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The Effectivity of Acetone Extract From *Carcia Papaya* on the *Leishmania trpica* In Vitro

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ABSTRACT

The current study was conducted to know and evaluate the efficacy Acetone extract of the seeds of the fruit papaya on the biological and molecular characteristics of the promastigote of cutaneous leishmaniasis parasite isolated from the skin ulcers of patients in leishmaniasis visiting the dermatological consulting clinic in Sharqat Hospital. The study dealt with the cultivation of the parasite isolated from the skin in the medium of NNN and RBMI 1640 where a biopsy was taken from the infested area and cultured in the appropriate culture media that and different concentrations of the plant extracts under study were prepared for each of the acetone, 10%,7.5%,5%,2.5%, and the observation of the effect of those concentrations on the parasite's vitality compared to the control group .Through the results that appeared using high concentrations of acetone, where death and direct destruction of the parasite appeared in promastigote at each of the concentrations where the results showed that the most effective concentration of the parasite in front of the flagellum acetone 10 on the fourth day of the at 0.01<p, where the average number of parasite treated with this concentration was (74.83), where Complete inhibition on the fifth day compared with the parasite numbers in the following concentrations 2.5-5, which showed phenotypic changes on the parasite's vitality, growth and preparation, as well as the loss of flagella or their appearance in a different form due to the lethal concentrations and toxicity of the substance used, where the relationship is inverse with Increasing the concentration, the results of the primers (4) used for all plant extracts of papaya seeds for each of acetone The acetone extract was distinguished by the appearance of the highest bundles in it, reaching (64) bundles, including (33) unique bundles and (31) bundles that were absent, absent after(22) Based on the results, the concentration of 7.5 for the acetone extract showed the highest unique and absent bands, then in the second place a concentration of 10% for acetone as well, and in the third place a concentration of 2.5 for acetone also came after 5% it in the four the place.

Introduction

leishmani infect millions of humans and other mammals in the tropical and subtropical regions of the world responsible for a spectrum disease of man, the leishmaniases, with symptoms ranging from self-healing cutaneous ulcers to disfiguring and progressive mucocutaneous lesions as well as the visceral disease which can be fatal if left untreated . The importance of leishmaniasis was highlighted by its selection by the World Health Organization as one of the six major diseases for study in the special program for Research and Training in Tropical Diseases In the Old World, cutaneous leishmaniasis (CL) is also known as"Oriental Sore", "Baghdad boil", "Delhi boil". CL is usually caused by *L. major*, *L. tropica(1)* . CL presents usually as a self-limited, ulcerative skin lesion Leishmaniasis represents a major and general health problem and a danger to people, especially to those who live in or travel to endemic areae The infection appears in the form of a skin lesion, papule, or papillae that is raised in the middle and covered with a thin layer of the skin where ulcers and infiltrates arise that cause skin infection. And lymph nodes in the area of the injury, leaving in its place an area dark in color, tending to brown, and irregular limbs in the areas of the body It is called by other names in a number of countries: the Delhi pill in India, the Aleppo pill in Syria, and the Odessa pill in Russia This disease is transmitted by a vector insect that is a female sand fly of the genus *Phlebotomus* in the Old World and *Lutzomyia* in the New World..(1).

the papain superfamily, as a proteolytic enzyme, papain is of critical importance in many vital biological processes in all living organisms. Papain shows extensive processes in all living organisms proteolytic activity towards proteins, short chain polypeptides, amino acid ester and amide links and is applied extensively in the fields of food and medicine(2).

The development of the outcome in the field of molecular genetics providedthe methods

Molecular examination has important benefits in discovering genetic variation in living organisms and resulting in mutations as a result of the RAPD-PCR

polymerase chain reaction as a result of the doubling and amplification that occurs during the reaction that results in bundles. Spectrophotometry and seeing the unique and absent beams and comparing these prefixes. It is considered one of the important methods in molecular diagnosis because it is a fast, economical and inexpensive method.(3).

Materials and methods

The study was conducted in the Parasitology and Molecular Laboratory at the College of Science, Tikrit University, from 12/1/2021 to 20/6/2022.The parasite was obtained from patients attending the consultative dermatological clinic at Al-Sharqat General Hospital. Samples were taken from the edges of the ulcers and cultured in the culture medium. RPMI 1640 with Fetal Cow Serum and Antibiotics (4).

The plant is under study.

The seeds of the papaya plant used in the study were taken at an amount of 200 g. They were washed well and purified from impurities and dust. It was placed in the incubator at 37 ºC with continuous stirring until it dried. It was crushed well to obtain a powder and was placed in a sterile and airtight box، and the name and address were written (5).

Extraction

The Acetone extract of papaya seeds was obtained using a Soxhlet apparatus according to the approved methods. The extract was lyophilized by Lyophilize through (Freeze-drying) technique, and the powder was collected, weighed and placed in a sterile, airtight container with the name and weight written on it (6).The following concentrations were prepared: - 2.5%,5%,7.5%,10%

Preparation of plant extracts according to the dilution law N1XV1=N2XV2.

Cultures of media in growth

There are two phases of this medium, liquid and solid, from the cultural media .Necessary for the development of the parasite are frontal flagella, ease of cultivation, and permanence of life.

Solid culture medium (NNN) Novy-MacNeal-Nicolle medium is called Biphasic Medium.

RPMI 1640 (Roswell Park Memorial Insttitute)

Liquid Culture Medium Prepared culture medium RPMI1640 was used, to which cow fetal serum FCS was added by 10% add to it the following antibiotics 0.2 mg/ml Gentamycin, 0.2 mg/ml Streptomycin, 0.2 mg/ml Ampicillin and 0.000514 mg/ml Nystatin. All antibiotics were added to prevent contamination in the culture media and ensure the safety of the implants (7).

Isolation and Cultivation of parasite

After a specialist diagnoses the skin lesion, the parasite is isolated.

The extracted liquid is cultured in two vials with a volume of 25 ml, containing 4 ml.

The bottles were kept from the gel culture medium at a temperature of 26 ºC, the optimum temperature for parasite growth. After sufficient isolation, approximately 24 hours, a drop is taken from the medium to ensure the sample is not contaminated. And it is left for 3-4 day of its cultivation, the parasite Promastigote is seen, and thus we made sure of the presence of the parasite .We take 0.5 ml of the RPMI 1640 liquid culture medium with a temperature of 26 ºC (8).

Counting of Parasites

- We take 1 ml of RPMI culture medium containing the Leishmania parasite Promastigote into a second vacuum tube and add 9 ml of distilled water and mix it well so that the dilution ratio is 1:10
- We take ten microliters by Micro Pipette from the tube and place it on the Hemocytometer box and then put the Cover Slide to calculate the number of parasites in 64 squares and at the magnification power of 40x according to the equation

Number of parasites in 1 ml = Number of parasites in 64 squares x 25 degrees of dilution x 10(9).

DNA extraction and molecular assay

DNA extraction of the Leishmania parasite was carried out on the samples with the control coefficient and according to the approved methods (10), and electrophoresis was carried out after extraction to ensure the presence of DNA (11).

Matarial and Methods

Results and discussion RAPID-PCR was used to evaluate the effect of Acetone plant extract on Papaya carcica seeds on the DNA of the promastigote parasite and compare it with control isolates not treated with the extract. PCR-PreMiX was used, and according to the instructions, 14IU=DW/PRIMER2IU /DNA 2IU was added, mixed well, and then placed in the PCR Thermal Polymerization Device according to the table.

Table 1

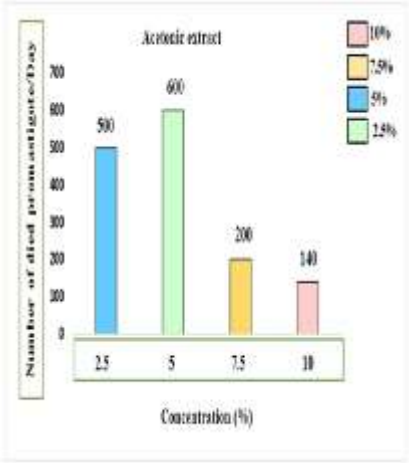
NO	Step	Tempter	Time	Cycle
1	Initial Denaturation	94 C	2 mint	1
2	Denaturation	94 C	30 second	40
3	Annealing	55C	30 mint	
4	Extent ion	72C	80 mint	
5	Final Extent ion	72 C	5 mint	1
6	Final had	4		

Four types of primers were used and detected by migration on agarose gel with the presence of the DNA Marker. After completing the PCR process, they were migrated to the electrophoresis device and with agarose gel. Watching these bundles and variations, identifying the binding sites and their absence, and viewing them on the UV device (12). During the results of the primers and the emergence of bands of different molecular weights is the result of the difference in the binding sites complementary to this primer in the genes of each isolate was used (4)Primer .

Sequence Primer No

1-OP D-03 GTCGCCGTCA
2-OP D10 GGTCTACACC
3-OP D -18 GGAGAGCAAC
4- OP G-14 GGATGAGACC

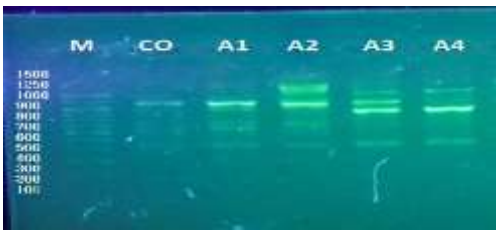
Molecular diagnosis: Molecular diagnosis is essential in diagnosing genetic mutations, as it depends on the differences resulting from the RAPD-PCR primers used with the volumetric index and comparing them with the control sample(13).

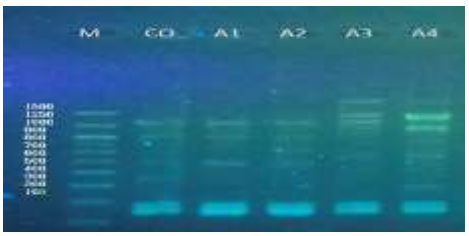


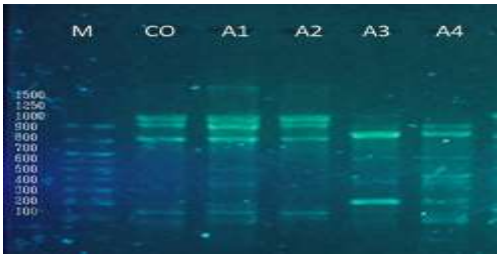
Mean± S.E day	Acetone Extract 10%	7.5%	5%	2.5%	السيطرة	Day
660.0± 260.0	400	400	800	1000	1600	1
800.0± 340.6	200	300	700	700	2100	2
820.0± 456.7	100	200	600	600	2600	3
620.0± 339.7	0	100	500	600	1900	4
520.0± 312.1	0	0	400	500	1700	5
	140.0± 74.83	200.0± 70.71	600.0± 70.71	680.0± 70.9	1980± 177.2	Mean± S.E conc

Effect of Acetonic extract of papaya on the vitality of promastigote

Use the following concentrations 2.5%, 5%, 7.5%, 10%. The current study also showed a high inhibition of this extract on the promastigote parasite at a concentration of 10% on the fifth day from the start of the experiment at a probability level between the average concentration of the concentration Also, phenotypic changes were observed in the external form of the loss of flagellaum and the occurrence of phenotypic changes on the third and fourth days of the experiment. Also, the results of the current study showed a high inhibition activity of 7.5% of the concentration on the fifth day and phenotypic changes in the parasite, such as the loss of flagellaum and a change in the external shape. This is evidence of the toxicity of the substances in the plant and their interaction with the parasite in the protein-making process either during its association with the ribosomes in the cytoplasm or with the enzymes involved in making the amino acids that make up the protein. This interaction is proportional to the content and active compounds found in the seeds of the plant or the effect on the enzymes Which help in the manufacture of nucleic acids or inhibit the work of the enzyme's polymerase DNA, RNA and prevent the work of protein or affect the process of excretion and cellular entry, or help in the breakdown of cell membranes. As for the concentration (5%-2.5%), there is no inhibition, only phenotypic changes in the parasite's vitality and morphology on the fourt







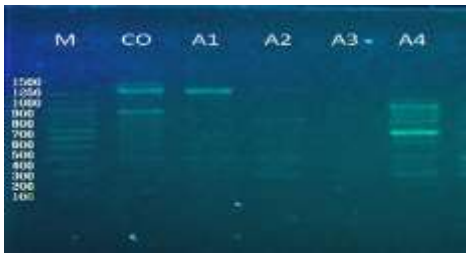


Table 2

no	2.5% U	2.5% A	5% U	5% A	7.5% U	7.5% A	10% U	10% A	S.U	S.A	SU+A
P1	1	6	1	0	4	3	2	4	8	13	21
P2	1	0	0	1	1	0	1	0	3	1	4
P3	3	1	1	1	5	3	4	2	13	7	20
P4	2	2	2	3	3	3	2	2	9	10	19
S	7	9	4	5	13	9	9	8	33	31	64
	U=Unique /A=Absent/S=Summation/P=Prime										

The tables (2) show the difference in the results of phase primers on an agarose gel. This difference resulted in distinct packages (unique packages and missing packages). The total number of these packages on which the study was conducted was (64) different packages compared to the primary sample (the control sample). The resulting unique packets were (33) individual and (31) missing: the initiator P-1 produced (21) packages, the highest number of special and absent packages. While the initiator P-3 produced a unique bundle of (13) bundles, the initiator P-1 produced the highest percentage of the absent bundles, amounting to (13) bundles. For the

four concentrations, the concentration percentage reached 2.5% in (16) bundles, of which (7) were single bundles and (9) were absent. As for the 5% concentration, the number of bundles in it reached (9), including (4) individual bundles and (5) absent bundles. As for the concentration, 7.5%, the number of packages in it reached (21), including the unique (13) packages, the missing packages amounted to (9) packages and the concentration of 10%, the number of packages in it (17) of them (9) unique packages and (8) missing packages as for the concentrations, the concentration was 7.5%, the highest number of bundles out of the (21) bundles, the unique ones were (33). In contrast, the absent ones were (31) bundles. Thus, the concentration of 7.5% obtained the most significant number of packages, and the second was a concentration of 10%, then a concentration of 2.5%, and finally, a concentration of 5%(14).

The research results show that acetone extract from papaya seeds affects the DNA of the Leishmania with all its concentrations(15). Indicators of the RAPD-PCR technique showed high efficiency in diagnosing these mutations due to the increased number of genetically mutated packets. It is concluded from this that the extract used in this research has an effect on the vitality of the parasite and that the increased concentration has led to the killing of the parasite despite the absence of any adverse side effects as it is a natural extract. Increasing the extract concentration destroyed the DNA sequences and the primer loci, thus giving rise to unique bundles. On the other hand, the position of the initiator identifier that had a space in the primary sample disappeared, and packages that were present in the original control sample disappeared, which is consistent with what the researchers mentioned in previous studies.

The susceptibility of the alcoholic extract from the papaya seeds led to the genetic transformation of the DNA in addition to the interference of other reasons in the inhibition process. The permeability of the cell membrane may have enabled the entry of toxic substances into the cells, or it may be due to the mixing of imports that entered with parasitic substances and compounds. This led to the spread and transformation into harmful substances and mixtures, or it was due to the permeability of the cell membrane, which ledfd to the release of necessary substances that were unable to come out, or because of an explosion in the parasite's membrane(16).

Conclusion. The above results show that Acetone extract significantly affects the vitality of the cutaneous leishmaniasis parasite. This effect damages the parasite's genetic map during the reproductive process, especially when the nuclear load is doubled, leading to a genetic transformation that significantly damages the parasite's genes. Thus, this prevents the parasite from multiplying and attacking the body. Finally, increasing the concentration of the extract increases the possibility of the termination of the parasite.

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