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Article

Study the Antibacterial activity and Immunomodulatory Effect of Aqueous *Commiphora myrrh Leaves* Extract *in vivo*

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Abstract: *Commiphora myrrh* demonstrated antibacterial efficacy against microorganisms. Myrrh stimulates the activation/maturation and differentiation of both (myeloid and lymphoid) cell types of WBCs during wound healing of caused stomach and skin injuries, and it has significant anti-inflammatory, analgesic, and anti-hyperlipidemia efficiency. The aim of this study is to investigate the antibacterial activity and immunomodulatory effect of aqueous *C. myrrha* extract**.** Pseudomonas aeruginosa was collected from the Microbiology Laboratory/College of Health and Medical Technology/Sawa University. C myrrh leaves were bought from the Iraqi market and dried for extraction. Concentrations of 25, 50, 75, and 100 mg/ml were created. The experimental design included two parts, in vitro and in vivo. The in vitro investigation estimated the antibacterial activity using the agar diffusion method and in vivo study was Included four groups. Group I (Control) received daily intraperitoneal injections of normal saline. Group II received intraperitoneal injections of 1.5 x 108 CFU/ml of P. aeruginosa. Group III received oral administration of 100 mg/ml extract. Group IV received intraperitoneal injections of 1.5 x 108 CFU/ml of P. aeruginosa followed by oral administration of 100 mg/ml extract for 14 days. then notice the clinical signs of each group followed by estimation , TLR-2 , IL-17 , IL 10 , arthus reaction and delayed hypersensitivity. The extract demonstrated strong antibacterial activity against the tested isolates. The maximum activity was seen at a dose of 100 mg/ml, producing an inhibition zone of 1.6 ± 0.01. The animals in group IV had the highest TLR-2, IL-17, and IL-10 levels with a significant difference ($p \leq 0.05$).The arthus test and DHT results showed that the group that received a mixture of extract and *P. aeurginosa* had significantly greater values than the other group ($p < 0.05$). *C. myrrh* aqueous extract leaves has immunomodulatory effect on the immune response ,innate immunity and acquired immunity as well as it showed the antibacterial activity *in vitro* .

Keywords: Immunomodulatory, *Pseudomonas aeruginosa*, *Commiphora myrrh*, aqueous l*eaves* extract, immune response.

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1. Introduction

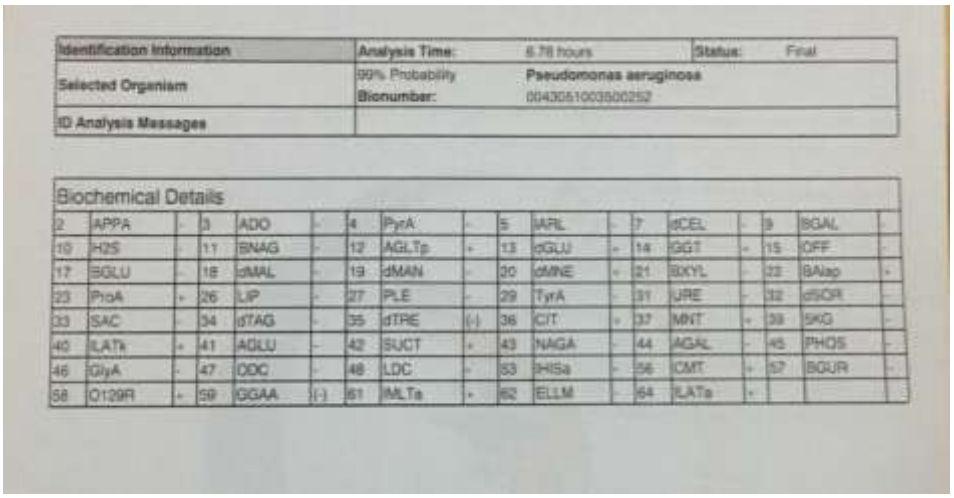
Commiphora species are small trees or shrubs with spinescent branches and bark that contains pale-gray discharge or reddish-brown resin. Resins produced by *Commiphora* are used in fragrances, bouquets, and as ointments in the embalming process, while their therapeutic applications are gaining popularity among humans [1]. *Commiphora myrrh* is often found in southern Arabia, northeast Africa, Somalia, and Kenya. It has traditionally been used to treat wounds, oral ulcers, pains, fractures, stomach problems, microbial infections, and inflammatory conditions. Research has demonstrated that it possesses numerous biological actions such as anti-inflammatory, antioxidant, anti-microbial, neuroprotective, anti-diabetic, anti-cancer, analgesic, and anti-parasitic [2].In addition, *C.*

myrrha resin shows antimicrobial activity against bacteria such as *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Fusobacterium nucleatum* [3], [4]. Both myeloid and lymphoid cell types of WBCs are activated, matured, and differentiated during wound healing of induced gastric and skin injuries [5]. It has strong anti-inflammatory, analgesic, and anti-hyperlipidemic properties, reduces body weight gain, and improves blood lipid profiles [6]. It also helps rats with induced ulcerative colitis by reducing the expression of inflammatory mediators and boosting endogenous antioxidative processes [7]. Furthermore, Shin., et al. [8] recorded that the C. myrrh suppress itchassociated histamine and IL31 expression in stimulated cultured mast cells .Other diseases such as aches, arthritis, and inflammatory diseases have been treated [9]. It is typically used in conjunction with frankincense to treat fractures, pains, edema, joint inflammation, and trauma [10]. It is widely used in dermatology to treat skin ulcers, lesions, and empyrosis the aim of this study is to investigate the antibacterial activity the vivo immunological effect of aqueous *C. myrrha* extract [11].

2. Materials and Methods

Bacterial Isolation

Pseudomonas aeurginosa was obtained from Microbiology Laboratory/College of Health and Medical Technology/Sawa University. and identified by Vitek2 system(Olympus, Japan).



Identification Information			Analysis Time:	6.78 hours		Status:	Final
Selected Organism			99% Probability Bionumber:	Pseudomonas aeruginosa 0043051003800252			
ID Analysis Messages							

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	+	13	dGLU	-	14	GGT	-	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	-	21	BXYL	-	22	BAlap	+
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	-	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	(-)	36	CIT	+	37	MNT	+	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	HISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	(-)	61	MLTa	+	62	ELLM	-	64	ILATa	+			

Figure 1. Identification of *Pseudomonas aeruginosa* by Vitek2 system

Aqueous Extract preparation

The plant leaves were purchased from an Iraqi market, dried, and 100 g of crushed leaf particles were extracted by immersing them in 1 L of distillate water in a conical flask and stirring them for 4 hours. The extract was separated using Whatman No. 1 filter paper once it had cooled to a powder, making concentrated water-based extracts [12], followed by 25, 50, 75, and 100 mg/ml concentrations.

Antibacterial Activity Determination

Utilizing the agar well diffusion method, the antibacterial activity of plant extracts was assessed. The isolates were adjusted to 1.5 × 108 colony-forming units CFU/ml, and they were subcultured on Mueller-Hinton agar with wells measuring 6 mm in diameter. 0.1 ml of prepared concentrations (25 mg/ml, 50 mg/ml, 75 mg/ml, and 100 mg/ml) were added to each well [13].

In vivo study

The laboratory animals

The Iraqi Center for Genetics and Cancer Research at Mustansiriyah University provided a total of 20 male mice. Male albino mice (Blab-c) weighing 22±3 g and aged between 6 and 8 weeks were housed in bio-clean hoods with alternating periods of light and darkness lasting 14 and 10 hours, respectively, at a temperature of 20 to 25ºC.

Design of the study

There were twenty animals in the current experiment, five mice in each group: Group I was given normal saline solution intraperitoneally every day, Group II received an intraperitoneal injection of 100 µL of 1.5 x 108 CFU/ml of *P. aeurginosa*, Group III received an oral dose of 100 mg/ml of extract for 14 days, and Group IV received an intraperitoneal injection of 100 µL of 1.5 x 108 CFU/ml of *P. aeurginosa* and an oral dose of 100 mg/ml of extract for 14 days. After that, each group's clinical symptoms were observed.

Animal Activity Observation

Note :-the study used 100 mg/ml of extract depending on the antibacterial results

Blood and serum collection

Following 14 days of treatment, a total of 20 blood samples (about 1.5 ml from each animal) were drawn from the face vein. The serum was separated and kept at 4C after the whole blood was collected and centrifuged at 1,000–2,000 x g for 10 minutes.

Arthus Reaction and Delayed Type Hypersensitivity

On the fifth day following treatment, each mouse in the groups received an injection of 50 ul of *P. aeurginosa* into its right foot pad, while the left foot pad received a normal saline injection. The delayed type hypersensitivity peak happened 24 and 48 hours after injection, while the arthus reaction was measured by assessing the increase in footpad edema after 4 hours. As advised by [14], the measurements were made with a digital vernia and in millimeters.

Measurements of TLR-2 IL-17 , IL-10 serum Level

TLR-2,IL-17 , IL-10, and IL-4 levels in male of albino mice were measured by means of ELISA. Procedures were conducted in compliance with Elabscience's manufacturer's instructions was provided with each Kit.and utilizing the standard curves shown below in figure 2,3 and 4.

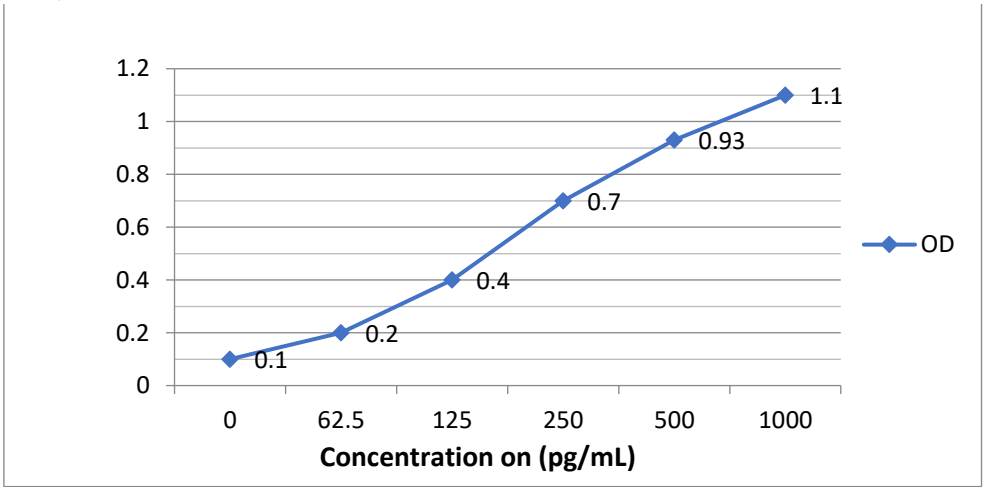


Figure 2. Standard curve of IL 17

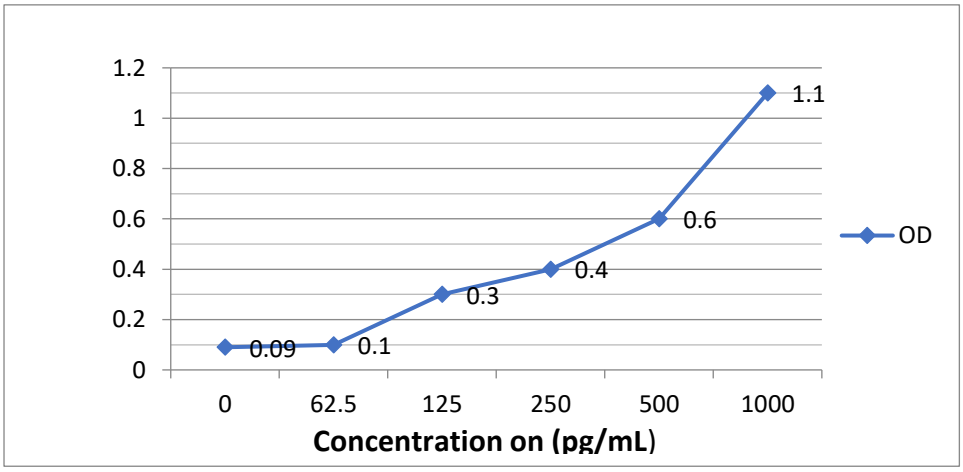


Figure 3. Standard curve of of IL 10

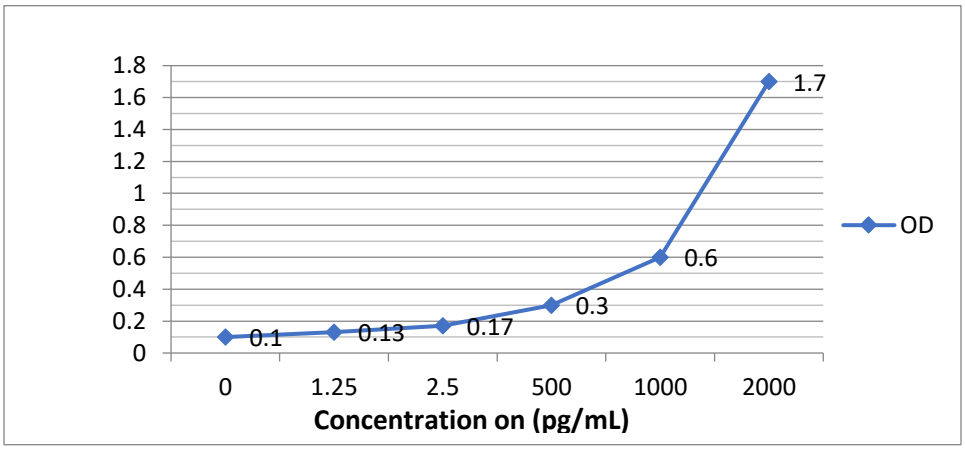


Figure 4. Standard curve of TLR-2

Statistical analysis

Using the program SPSS statistic, the findings were reported as the difference between means and statistically assessed using LSD; the differences were deemed significant when P≤0.05 [15].

3. Results

Antibacterial activity

According to the findings, the extract significantly reduced the antibacterial activity of the isolates under study. At a dosage of 100 mg/ml, the greatest action was detected, resulting in an inhibition zone that measured 1.6 ± 0.01. Then, at dosages of 100 mg/ml and 150 mg/ml, the inhibition zones were 1.9 ± 0.31 for 50 mg/ml and 3.7 ± 0.14 for 75 mg/ml, as shown in f was 5.00 ±0.00 in figure 5.

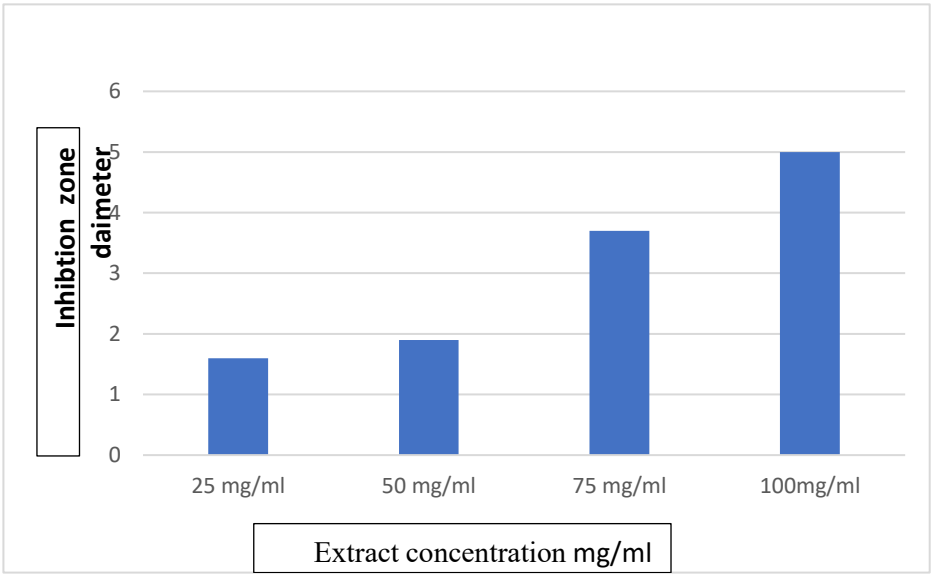


Figure 5. The Antibacterial activity of extract against the bacteria

The findings concurred with [16], which examined the antibacterial properties of methanolic extract. With inhibitory zone diameters ranging from 6.17±0.28 mm in A. baumannii, 9.67±0.57 mm in S. aureus, 12.17±0.28 mm in *K. pneumoniae*, 12.67±0.57 mm in E. coli, and 12.67±0.28 mm in *P. aeruginosa*, all test bacteria were susceptible to methanolic extract. Additionally, the methanolic extract was selected for additional testing since it showed the most inhibitory effect. For *E. coli*, *K. pneumoniae*, and *P. aeruginosa*, the methanolic extract's minimum inhibitory concentration (MIC) varied from 3.12 mg/mL. Additionally, the results corroborated [17], which documented the antibacterial properties of C. myrrha extracts in a variety of solvents (hot water, hexane, DMSO, methanol, and

ethanol) against isolated airborne bacteria using the disk diffusion technique assay in agar plates. Micrococcus *yunnanensis, Deinococcus radiodurans, and Paenibacillus pasadenensis* were all inhibited by C. myrrha that was extracted using methanol and ethanol.Furthermore, *C. myrrha* resin extracts made with only methanol showed antibacterial activity against *Rhodococcus qingshengii* dj1-6-2. However, *C. myrrha* (T. Nees) Engl. resin extracted with hot water, DMSO (apart from *Micrococcus yunnanensis*), and hexane showed no antibacterial activity. Because their effective components differ, the different extraction solvents employed to generate C. myrrha extracts are likely to affect their antibacterial activities.In general, the antibacterial effect may be related to the cell wall structure [17].

Animal Activity Observation

For seven days, the mice in the *P. aeurginosa*-treated group showed clinical signs of illness, including noticeable weight loss, diarrhea, and decreased movement activity throughout the cage area. In contrast, the mice in the extract-treated group showed clinical signs of illness for three days, and the mice in the normal saline and extract group showed no changes in activity or behaviors, which was also agreed upon [18]. that examined the effectiveness of extract from *Communiphora Myrrh* as a substitute treatment for giardiasis in hamsters. *Giardia Lamblia* Histopathological analysis showed that the intestinal mucosa had completely healed following the combination treatment, whereas metronidazole and either myrrh plant treatment had caused partial healing of the intestinal lining epithelium. In addition to its many biological properties, this pair has anti-inflammatory, antioxidant, anti-microbial, neuroprotective, anti-diabetic, anti-cancer, analgesic, and anti-parasitic properties. More recently, it has been shown to be effective against respiratory infections [19].

Immunological assay

The findings of TLR-2 reported the animls in group 4 showed highest value (19.500±0.25) with a significant difference at p≤ 0.05. On the other side , IL-17and IL-10 finding in table 4 showed the group 4 that received extract with *P. aeurginosa* given a high value (253.504±0.673, 73.606±0.234,) respectively which higher than other groups with a significant difference at p≤ 0.05.

Table 1. Serum TLR-2, IL-17, IL-10 level in studied group ((Mean ±SE)

Groups	TLR-2	IL-17	IL-10
Group I	5.531 ±0.02 c	156.150±0.04 d	65.621±0.06 b
Group II	6.126±1.03 b	169.515±0.984 c	66.267±2.93 b
Group III	3.511±1.14 c	191.745±0.342 b	72.325±6.03 a
Group IV	19.500±0.25 a	253.504±0.673 a	73.606±0.234 a

Arthus Reaction and Delayed Hypersensitivity Test (DHT)

The results of the Arthus test and DHT revealed the group that had a combination administration of extract and *P. aeurginosa* showed a higher value than the other group, with significant differences at p ≤ 0.05 with other groups as in table (2 and 3).

Table 2. DHT and Arthus reaction results (Mean±SE)

Groups	Arthus reaction	DHT		
		24 hr	48 hr	72 hr
Group I	3.25±0.25	3.25 ±0.25	3.25±0.25	3.25±0.25
Group II	5.15 ±0.15	5.25±0.25	4.25±0.25	4.15±0.15
Grorup III	4.25±0.25	4.25±0.25	4.00±0.00	4.00±0.00
Group IV	5.25±0.25	5.25±0.25	4.25±0.25	4.00±0.25

A few research have looked into how myrrh affects the immune system. In mice, its dietary supplementation stimulated lymphocyte transformation, phagocytic activity (PA), and phagocytic index (PI), which resulted in a marked improvement of the cellular immune response [20]. Additionally, it raised IL-4 levels in a fascioliasis patient (Massoud et al., 2004). Additionally, it raised IL-2 and IFN-γ levels in Schistosoma-infested mice [21]. The results corroborated [22] investigation of the Myrrh Inhibits LPS-Induced

Inflammatory Response using LPS from Escherichia coli, which discovered that pretreatment with Myrrh extract decreased LPS-induced production of TNF-α but not IL-1β and IL-6. and discovered yrrh on the activation of MAPKs and the transcription factor NF-κB, which control the expression of several inflammatory and immunological genes. In addition to coordinating the expression of proinflammatory enzymes and cytokines including iNOS, COX-2, TNF-α, and IL-6, NF-κB is known to be essential for the control of genes involved in cell survival [22]. Iκ-Bα is an inhibitory protein that controls NF-κB activity. The inhibitory proteins become phosphorylated with cell activation, which in turn causes Iκ-Bα to degrade. Free NF-κB units are released when Iκ-Bα is lost, and these units go from the cytoplasm to the nucleus, where they may cause the transcription of particular target genes. There are toll-like receptors (TLRs). The TLR family can identify pathogen-associated chemicals carried by bacteria, viruses, parasites, and fungi, triggering an immune response [23]. The immunological mechanism of polysaccharides used in traditional Chinese medicine has been most thoroughly investigated through the TLR4 signaling pathway. NF-κB and MAPK are downstream of the TLR4 signaling pathway, which must be activated for immune function. There are two methods for triggering the TLR4 signaling pathway: MyD88-dependent and non-MyD88-dependent methods [24]. TRAF6 is necessary for downstream IRAK4 and IRAK1 pathways that rely on MyD88 [25]. Nevertheless, a number of research examined the immune impact of myrrh against parasites, and their findings concurred with ours. One such study examined the M-RNA Gene Expression of INF-Γ and IL-10 during the Intestinal Phase of Trichinella spiralis following Myrrh and Albendazole Treatment [26]. After three consecutive days post-infection (pi), the mice were infected with 300 T. spiralis larvae and treated with either albendazole (50 mg/kg per day) or myrrh (500 mg/kg per day). The intestinal tissue was examined using reverse transcription (RT) PCR to detect the expression of INF-γ and IL-10, which was found on days three and twenty pi in the groups treated with albendazole and myrrh, respectively. On days 5 and 20 pi, IL-10 expression was seen in the control group. as well as the findings concurred with [27], which was examined Artesunate and myrrh's impact on S. mansoni infection and the amounts of certain Th1 and Th2 cytokines that were discovered Interleukin 2 (IL-2) levels were dramatically reduced following artesunate therapy, but there was no discernible difference between the myrrh-treated and infected groups. However, following treatment with artesunate, the level of IL-10 did not considerably decrease; however, following treatment with myrrh, it did dramatically increase. According to the arthus reaction data in Table 4, T helper cells mostly differentiate into Th1, Th2, and Th17 cells, as evidenced by an increase in serum IL-17 levels and an increase in antibody production. However, according to the DHT table, the immunologic reaction, which is mostly mediated by T cells and monocytes and manifests hours to days after the antigen crosses, caused the group IV to grow [28].

4. Conclusion

The results showed that the *C. myrrh* aqueous extract leaves has immunomodulatory effect on the immune response *in vivo* by measuring several parameters related to innate immunity TLR-4) and acquired immunity(IL-17,IL-10, Arthus reaction ,DHT), and finally, it shows the antibacterial activity of the extract in an *in vitro* .

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