

# **Biochemical Study for Evaluated Three Culture Media to Cultivation *Neagleria fowleri***

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**ABSTRACT:** Three new culture media were prepared from aqueous extract of *Fragaria ananassa* fruit, *Helianthus tuberosus* tubers and sheep testes, experimented their efficiency in isolation and growth of *Neagleria fowleri*. The results showed variation in the numbers of trophozoites and cysts of this parasite on the three culture media, ahead sheep testes extract medium in increasing growth rates during the incubation period as it was reached in the fourth day to 2925 amoeba/ml and then followed by the *Fragaria ananassa* fruit extract medium reached the parasite numbers to 2375 amoeba/ml, and the *Helianthus tuberosus* tubers extract medium which appeared less efficient in the growth rates where the numbers of parasite reach to 1875 amoeba/ml. Results of chemical analysis suggested highest content of moisture percentage in the fruit of *Fragaria ananassa* with 46%. Highest percentage of carbohydrates was found in tubers of *Helianthus tuberosus* 86.3% and outperformed sheep testes in the percentages of oils 30%, carbon 16%, fiber 12%, nitrogen 8.75% and proteins 54%.

**KEYWORDS:** biochemical- efficiency, new culture media, *Neagleria fowleri*

## **I. INTRODUCTION**

The study of microbiology has begun including parasites detailed in a scientific manner after World War II, referred [1] to industrial and commercial development has helped in the transmission and spread of these microorganisms to places far apart from the world. The most of studies on parasite cultivation and development specially *Neagleria fowleri* required to provide different laboratory cultivation environments this of environments that were not completely identical with the environments in which they live such as pool, fresh water, sewage, sludge or by these organisms in nature or within the human body example in central nervous system, but its close resemblance them closer may provide needs and nutrition requirements for cultivation [2]. The basic components of any culture medium conveniently carbonaceous and nitrogenous source, water and solidifying materials such as Agar, depending on structures of culture media divided into several types, including living culture media. They consist of plant tissues such as water agar or animal tissues like fetal calf serum medium [3]. In parallel to the study of microbiology flourished and spread the cultivation of plants and herbs in most parts of the earth and the varied uses either used in whole plants, single parts, in powders or extracts as its formation these plants are distinctive and significant position in the world's agricultural production [4]. Including these plants ***Fragaria ananassa*** and ***Helianthus tuberosus*** which contains natural chemical substances that are of great importance in the physiological and medical effects the possibility of use as living culture media for cultivation various microorganisms. Also these two plants consider one of the most important food plants edible in different parts of the world, including United States, Japan, Spain, Turkey, European states, Egypt and Iraq [5,6,7]. Meat of sheep being rich in proteins and amino acids, vitamins and elements such as nitrogen, carbon and potassium beside of they contain fat, salt and fiber [8] (Oler *et al*, 2015).

In Iran [9] isolation and identification free living amoeba *Neagleria* and *Acanthamoeba* by use (NNA) non-nutrient agar that has been seeded with lawn of *Escherichia coli* (Amoeba food source) but the results show the medium non-selective for *Neagleria fowleri*. The study of [10] using the medium of soil for the primary isolate, but it shows a high rate of pollution in the medium because it is used to isolate the most other organisms such as fungi, bacteria and actinomycetes. But the limited studies that deal with cultivation pathogenic amoeba *Neagleria fowleri* on living culture media there for **aims** this study uses tissue extracts for ***Fragaria ananassa***, ***Helianthus tuberosus*** and testes of sheep due to the higher chemical contents. So we decided to use extracts as alternatives ordinary culture for support, cultivation and development of pathogenic amoeba as well as being among the cheap and locally available.

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## II.MATERIALS AND METHODS

### 2.1 preparation study samples extracts

Collected samples (**Fragaria ananassa fruit, Helianthus tuberosus tubers** and testes of sheep) from the local market, brought to the laboratory, washed and cut it weight 4 gm of fresh samples separately and then add each with distilled water put it in a blender for a quarter of an hour and then filtration it [11]

### 2.2 Prepare three culture media

were taken 100 ml of (**Fragaria ananassa fruit, Helianthus tuberosus tubers** and testes of sheep extracts each sample individually, add 2 gm agar sterilization media by Autoclave was pouring all it in the petri dishes, for cultivation the *Neagleria fowleri*

### 2.3 Prepare the water agar medium(control Medium)

weight of 5 gm of agar to 250 ml of distilled water sterilization by Autoclave was pouring in the petri dishes for cultivation the *Neagleria fowleri*

### 2.4 preparation solution page

Page solution prepared based on [12]

### 2.5 parasite inoculum preparation

collected parasite *Naegleria fowleri* containing phases in a sterile tube .put it in the centrifuge, repeated this process several times, collect sediment and added a 1 mL of sterile distilled water, after a good shake it took a drop of suspension so placed on a glass slide covered with a lid slide and examined using a light microscope to check for the presence of the parasite.so took 1 ml of this concentration with 4 ml of page liquid medium and cultivated on the four culture media calculated numbers of parasite after 24 hours [13]

### 2.6 Estimation the percentage of total nitrogen

It adopted the method [14] to estimate the percentage of nitrogen study samples where they were taking 0.2 gm weight of each sample is dried, placed in a beaker digest 100 mL, add a 5 mL of concentrated sulfuric acid and left samples overnight and then preheat the beaker digestion for half an hour until boiling then cold and add a 3 ml of the mixture of acid (4 ml per chloric acid concentrated +96 ml of concentrated sulfuric acid), then preheat the beaker until he became the solution clear, it finished size to 50 ml with distilled water after that as much as the total nitrogen in plant samples using Kjeldhal digestion techniques

### 2.7 Estimation the percentage of total proteins

The value is multiplied by the percentage of total nitrogen by a factor of 6.25 according to [15]

### 2.8 Estimation the percentage of total fat

taken every 5 gm of dry samples and add a 100 ml hexane in conical flask to extract oil, left 24 hours on the magnetic stirrer, then the sample was dried oil percentage calculation by the method [16]

### 2.9 Estimation the percentage of total fiber

8 gm were taken from each sample of the study samples of the oil after removing them and add them sulfuric acid, sodium hydroxide concentration of 1.25%, after washed it, dried in oven so weighed, then placed the dried materials in furnace to burn and calculate the percentage of fiber [16]

### 2.10 Estimation the percentage of total carbon

1 gm was taken from each samples and placed in furnace dry at a temperature of 550 ° C for 4 hours, then calculated the proportion of carbon depending on [17]

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### 2.11 Estimation the percentage of moisture

It was taken 3 g fresh samples of **Fragaria ananassa fruit, Helianthus tuberosus tubers** and testes of sheep and put in the oven at 65 degrees of heat until the drought was calculating the moisture ratio by the method [17]

### 2.12 Estimation the percentage of Carbohydrates

It is calculated as follows

$$\% \text{carbohydrates} = 100 - (\text{percentage of oils} + \text{percentage of Carbon} + \text{percentage of proteins}) \quad [17]$$

### III. Results

**Table( 1) test the direct effect of the sheep testes extract medium, Fragaria ananassa extract medium and Helianthus tuberosus extract medium on the growth rates of Naegleria fowleri in vitro**

Results suggested sheep testes extract medium has significantly increased in the preparation of parasite growth, which reached a maximum of 2925 amoeba/ml, followed by the Fragaria ananassa extract medium, which showed an increase in the preparation of the parasite reached 2375 amoeba/ml

While it was the medium of the least efficient Helianthus tuberosus extract medium which reached the numbers of the parasite in 1 ml to 1875 amoeba compared with the control medium(water agar)

**Table( 1) test the direct effect of the sheep testes extract medium, Fragaria ananassa extract medium and Helianthus tuberosus extract medium on the growth rates of Naegleria fowleri in vitro**

S	Time Culture media	Rates of amoeba numbers in 1 ml			
		24 hours	48 hours	72 hours	96 hours
1	sheep testes extract medium	2000	2325	2675	2925
2	Fragaria ananassa extract medium	1150	1375	2100	2375
3	Helianthus tuberosus extract medium	725	825	1475	1875
4	Control	450	350	925	1375

\*control=water agar

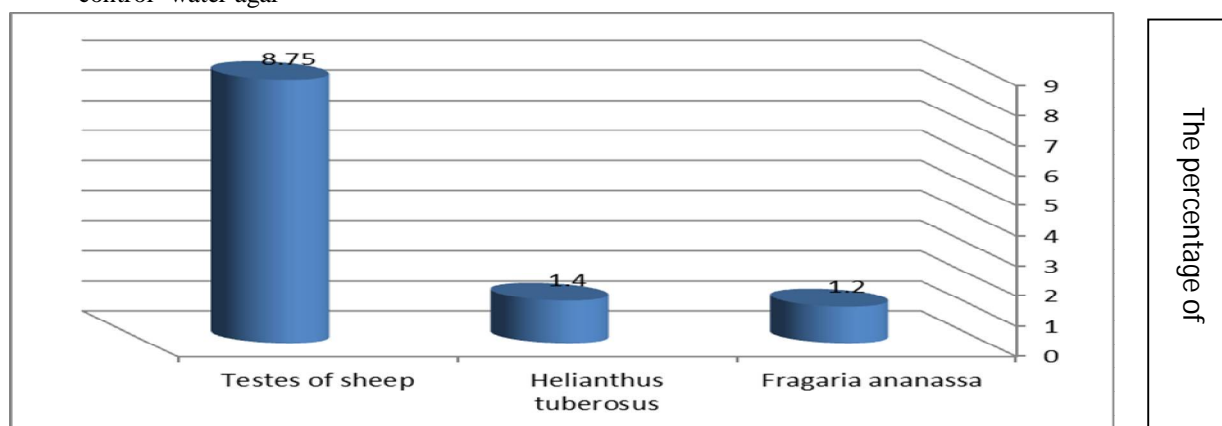


Figure (1) the percentage of nitrogen for the fruit of the **Fragaria ananassa** and tubers **Helianthus tuberosus** and sheep testes

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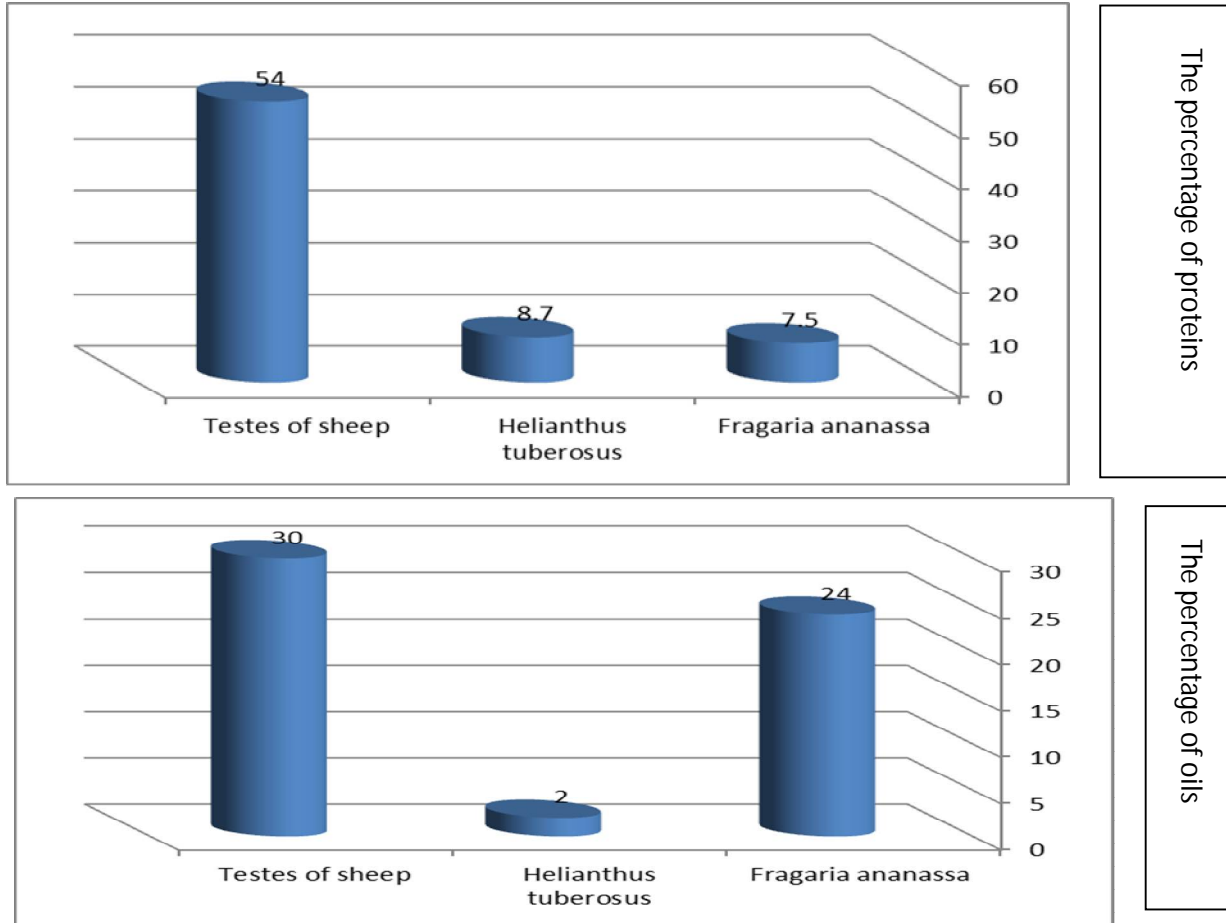


Figure (3) the percentage of oils for the fruit of the **Fragaria ananassa** and tubers **Helianthus tuberosus** and **sheep testes**

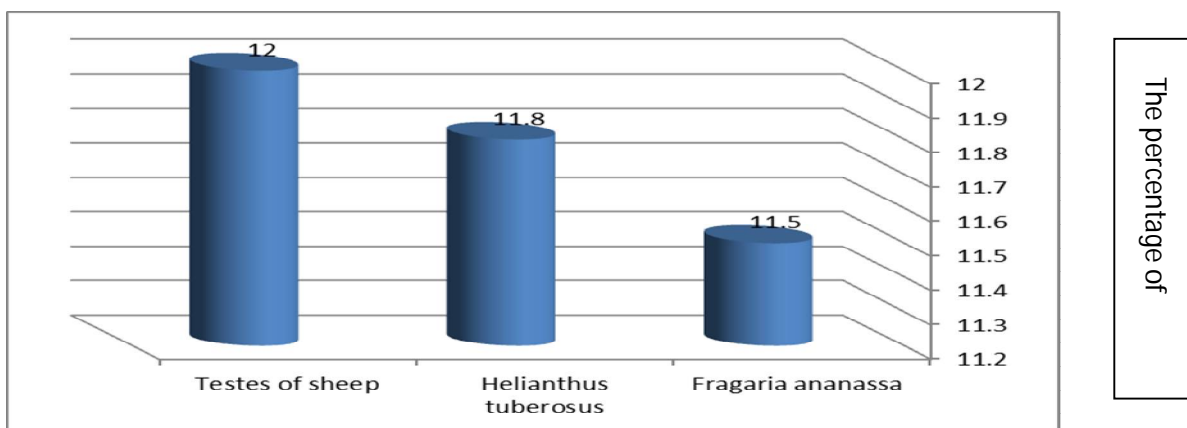


Figure (4) the percentage of fibers for the fruit of the **Fragaria ananassa** and tubers **Helianthus tuberosus** and **sheep testes**

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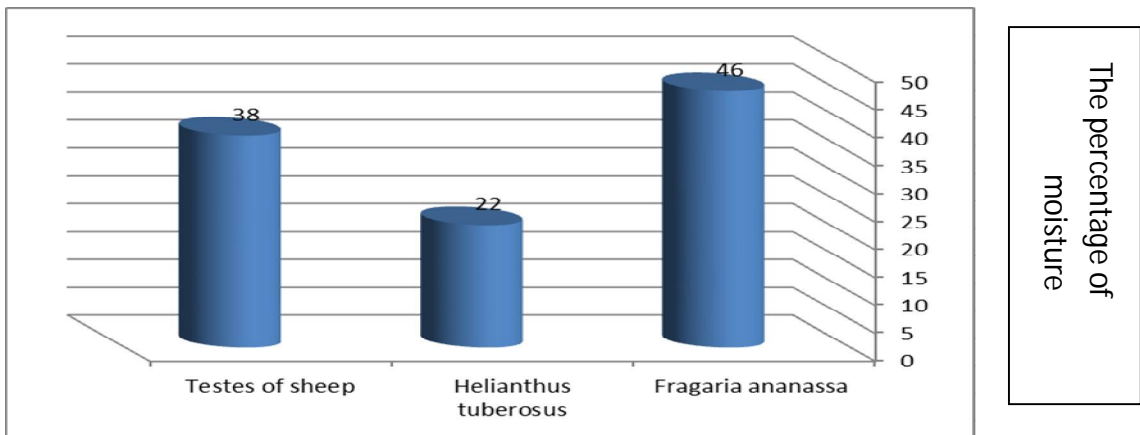
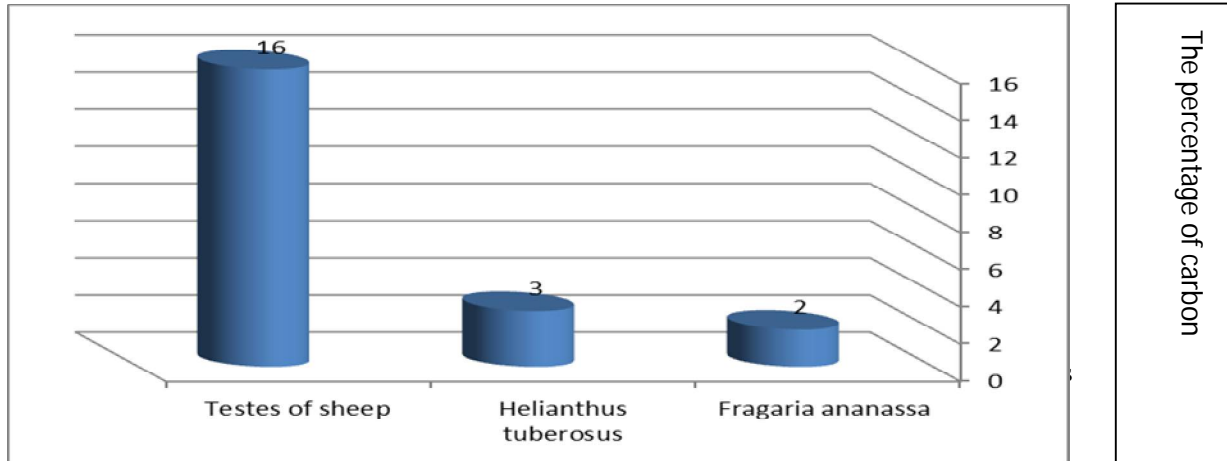
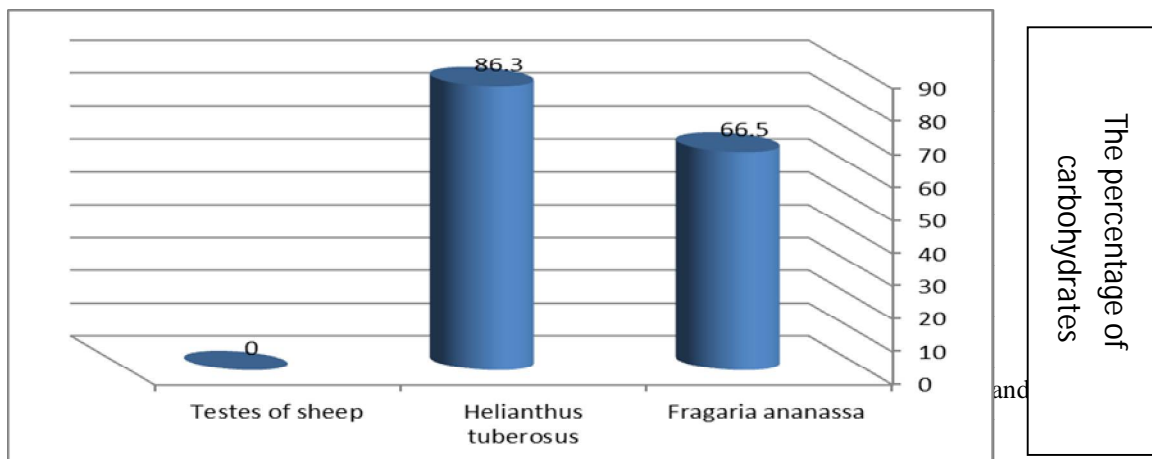


Figure (6) the percentage of moisture for the fruit of the **Fragaria ananassa** and tubers **Helianthus tuberosus** and **sheep testes**



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## IV.DISCUSSION

several previous studies performance for obtained on the suitable solid culture medium for the cultivation of the parasite *Naegleria fowleri* in vitro by preparing extracts of tissue plant and animal, such as the medium of the beef extracts, bovine serum, yeast extract and water agar but these media were not selective or supportive for the growth of the pathogenic parasite where it is among the ordinary media that use of a wide range of microorganisms, bacteria, fungi and actinomycetes so during the current study was to prepare three new solid culture media from plant sources and animal showed a highly efficient and degrees in isolation and cultivation of the parasite this is cooperate with a study[18] that Solid media better than the liquid in the parasite's cultivation.

The results showed a table (1) exceeds the medium of testes sheep in the cultivation and increase the number of parasite purity high and the reason may be due to higher chemical content of the testes necessary for the growth and reproduction of the *Naegleria fowleri*, as referred[8] to contain sheep meat amino acids, the content of protein high, other substances making it a suitable to isolate most of the parasites

[19], a study similar to the current study to isolate and purify some of bacteria, but researcher used the extra materials to support the medium of the testes, including the peptone, glucose, sodium chloride and dye promothymol blue Unlike the current study since been testes sheep without any additives Maybe it's because being a medium of animal tissue extract gives a large number of cells and the rapid advance of generations

Or maybe its nitrogenous content 8.75% and higher protein 54. As shown in the figure of (1.2) if refer to[20] The role of nitrogen in the composition of cell membranes, nucleic acids, proteins of the parasite, increases the speed of growth, vital processes and works parasite to produce an enzyme protease to break down proteins, leading to assemble amino acids, leading to a rapid growth of the parasite

Also plays a lipid Higher testes sheep, which reach to 30% as shown in figure (3) is very important the role of parasite cultivation and make medium testes sheep cultivation, development and support of the fact that the pathogenic *Naegleria fowleri* live in the brain Which has unique feature of myelin sheath is the large amount of specific lipids. Demyelination has been described in human primary amebic meningoencephalitis because the action of a specific phospholipolytic enzyme produce by *Naegleria fowleri*. perhaps lipids are nutritional substrates for *Naegleria fowleri* on they act as binding or receptors sites for toxins or enzymes produced by amoeba.

Since amoeba grew better with lipids present in the medium.

[21]

Also the medium of the testes content of carbon-16% and higher fiber 12% as shown in Figure(4.5) as it is a carbon-source in the culture medium of the most important elements necessary for the growth of the *Naegleria fowleri*, it is known that the protoplasm, enzymes, cell wall and food stored are considered carbon structures [20]

It was observed that the medium of the fruits **Fragaria ananassa** extract second place in efficiency of isolation and identification of this parasite the reason is due to the water content higher this figure (6) and he needs the *Naegleria fowleri* to grow, to complete its metabolic and his movement.

Where [22] study on the isolation of different types of protozoa such as amoeba from fruits **Fragaria ananassa**, which cooperate with the current study, the fact that **Fragaria ananassa** encouraging medium for the cultivation of pathogenic parasites

The medium of **Helianthus tuberosus** came third place was the reason is due to the different parasites in the possibility of the use of carbon sources, whether simple or complex Because carbohydrates **Helianthus tuberosus** high proportion reached 86% as shown in figure (7) compared testes sheep medium and **Fragaria ananassa** may be glucose concentration increased growth, increased parasite that up to focus optimization which ... then we find that the increase in the concentration of sugars is working to increase the pressure osmosis where the central pressure is higher than the cell pressure so it will be water exit rate faster than enter them leading to protoplasm drying occurrence cell plasmolysis [20]

[23] also a study on the preparation of the medium tubers **Helianthus tuberosus** extract with the addition of monosaccharide to support the growth of bacteria as the medium tubers **Helianthus tuberosus** extract was not suitable because of the complex sugars that the parasite could not be used it is not having to enzymes suitable for the consumption of these sugars



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## VI. CONCLUSION

Therefore, we conclusion from the current study that living culture media from animal sources were more efficient than the living culture media from plant sources to isolate the parasite *Naegleria fowleri*

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