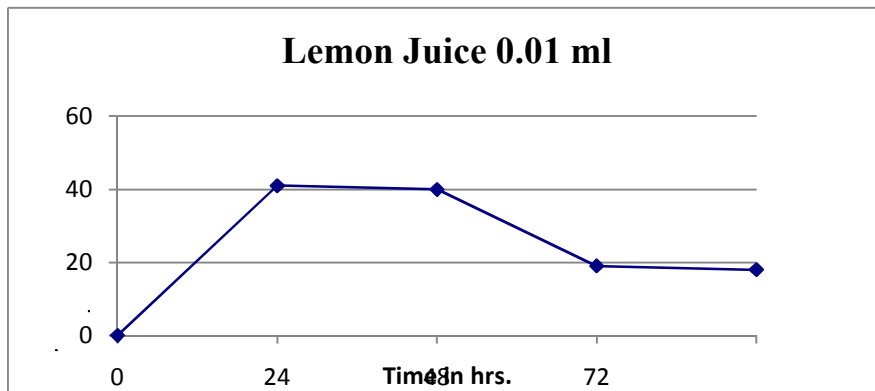


Figure 2 Show table 3 results ( Graph plotted between Cell growth and Time )

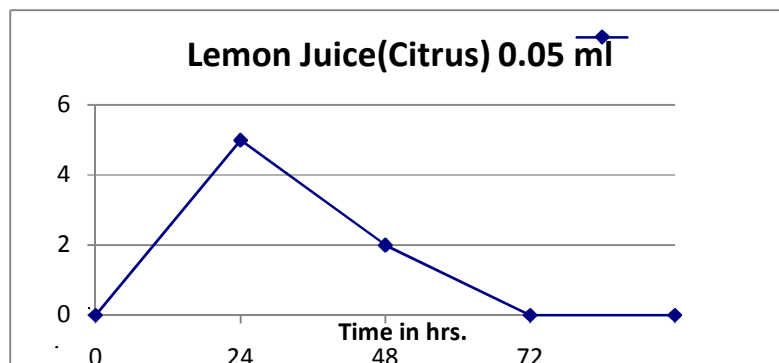


Figure 3 Show table 5,6,7 results ( Graph plotted between Cell growth and Time )

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