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Research Article

Antibacterial and Antiparasitic Biosurfactants Produced by Candida Cruzi in Different Nitrogen and Carbon Sources

AHMED ABD BURGHAL*[1], WIJDAN HUSSEIN AL TIMIMI[2] AND ATHRAA ABD-ULAMEER AL-HILFI[3]
[1]Biology Department, College of Science University of Basrah, Basrah, Iraq.
[2]Biology Department, College of Science University of Basrah, Basrah, Iraq.
[3]Biology Department, College of Science University of Basrah, Basrah, Iraq.
*Corresponding Author
Email ID: ahmed.burghal@uobasrah.edu.iq
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ABSTRACT
One of the most remarkable products that have important in the medical application is biosurfactants. In the present study biosurfactants produce by Candida cruzi partially purified. The result of Infra-Red Spectroscopy showed the presence of different chemical groups. The antibacterial activity of optimized biosurfactants was performed with different types of Gram-positive and negative clinical isolates. The biosurfactants produce from media supplied with glucose has the more potent inhibitory activity against all isolates, the maximum inhibition zone diameter was 22mm on *E. coli* while nitrogen source the largest inhibition zone is 20mm when the produce of biosurfactants with asparagine and peptone of all isolates. The result of antiparasite against *Naegleria fowleri*, by biosurfactants suspension, showed a high decrease in numbers of parasites after 72h at 250µl (50% activity) of biosurfactants extracted from lactose media and (40% activity) from asparagine media at 500µl after 72h. The antioxidant show the biosurfactants from different source have an activity to inhibition of DPPH free radicals, the percentage of activity was 46.5% at 10mg/ml compared with ascorbic acid. The significant inhibitory effect of biosurfactants against microbes indicates the possibility of using it in the medical and pharmaceutical.

Keywords: Biosurfactants, Antibacterial, Antiparasitic, Antioxidant, Candida cruzi.

1. INTRODUCTION

Microorganisms produced Biosurfactants which are surface-active agents with lower toxicity, higher efficiency, stability, higher biocompatibility, and biodegradability than chemical surfactants [1]. Several biosurfactants in much biochemical application have potential alternatives to the conventional therapeutic agent because they exhibit antifungal, antiviral, antibacterial, and anti-tumor activities [2-3]. There is a high possibility that researchers in the pharmaceutical field could potentially use biosurfactants to treat respiratory infections as antiviral agents, especially towards COVID-19 [4]. Biosurfactants have significant medicinal uses because of their antimicrobial activity and low toxicity [5-6]. Van Bogaert et al. [7] showed that Sophorolipids synthesized in high concentrations by nonpathogenic yeasts. They are glycolipids contain a dimeric carbohydrate sophorose connected to a long-chain hydroxyl fatty acid via a glycosidic linkage [8]. Díaz De Rienzo et al. [9] established that sophorolipids are hopeful compounds as antibiofilms formed by bacteria [Gram-positive or Gram-negative). Even for *Bacillus* sp. P5 has shown the highest potential for surfactin production as antimicrobial agents [10], Lipopeptide compounds are amphiphilic biosurfactants produced by produced by Acinetobacter junii showing numerous biotic activities [11], Rhamnolipid from Pseudomonas species [12]. Biosurfactants may use as antiparasite, the supernatant culture of a surfactin produced by *B. subtilis* strain has the highest activity to an inhibiting the growth of the Plasmodium falciparum intra-erythrocytic [13]. Spores of Nosema ceranae causes several frequent parasitic infections in Apis mellifera, the study showed a clear reduction in the infection when the surfactin was added [14]. Free-living amoeba (FLA) is widespread in the environment, which is found in freshwater, saline, and soils, it can infect humans, animals, and parasites in tissues [15]. The amoeba of Naegleria fowleri infect the central nervous system and cause primary amoebic meningoencephalitis symptoms appear within seven days of exposure to the parasite and lead to death [16]. Intricate diseases such as atherosclerosis, stroke, diabetes, Alzheimer's are treated or prevention by Antioxidant-based drug [17] . Newly it has been clarifying that some of the biosurfactants are

efficacious antioxidants auspicious to be complete replacement or alternative for synthetic antioxidants [18]. This study aimed to estimate the biosurfactants produce from Candida cruzi as antibacterial, antiparasite, and antioxidant.

2. MATERIAL AND METHODS

2.1. Biosurfactants production

The *Candida cruzi* producing biosurfactant was isolated from produced water samples collected from the oil field in Basra, Iraq. The yeast was identified using Hicrome candida Differential Agar (Hi-Media Laboratories Pvt. Ltd., India) according to manufacturer instructions. Then screened to produce biosurfactants on Mineral Salt Media supplemented with 2% vegetable oil. Biosurfactants potential assay determined by using oil spreading method, emulsification activity and oil collapse. For optimization of the biosurfactant using the mineral salt medium supplemented separately with carbon sources (Glucose, Lactose, Fructose, and Dextrin] and nitrogen sources (Asparagine, L-Arginine, peptone, and Sodium nitrate) [19] . In this study, the free cell supernatants of each optimized culture was used for the antimicrobial and antiparasite assay.

2.2. Biosurfactants recovery

To partial purify of biosurfactants, the pH of supernatants was changed to 2 by added drops of 6 N HCl, mixture incubated at 4°C until the complete precipitation of the biosurfactants for 24h. The crude products were extraction with chloroform/methanol (2:1 v/v), after two layers of a mixture is formed, the organic layer was collected for dried at room temperature for 96h [20].

2.3. Fourier transform infrared spectroscopy

The functional groups of the partially purified product identified by (FT-IR) analysis in the Department of Chemistry /College of the Science / University of Basrah, using (KBr) pellets at a standard wavenumber (4000-400 cm^{-1}) [21].

2.4. Determination of antibacterial activity

Antibacterial activity of eight optimized crude biosurfactants (free cell supernatant) estimated against several bacteria isolated from pathogenic sources that included *Staphylococcus aureus*, *Bacillus cereus* (G+ve) and *E.coli, Pseudomonas aeruginosa* (G-ve). Agar well diffusion method was used after inoculated of bacterial isolates on Mueller Hinton agar Petri dishes by spreading at a concentration of 108 Colony Forming Unit (CFU)/ml (0.5 McFarland turbidity standards). The wells were punched over the agar plates using a sterile core borer with 6 mm in diameter, 50 μl of cell-free supernatant biosurfactant was dropped into wells and incubated overnight at 37 °C, the results were recorded by measured the inhibition zone (mm) [22].

2.5. Determination of antiparasite activity

The parasite *Naegleria fowleri* was isolated and identified in the parasitology Lab. in the Biology department in our college (University of Basrah). The parasite was activated on water agar, incubate at 35°C for 24h. The page solution was prepared according to [23]. Pure culture of parasites centrifugation at 4000 rpm for 10 min, the precipitate was resolved with 1ml of distilled water, shaking well, tested to ascertain the vital presence of parasite [24]. The medium was inoculated with 1ml of parasite suspension and added 2ml of page nutrient solution, the biosurfactant was added at [250 and 500 ml] both alone at three replicate for each group the survival number of the parasites was calculated after 24, 48 and 72h under Neubauer Haemocytometry [25].

2.6. DPPH assay:

Antioxidant activity of biosurfactant after partially purified was evaluated by the Inhibition of diphenyl-2-picrylhydrazyl (DPPH) radicals measured according to Goodarzi et al. [26]. The biosurfactant was prepared at following concentration (2, 4, 6, 8, 10) mg /ml, by dissolved in Dimethyl Sulfoxide (DMSO), 1ml of each concentration was added to 2ml of DPPH methanolic solution (40 mg/ml), agitated quickly, and left in the dark at 30min. The same procedure was used for methanol and DMSO as blank, while the standard solution used ascorbic acid. The Optical density (OD) of the solutions was determined at 517 nm by a spectrophotometer [27]. The percentage of DPPH inhibition calculated from the following equation: (%) inhibition = OD of DPPH – OD of the sample /OD of DPPH × 100.

IC_{50} of Antioxidant activity of each sample was then expressed as the half-maximal inhibitory concentration

3. RESULT AND DISCUSSION

3.1. FTIR analysis

FTIR analysis (Figure 1) shows present different groups, stretching amine (N-H) groups near 3800–3000 cm^{-1}. Stretching of alkane (C-H) at 2922–2671 cm^{-1}. Between 1741 and 1712 cm^{-1}, The existence of expanding peaks for ester groups(C=O), with the bands presumably present to the hydroxyl group (O-H) at 1367 cm^{-1}, while the band showed at 1645 cm^{-1} refers to the alkene (C=C) group, also the bands at 1165 and 1097 cm^{-1} refer to ester, while the bands present at 723 to 588 cm^{-1} detected as alkene. The functional groups of partially purified biosurfactant were evaluated by the FT-IR

spectrum. The results function groups depended of the FT-IR were commonly found in glycolipids Biosurfactant produced by yeasts [28]. They found absorption bands at 723, 1367, 1383, 2924, 2852, and 3007 cm^{-1} explained the aliphatic long fatty acid chain is the presence [29-30]. The other functional groups were presented in glycolipid biosurfactants by Singh and Tiwary [31].

3.2. Antibacterial activity of biosurfactants

In the last years, the used of biosurfactants in commercial application special medicine field have increased considerably. They used in treating many infections and as a therapeutic agent because of antimicrobial and antiadhesive properties [32]. Biosurfactants belong to Lipopeptide (LPBs) are broad range of molecules that showed antimicrobial activity for bacteria, fungi, viruses, and biofilm forming microorganisms [33]. The bioactivity of extraction biosurfactant evaluating against bacteria isolated from clinical sources shown in (Table 1 and Figure 2). The zone of inhibition obtained represents of biosurfactants are the antimicrobial agent. The results showed a high effect for all bacteria this was agreed with Garg et al. [34] produce biosurfactant has significant antibacterial activity against pathogenic *Escherichia coli* and *Staphylococcus aureus* strain. Poomtien et al. [35] showed that biosurfactant produce by *Candida mucifera* NJP25 had a maximum effect against different species of bacteria.

The results showed that all crud biosurfactants (free cell supernatant) of different carbon and nitrogen sources gave close results the good inhibitory activity was against the all isolate by the free cell supernatant produce with glucose, the maximum effect was on bacteria of *E. coli* 22mm while the lowest was 15mm with free cell supernatant produce with dextrin this results were confirmed with Samadi et al. [36]. Das et al. [37] demonstrated that the biosurfactant's activity and the structure altered with the culture medium's composition, in contrast to one substance in the culture medium, the biosurfactant produced by *Bacillus circulans* using culture media with more than one carbon source demonstrated high antimicrobial activity.

For nitrogen sources, the inhibitory zones were found to be the largest 20 mm in the case of produce free cell supernatant with asparagine and peptone for all isolates. This was agreed with Kiran et al. [38]. That nitrogen source as acrylamide increases the creation of biosurfactants significantly followed by beef extract as an alternate nitrogen source. The inhibition zone of lowest activity was observed in free cell supernatant with L- Arginine in all isolate of bacteria range 16–15 mm. The biosurfactants have the adhering property to the cell surfaces led to a deterioration in the integrity of cell membranes and breakdown in the nutritional cycle, this one explanation of the antibacterial effect of biosurfactants. This feature led to a change in the properties of cell membranes and their nutritional functions, it works as an antibacterial agent [39]. Sambanthamoorthy [40] showed that the biosurfactants produced by *Candida sphaerica* have a potential activity of antimicrobial with concentrations less than 10 mg/l when used against *S. aureus* and *C. albicans*. Cameotra and Makkar [41] reported that change of membrane arrangement due to the interaction of biosurfactants with membrane proteins as well as phospholipids. The increase in permeability of membrane is induced by interaction between Lipopeptides biosurfactants and the cell membrane [42]. This finding was reported by Rufino et al. [43] that *Candida lipolytica* UCP0988 produces biosurfactant ruffian which has antibacterial potential.

3.3. Antiparasite activity

The current study demonstrated of free cell supernatants on the *Naegleria fowleri* parasite which caused a significant decrease in the number of parasites compared to control, the decrease in numbers were found to be largest in biosurfactant producing with lactose carbon source from 2500 to 1250 in Mm^3 after 72h at 250µl the efficiently killing of 50%, while with nitrogen sources the highest effect was in case of produce biosurfactants in asparagine media, the number decreased from 6620 to 4000 in Mm^3 after 72h the efficiently killing of 40% (Table 2 and Figure 3). The numbers of parasites were decreased from 2000 to 1000 in Mm^3 after 72h the efficiently killing of 50% at 500µl of biosurfactant extracted from lactose media, on the other hand, fewer activities appeared by biosurfactant from nitrogen source media, it was the highest decrease from 5870 to 3620 in Mm3 after 72h the efficiently killing of 39% (Table 3 and Figure 4). The decrease in number may be due to the effect of biosurfactant on cell proteins thus destroying the cellular structure of the cell and the death of the parasite. The studies for biosurfactant production from microorganisms such as Candida are very littlest, once a study by Al-Jubury [44] isolated Biosurfactant from *Pseudomonas* has a potential effect as antiparasitic especially for ciliate *Ichthyophthirius multifiliis* which are fish pathogenic.

3.4 DPPH assay:

A realistic and accurate strategy for evaluating radical scavenging action is the DPPH method, which is focused on the capacity of DPPH to decolorize in the presence of antioxidants.

Ascorbic acid was selected as the standard antioxidant. In the DPPH assay, an antioxidant acts as a hydrogen donor, therefore, reduces DPPH free radicals (the color transform from purple to yellow). The partially purified biosurfactants produce by candida cruzi showed the highest Scavenging of DPPH radical was observed 46.5% at 10mg compared with ascorbic acid 90.9% (Fig. 5). The result of Scavenging activity was found to decrease with decreased concentration of biosurfactants. The lowest activity of biosurfactants as an antioxidant was 10% at 2mg as opposed to the value of ascorbic acid 88.2%. This agreed with Ribeiro et al. [45] who noticed the antioxidant efficacy of the biosurfactants produced from *Saccharomyces cerevisiae* URM 6670.

da Silva et al. [46] showed antioxidant activity 25.47% at a 2000 μg/ml of *Candida bombicola* biosurfactant, also Abdollahi et al. [47] showed the antioxidant activity of surfactin and rhamnolipid biosurfactant. Biosurfactant offered the ability to donate hydrogen, so show DPPH scavenging activity. Further, the reducing vigor of biosurfactants was denoted that some functional groups found in biosurfactants were both electron donors and electron recipients to alter them into high stable compounds [48]. The glycolipid biosurfactants with unsaturated fatty acids were really powerful antioxidants because of unsaturated lipids were able to and prevent reactions of lipid peroxidation [49].

4. CONCLUSION

To sum up, the glycolipid biosurfactant was extracted from *Candida cruzi* and partially characterized. The biosurfactants showed the potential effective in inhibiting bacteria (Gram-positive and Gram-negative) as well as parasites. It also has a high ability to scavenge free radicals. The results of this study suggested that biosurfactants extracted from *Candida cruzi* can be uses in the pharmaceutical application.

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TABLES

Table 1: Antimicrobial activity of cell-free supernatants from varies sources

C and N sources		Inhibition zone diameters (mm)			
No.	Sources	*S. aureus*	*E coli*	*B. cereus*	*P. aeruginosa*
1	Glucose	21	22	20	20
2	Lactose	16	20	20	20
3	Fructose	20	20	20	20
4	Dextrin	16	16	16	15
5	Asparagine	20	20	20	20
6	Sodium nitrate	20	20	16	20
7	Peptone	20	20	20	20
8	L-arginine	20	20	16	20

Table 2: Effect of Biosurfactants at 250 µl on the number of Naegleria fowleri

Biosurfactants Sources	Rate of the parasite numbers / Mm^3			
	Initial time	After 24 h	After 48 h	After 72 h

Control (NS)	8750	8250	9500	10750
Glucose	1875	1500	1375	1000
Lactose	2500	1875	1500	1250
Fructose	4870	4370	3250	3120
Dextrin	7370	5750	5000	4370
Asparagine	6620	5500	5120	4000
Sodium nitrate	8250	7250	6000	5600
Peptone	7750	5820	5620	4870
L-arginine	5620	4250	3870	3500

Table 3: Effect of Biosurfactants at 500 µl on the number of *Naegleria fowleri*

Biosurfactants Sources	Rate of the parasite numbers Mm^3			
	Initial time	After 24 h	After 48 h	After 72 h
Control (NS)	8750	8250	9500	10750
Glucose	1125	1000	750	625
Lactose	2000	1625	1375	1000
Fructose	3870	3620	3500	3000
Dextrin	7120	5620	4750	4120
Asparagine	5870	5250	4750	3620
Sodium nitrate	7500	5250	5700	5000
Peptone	7250	5620	5120	4500
L-arginine	4750-	4120	4000	3370

FIGURES

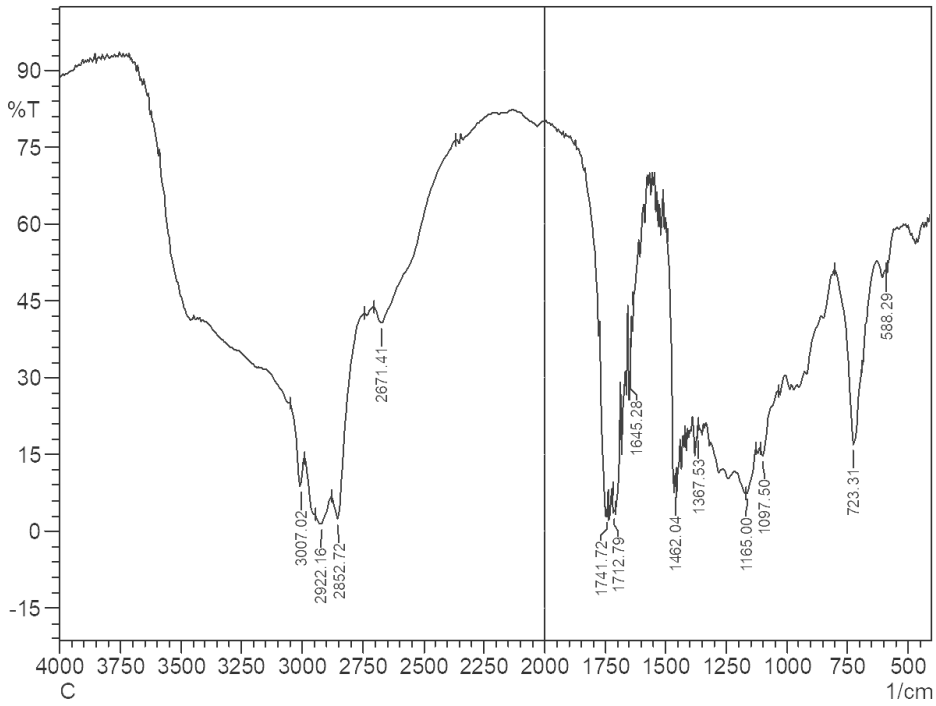

Fig.1: FT-IR spectrum of biosurfactant from *Candida cruzi*

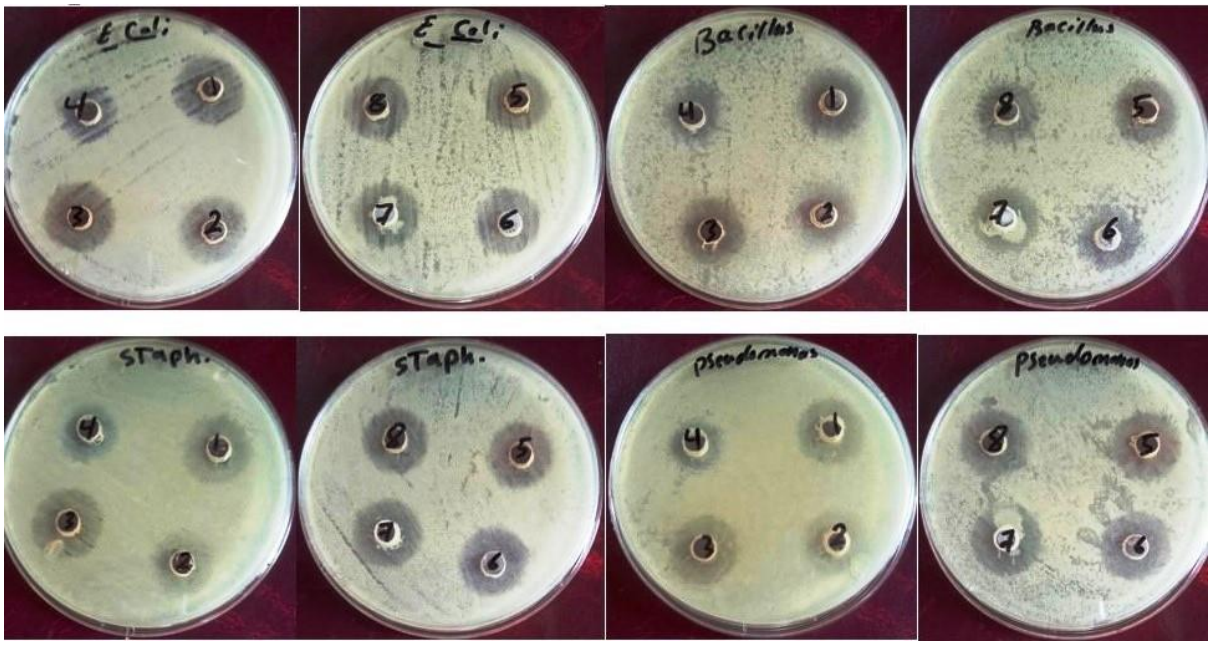

Fig.2: Antibacterial activity of supernatants from different media, 1 Glucose, 2 Lactose, 3 Fructose, 4 Dextrin, 5 Asparagine, 6 Sodium nitrate, 7 Peptone, and 8 L-arginine

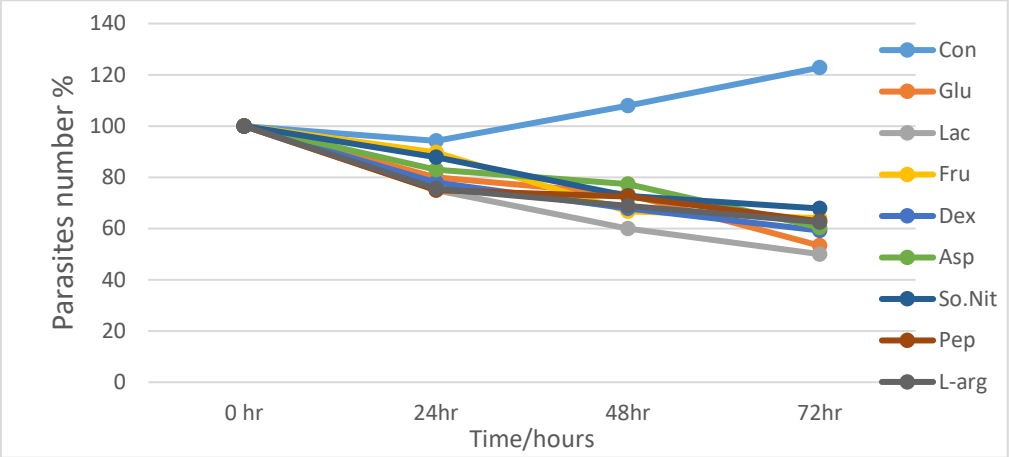

Fig.3: Percentage activity of Biosurfactants at 250 µl against *Naegleria fowleri*

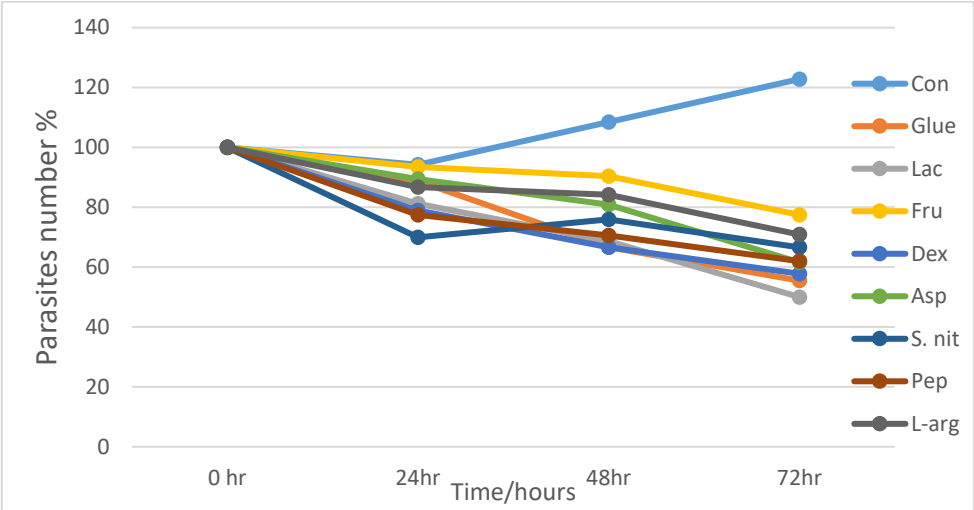

Fig.4: Antiparasite activity of Biosurfactants at 500 µl against *Naegleria fowleri*

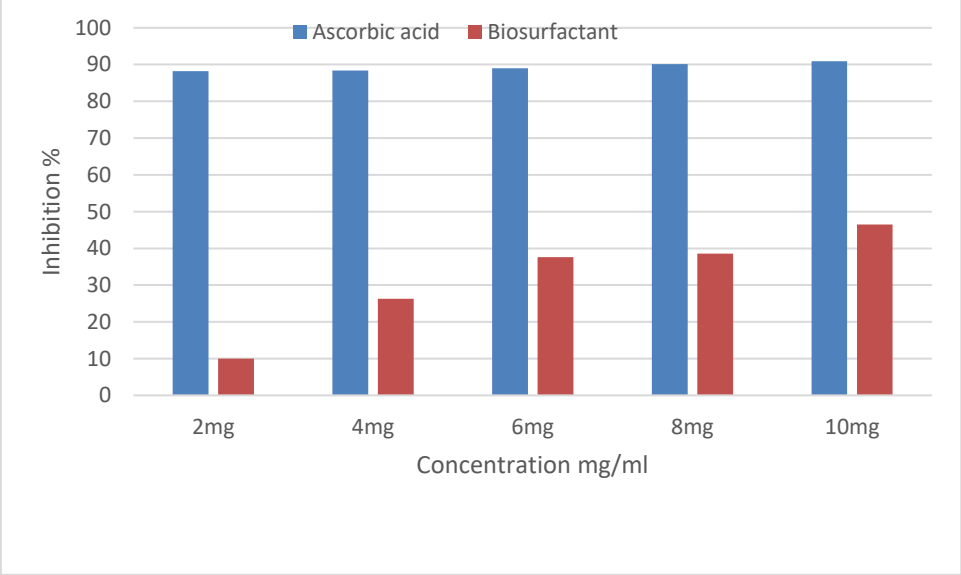

Fig.5: DPPH free radical scavenging activity of Biosurfctant and ascorbic acid