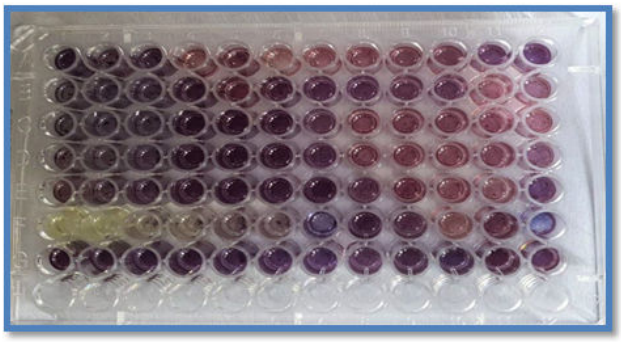

The % inhibition rate was calculated for each concentration of each fraction and compared to the computations of the positive and negative controls, as well as ANOVA analysis to determine the concentration that there is no significant mean difference between this and the positive control (the null hypothesis is to be achieved) at ($p>0.05$).

Fraction 1 (EAL): The calculated inhibition rate and mean for Ethyl Acetate Leaves' five concentrations revealed that there is low activity as antileishmaiasis comparing with the positive control inhibition rate that is 56.25% (Table 1).

Table 1: ea ea e a a e a

Sample name	Concentrations	% IR Means ± SD
A1	1 mg/ml	9.745471 ± 0.279610443
A2	0.5 mg/ml	24.84904 ± 0.085359371
A3	0.25 mg/ml	37.38439 ± 0.030214051
A4	0.125 mg/ml	36.4366 ± 0.123776681
A5	0.625 mg/ml	38.88252 ± 0.090691173

Fraction B (EAR): The % IR and mean comparison calculations revealed that concentration at 1 mg/ml (B1) has killing effect to the cells more than that of positive control (% IR 56.24856684) and % IR for B1 is 71.32156233% (Table 2).

Table 2: Mean of % IR for each concentration gradient for fraction B.

Sample name	Concentrations	% IR Means ± SD
B1	1 mg/ml	71.32156233 ± 0.162735641
B2	0.5 mg/ml	17.23610793 ± 0.245993677
B3	0.25 mg/ml	21.94450814 ± 0.562203403
B4	0.125 mg/ml	2.835741038 ± 0.038212854
B5	0.625 mg/ml	7.360697088 ± 0.043135446

Fraction C (SSL): The % IR and mean comparison calculations revealed that concentration at C1 (1 mg/ml) has the inhibition rate greater than that of positive control, the %IR of +ve is 56.24856684% and % IR of C1 is 73.40059619% also at concentration C3 (0.25 mg/ml) and C4 (0.125 mg/ml) show activity close to that of positive control (Table 3).

Table 3: Mean of % IR for each concentration gradient for fraction C.

Sample name	Concentrations	% IR Means ± SD
C1	1 mg/ml	73.40059619 ± 0.072350996
C2	0.5 mg/ml	28.05931361 ± 0.117252813
C3	0.25 mg/ml	55.78995643 ± 0.183798803
C4	0.125 mg/ml	52.7325537 ± 0.283033959
C5	0.625 mg/ml	24.3598563 ± 0.430743801

Fraction D (SSR): The % IR and mean comparison calculations revealed that the concentrations at D3 (0.25 mg/ml), D4 (0.125 mg/ml) and D5 (0.625 mg/ml) showed greater activity than positive control rate. And at concentration D2 (0.5 mg/ml) displayed very close effect to positive control (Table 4).

Table 4: Mean of % IR for each concentration gradient for fraction D.

Sample name	Concentrations	% IR Means ± SD
D1	1 mg/ml	34.4187113 ± 0.173305126
D2	0.5 mg/ml	56.61545517 ± 0.203592731
D3	0.25 mg/ml	65.81823741 ± 0.024931016
D4	0.125 mg/ml	66.24627379 ± 0.116610463
D5	0.625 mg/ml	61.99648399 ± 0.296154614

In figure 2 we can see the inhibition rate % IR against concentrations and comparing the result with the positive control used (Figure 2).

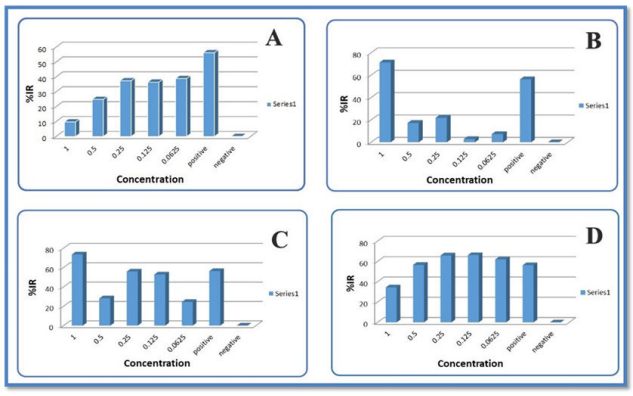

Figure 2: % IR of the four fractions against concentration (A for ethyl acetate leaves, B for ethyl acetate root, C for steroidal saponin leaves and D for steroidal saponin root).

ANOVA analysis revealed equivalent and non-significant differences between fractions and positive control, which is the goal in this study to achieve a similar effect to that of the control (Figure 3).

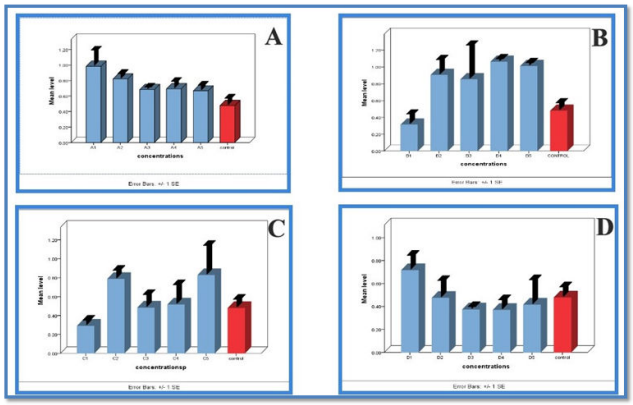

Figure 3: ANOVA diagram for different concentrations of four fractions (A for ethyl acetate leaves, B for ethyl acetate root, C for steroidal saponin leaves, D for steroidal saponin root).

IC_{50}: The IC_{50} of each analytical sample was calculated using the following procedure: At each of the five locations, inhibition ratios (y) were plotted against sample concentrations (x), and a regression line (y=ax+b) was generated.

Unfortunately, the computed IC_{50} for fraction ethyl acetate leaves EAL did not exist since the fraction lacks the ability to kill 50% of infected cells.

According to the straight line equation, the computed IC_{50} for second fraction ethyl acetate root EAR is 0.768 mg/ml.

The IC_{50} for steroidal saponin leaves SSL is 0.48 mg/ml calculated from straight line equation.

IC_{50} for the last fraction, steroidal saponin root SSR, is 0.6 mg/ml (Figures 3 and 4).

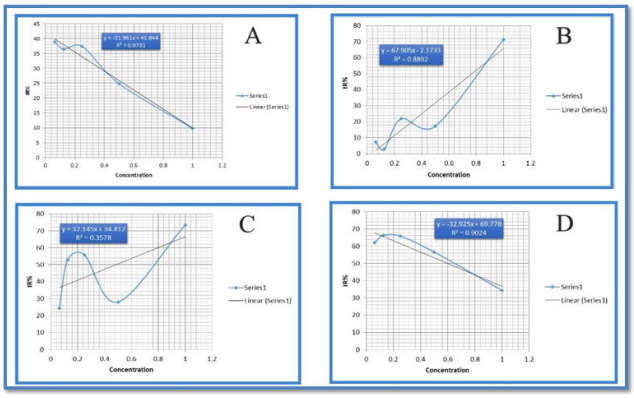

Figure 4: IC_{50} for the four fractions (A for ethyl acetate leaves, B for ethyl acetate root, C for steroidal saponin leaves and D for steroidal saponin root).

For the first time, antileishmanial activity was estimated for the plant *Agave attenuata* leaves and root for fractions rich in phenolic phyto constituents (ethyl acetate for leaves and root), as well as because *Agave* species are full of steroidal saponin and the activity of saponin as an anti-parasitic caught our attention, so we extracted steroidal saponin in a special method for leaves and root and tested for antileishmania effect. These fractions have antileishmanial activity, and in certain amounts (Table 5).

Table 5: Concentration of each fraction that showed best antileishmanial activity.

Fraction name	Fraction content	Concentration used	% IR

A	EAL	0.625 mg/ml	38.88%
B	EAR	1 mg/ml	71.32%
C	SSL	1 mg/ml	73.40%
D	SSR	0.125 mg/ml	66.25%

As previously stated, the antileishmanial activity of steroidal saponin in leaves and root is very good and phenolic phytoconstituents in root have a significant effect, making them a suitable candidate for further research as antileishmanial substances.

CONCLUSION

In this study, the Iraqi *Agave attenuata* leaves and root were discovered as a natural medicinal plant that contain many phyto constituents that proved their ability to act as antileishmania identical to that of pentostam treatment.

Four fractions used for this experiment depending on the high concentration of phenolic constituents that found in ethyl actate fraction for leaves and root. The other two fractions, we focused on steroidal saponin content of *Agave attenuata* that the saponin has anti-parasitic activity. The result was the phenolic content in ethyl acetate fraction for root has a considerable activity at 1 mg/ml, and has IC_{50} at 0.768 mg/ml. Both steroidal saponin fractions also showed a significant effect, for leaves the maximum activity was at 1 mg/ml and the IC_{50} appear at 0.48 mg/ml and about the steroidal saponin fraction for root, the highest activity was at 0.125 mg/ml and IC_{50} was at 0.6 mg/ml.

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