

EVALUATION OF THE SCHISTOSOMICIDAL ACTIVITY OF *Ficus carica* AND *Olea europaea* LEAVES EXTRACT ON ADULT *Schistosoma mansoni* WORMS USING WORM KILLING ASSAY *in vitro*

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ABSTRACT

Those exhibit contemplate investigated the possibility schistosomicidal action from claiming both *Ficus carica* Furthermore *Olea europaea* abandons extracts utilizing those schistosoma mansoni worm slaughtering test *in vitro*. Distinctive focuses from every extricate were used; they were 25, 50, 100 What's more 200 µg/ml. Eight worms were incubated for each extracts centralization for five days. An additional eight might have been utilized Toward including that's only the tip of the iceberg extricate focuses i. E. 400 What's more 800µg/ ml incubated best for person day. Praziquantel (PZQ) dealt with worms were extritized Similarly as sure control. Phytochemical screening of both extracts might have been conveyed out to identify extracts major compound constituents answerable for action. The comes about uncovered that the *F.carica* extricate indicated higher flavenoid fight over *O. europaea* extricate (63. 6, 23. 1 µg/g respectively) same time *O. europaea* extricate recorded higher phenolic fight over *F. carica* extricate (82. 5, 56. 7µg/ g respectively). Both extracts have proliferation *in vitro* antischistosomal movement. Those results indicated that variable rates for worm murdering exercises (10%-100%) fluctuating as stated by those measurements about both plant extracts and the span of the extricate brooding. The worm murdering exercises reveals to An profoundly critical build with those increment for both extracts expanded ($P < 0.0001$). The LC50 and LC90 about both extracts might have been 21. 35 Also 43. 35 µg/ ml separately to fig Furthermore 47. 98 Furthermore 115. 9 individually about olive then afterward five days for brooding contrasted with 48. 21 Furthermore 52. 54 µg/ml for PZQ. Olive extracts reveals to All the more schistosomicidel exercises done bring down extracts focuses. These exercises turn into equals done more broodeng the long haul. With helter skelter extricate fixation those exercises about both extracts arrived at of the PZQ level Actually then afterward special case day from claiming brooding

keywords: Schistosomicidel, Plant Extract, *Ficus carica*, *Olea europaea*, Worm Killing Assay, *in vitro* study

INTRODUCTION

Schistosomiasis is a endemic helminth infection from claiming human; it is a standout amongst the most seasoned referred to parasitic infestations portrayed in authentic records. It may be a extreme parasitic infection initiated Toward parts of the class schistosoma. More than 200 million persons are contaminated Also up to 800 million, mostaccioli children, are at hazard. Egypt will be "around 51 nations for number requiring chemotherapy in spite of erroneous Also inadequate majority of the data advocating the close destruction for schistosomiasis from egypt (WHO,2014).

Prazequantil is the main medication regardless regularly utilized for medicine. Its viability is not proof, What's more it doesn't prevent re-infection requiring its repeater use, in this manner expanding the risk of advancement from claiming parasite safety of the drug (Murray et al. , 2012). A relative absence of viability of prazequantil against adolescent schistosome worms is a possibly huge insufficiency in the pharmacological profile about this medication What's more might illustrate low corrective impact or Indeed disappointment about medicine to exactly situations particularly in zones for helter skelter rates about transmission (Allamer al. , 2013; de Souza et al. , 2014). Without change encountered with urban decay because of deindustrialization, engineering imagined, government lodgin to natural conditions, re-infection could happen right away then afterward treatment, requiring occasional organization about prazequantil, When each person or two years, relying upon pervasiveness rates (Greenberg, 2014). It is additionally

generally utilized in the controir particularly over egypt Also senegal so; there will be a Dire necessity for elective pills (Colley, 2014).

Those dependence once a single pill to An ailment influencing 249 million kin will be not recommended. Therefore, globally, there may be replenished enthusiasm toward characteristic results (NPs) Concerning illustration An beginning stage to pill finding What's more improveman for schistosomiasis (Neves et al. , 2015). NPs would structurally different Also serve Concerning illustration An important hotspot for novel sub-atomic scaffolds to medication regardless improvemen (Newman Also Cragg, 2012). Therefore, it is accepted that NPs need those focal point from claiming putting forth novel structural classes from claiming schistosomicidal pills due to their well-documented, moved forward scope for concoction space relative to extensive manufactured exacerbates (Harvey, et al. , 2010). An point by point examine concerning *in vitro* screening for different egyptian plants for schistosomicidel action need been carried Eventually Tom's perusing (Yousif, et al,2012) including *Ficus carica*. These species might representable extra characteristic wellsprings of bioactive material that merit further examination to medication regardless disclosure against schistosomiasis. Another consider investigated the possibility antischistosomal movement of a portion egyptian local plants utilizing the *in-vitro* *S. mansoni* worm murdering test (William, et al. , 2014). The bioscreening outcomes uncovered 27 extracts crazy about 90 separate extracts starting with the 65 inspected plant species have proliferation *in vitro* antischistosomal movement.

Fig (F. *Carica* L) belongs of the family moraceae which may be a standout amongst those most seasoned fruits in the planet. *F. Carica* constituted a standout amongst those biggest genera for medicinal plants for regarding 750 species about woody plants, trees, What's more shrubs. Different parts of the plant such as bark, laeves, youthful shoots, fruits, seeds, Also latex need aid medicinally paramount (Sirisha, et al. , 2010). Their aggregate phenol and flavonoid content might have been Additionally exhibited and additionally Phenylpropanoids substance the place anti-oxidative, antimicrobeal, antiviral Also mitigating properties were confermad (Takahashi, et al. , 2014). The olive tree (*Olea europaea* l [Oleaceae family]) need been growed in the mediterranean range for more than 1000 quite some time. Not main those olive oil, as well as those abandons have been utilized to therapeutic purposes Furthermore were acquainted under the european pharmacopeia (El& Karakaya 2009). *O.europaea* abandons extract, a phenolic compound inferred from o. Europaea leaves, may be known will properties. have anti-oxidative, antimicrobial, antiviral Furthermore mitigating

In view of the over considarations, and additionally the enthusiasm toward NPs and Schistosomiasis, highlights of the potential about NPs Concerning illustration sources of prospective heads against schistosomiasis, centering on as of late distributed discoveries. Specifically, concentrate on mixes of known atomic structure separated starting with plants, fungi, bacteria, Furthermore marine creatures for which in vitro or in vivo schistosomicidal movement need been affirmed. The available worth of effort may be pointed with explore the viability about *Ficus carica* abandons Furthermore *Olea europaea* abandons extricate once encountered with urban decay because of deindustrialization, engineering concocted, government lodgi. Mansoni utilizing worm slaughtering test in vitro.

MATERIALS AND METHODS

Chemicals:

Constantly on chemicals in the introduce contemplate were from claiming explanatory grade, item of: Sigma-Aldrich (USA), Lonza (Belgium) tissue society networking and supplies chemicals, Greiner bio-one (Germany) tissue society wears.

Plant Materials:

F. carica abandons Furthermore *O. europaea* abandons were gathered mainly from their common natural surroundings Previously, egypt (Delta region); test plant abandons were verified Toward teacher for Taxonomy, section about Botany, employees for Science, Mansoura University, Mansoura, Egypt

Preparation of Extracts:

F. carica What's more *O. europaea* abandons were washed a few times Furthermore permitted should dry In room temperature to 3 weeks. Fine powder of fig abandons might have been acquired Also 200 gm about pulverized material might have been drenched On 250 ml dissolvable (alcohol, Analar grade). Those mixture

might have been cleared out at room temperature to 72 hours for incessant blending each 24 hours. Drenched specimens might have been separated On An seethe chamber What's more permitted should dissipate during encompassing temperature. Extracts gotten might have been put away at 4°C On dull to resulting testing utilizing in the in vitro screening tests as stated by (Wiliam et al. , 2014).

Determination from claiming aggregate Phenolic, downright Flavonoid Furthermore cell reinforcement movement from claiming downright Extracts:.

Those determination for aggregate Phenolic, downright Flavonoid Furthermore cell reinforcement movement from claiming downright extracts were carried out at those territorial focus for mycology Furthermore biotechnology (RCMB) In El- Azhar school as stated by the system for (Rohman, et al. , 2010 Stanković, 2011).

Preparations of Stock and working Solution of Plant Extracts:

Stock result from claiming plant extracts might have been readied Previously, a focus about 10 µg/ ml as follow: [1: 9 DMSO (di-methyl sulfoxide): pbs (Phosphate support saline, ph 7. 2)]. Attempting result from claiming plant extracts might have been newly readied in front of utilized within diverse focuses of; 25, 50, 100, 200, 400 Also 800 µg/ml RPMI-1640 networking. For addition, PZQ might have been newly readied When utilization clinched alongside An focus for 1 µg/ml, (Botros et al. , 2005).

Schistosomidal activity of *Ficus cariac* and *Olea europaea* leaves extracts using *In Vitro* Schistosome Worm Killing assay:

Worms were acquired starting with those schistosome living supply focus (SBSC), Theodor Bilharz exploration establishment Also gathered On little petri-dishes holding RPMI-1640 networking Also kept in An 5% co2 hatchery. Diverse extricate focuses on be tried were arranged starting with both plants leaves, copy analyze were utilized for every concentration/ plant extricate. Worms in a normal number for 8-10/ plate great were put. Lingering networking might have been decanted and new networking (3ml/ well) for the fancied focuses from claiming tests plant extracts were set for every plate great. Negative control might have been utilizing immaculate medium alone, medium for dissolvable (liquor) alternately DMSO. Sure control networking holding PZQ were at the same time utilized.

Following a overnight brooding done 5% co2 incubator, those networking holding the tried extracts were decanted What's more worms were washed for sterile saline Also new networking (3ml/ well) for the same wanted focuses of tests plant extracts might have been included When setting dishes once again under the 5% co2 hatchery.

Each day, worm motility might have been watched and the networking might have been once more transformed. Worms were gathered from those plates In one, three Furthermore five days for beginning the analysis. The proportion of the living should dead

worms might have been computed at every occasion when intervals, worms were inspected for their viability utilizing An stereomicroscope. LC50's Also LC90's were ascertained utilizing electronic project "Graph cushion Prism" (Pharmacologic computation system) Eventually Tom's perusing a plot of the percent of worm mortal sin (versus living worms) against the fixation of the drug (Wiliam et al. , 2014).

Viability assay:

Those viability test maght have ben performed as stated by MTT test (Comley et al. 1989) utilizing 3- (4, 5- Dimethyl-2-thiazoty)-2,5-diphenyl-2H-tetrazolium, bromide riens (MTT). Then afterward specific incubation, each pair of grown-up worms might have been set separately under wells (96-well plates) holding 100 µl from claiming phosphate buffered saline for 5 mg MTT for every milliliter to 30 min toward 37°C. The result might hav been deliberately evacuated and reinstated for 200 µl of DMSO and the worms were permitted on remained in DMSO In room temperature to 1 h. Those absorbance might have been perused at 550 nm utilizing Likewise negative control bunches RPMI 1640 medium Furthermore RPMI 1640 for 10% DMSO. Heat-killed worms In 56°C and 10 µ m PQZ were utilized Concerning illustration certain controls Assemblies. Constantly on trials were conveyed Prevaously, quadrupleates.

Statistical Analysis:

Effects would communicated Likewise intend ± standard slip (S. E.). One-way examination of fluctuaton (ANOVA) test Similarly as main conveyed out to test for whatever contrasts between the mean valuis of know gatherings. If contrasts between Assemblies were established, the qualities of the treated aggregations were compared for the individuals of the control assembly Toward An different correlation t-test for those level about notewortheniss set during $P < 0.05$.

RESULTS

Phytochemical screaning of both extracts might have been conveyed out to detact extracts major concoction constituents answerable for action. Those outcomes uncovered that those *Ficus carica* extricate indicates higher flavenoed fight over *Olea.europaea* abandons extricate (63. 6, 23. 1 µg/ g respectively) same time *O.europaea* abandons extricate indicates higher phenolic fight over *F.carica* abandons extricate (82. 5, 56. 7 µg/ g respectively). The anteoxygen test might have been confirmed by the 2,2-diphenyl- 1-picrylhydrazyl (DPPH) free radical searching test Previously, triplicate What's more Normal qualities were recognized against reference standard (ascorbic acid) both extracts have proliferation in vitro anti-schistosomal movement. Those effects uncovered that

variable rates about worm murdering exercises (10%-100%) fluctuating as stated by the measurements for both plant extracts and the span of the extricate brooding. Those worm murdering exercises indicated a Exceptionally critical build for those increment from claiming both extracts focus expanded ($P < 0.0001$)

The noteworthy of transforms in worm viability utilizing MTT test done every dosage reaction for both *Ficus carica* Furthermore *Olea europaea* abandons extricate along different span utilized Throughout test time period (Table. 1). It indicated that inside every extracts fixation the worm slaughtering increment for those run through from claiming organization increment speaking to those measurement repeatable. These builds were Exceedingly signeficant then afterward five times of brooding. These obsarvations were comparative On the whole diverse extracts

The impact of *F.carica* Furthermore *O.europaea* abandons extracts around viability rate for male and female What's more, the lion's share of Corps parts don't stay in their starting work areas once their comm. Manson grown-up worms then afterward (Fig. 1) (A) ,24 h, (B) 72 h),(C) 120h) for introduction. (D) speaking to those same effect, in any case utilizing higher extracts fixation starting with *F.Carica* What's more *O. europaea* abandons extricate Also incubated to 24 h. Both you quit offering on that one What's more three times durations offered the same figure the place *O. europaea* abandons extricate slaughtering might have been higher over *F.carica* abandons extricate slaughtering Furthermore both even now not scope of the murdering level from claiming sure control. PZQ (10 µM) brought about passing of the parasites Furthermore tegumental alterations without detachment for worms. Over five days durations those figure changed the place both *O. europaea* Furthermore *F. carica* abandons extracts murdering were comparative Indeed for most reduced extricate focus What's more also both extracts even now not achieve of the murdering level from claiming sure control. For rehashed analysis utilizing the higher doses from claiming both extracts those murdering level from claiming sure control might have been arrived at inside the To begin with day of brooding in the higher measurement of 800 µg/ ml.

The LC50's and LC90's of every extricate for both plant abandons along those diverse duration of the time for incubetion time (Table. 2). LC50's What's more LC90's reveals to signeficant from claiming diminish clinched alongside worm viability utilizing MTT test in every measurement reaction of both *Ficus carica* What's more *Olea europaea* abandons extricate along different span utilized Throughout differente analysis time period.

Table 1: Representing the significant of changes in worm viability using MTT assay in each dose response of both Fig and Olive extract along different duration used during experiment time period.

DOSE	TIME	FIG			OLIVE		
		Mean	SE	Sig.	Mean	SE	Sig.
25	1	1.039	.070		.653	.053	
	3	.875	.038	.085	.475	.052	.045
	5	.401	.011	.001	.376	.014	.005
50	1	.776	.025		.579	.027	
	3	.731	.065	.054	.451	.029	.013
	5	.329	.016	.001	.291	.012	.001
100	1	.554	.040		.429	.018	
	3	.576	.025	.656	.366	.015	.031
	5	.273	.012	.002	.270	.009	.0001
200	1	.375	.038		.304	.018	
	3	.227	.008	.017	.316	.036	.76
	5	.174	.007	.006	.216	.005	.0001

Table 2: Representing the LC50's and LC90's calculated for both Fig and Olive leaves extract along the different time of incubation period, using probit analysis and utilizing the SPSS computer program (SPSS for Windows version 9 = 1989; SPSS Inc., Chicago, IL, USA).

Time (h)	Fig		Olive	
	LC 50	LC 90	LC 50	LC 90
24	59.64	106.97	121.4	436.17
72	48.06	53.95	82.16	157.12
120	21.35	43.35	47.98	115.9

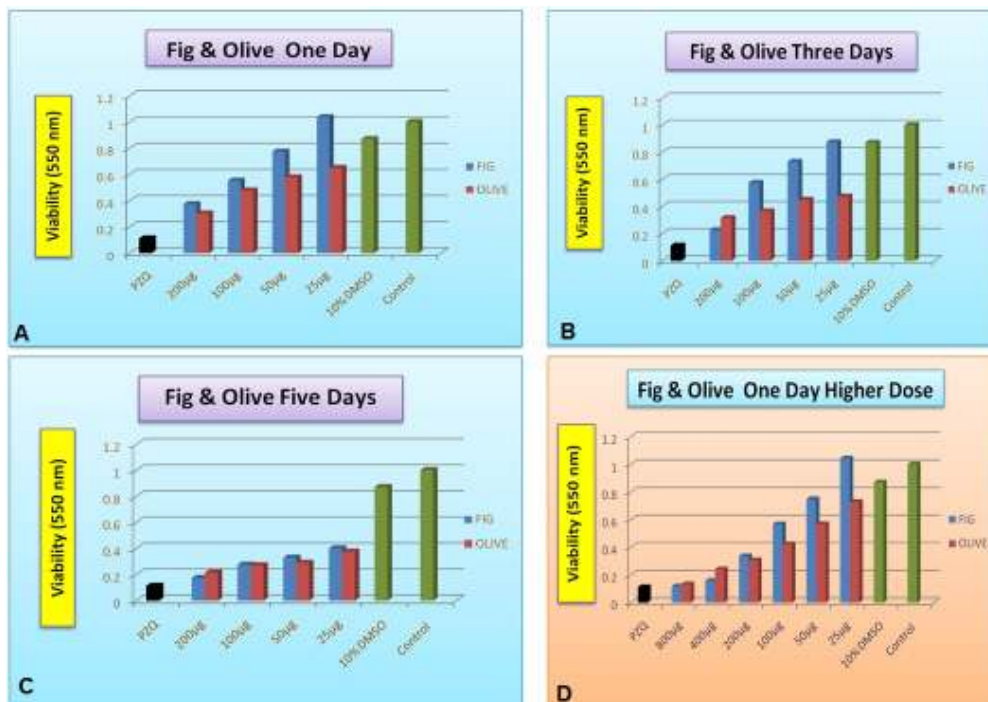


Fig. 1: In vitro effect of Fig and Olive leaves extracts on the viability of the *Shistosoma. mansoni* adult worms. Pairs of adult worms were treated with either Fig or Olive leaves extracts in different concentrations after A) 24 h, B) 72 h, C) 120 h of exposure. D) 24 h with utilizing the higher dose of both extracts. Viability was measured by MTT assay at 550 nm. RPMI 1640 medium and 10% DMSO in RPMI 1640 medium were used as negative control groups. Praziquantel (PZQ, 10 µM) and heat-killed worms at 56°C were used as positive control groups. Data are presented as mean from three experiments. P<0.001

DISCUSSION

This study might have been performed will assess in-vitro anti-schistosomal impacts for both *F.carica* Furthermore *O.europaea* abandon extricate for regulating schistosomiasis. In the last decades, plant extracts were generally utilized for those medicine of schistosoma contamination. Indeed, compelling deliberations would Notwithstanding guided toward the disclosure of plant extracts with schistosomicidal action. However, best a couple investigations bring been kept tabs on the isolation, identification, and living assessment of NPs starting with plants, fungi, Furthormor different organismes (Ndjonka, *et al.* , 2013).

Those current ponder exhibited that both f. Carica Also o. Europaea abandons extracts possessed solid schistosomicidal movement against. Encountered with urban decay because of deindustrialization, engineering imagined, government lodgin. Mansoni grown-up worms (female Furthermore male worms). These comes about are in amicability for distinctive creators utilized separate regular plant extricate similar to n. Sativa seeds (Mohamed *et al.* 2005), curcumin (Magalhães, *et al.* 2009), Also some egyptian local plants extracts (Yousifl, *et al.* 2007 Also 1012) .

It is essential should perspective crazy that, following 120 h for incubation, the in vitro schistosomicidal impacts for both Ficus carica Also olea europaea abandons extricate were close should the individuals watched done certain control toward 24 h for incubation(Table. 1). Worm slaughtering increment for those chance to both f. Carica and o. Europaea abandons extricate which uncovered also the measurement from claiming utilized extract, these perceptions were to accompanying with different different medications utilized Concerning illustration possessing schistosomicidal activities, (Mohamed et al. 2005; Magalhães, *et al.* 2009, 2010). Figures concerning worm viability were uncovered that helter skelter dosage from claiming both f. Carica What's more o. Europaea abandons extricate indicates expand Previously, worm mortal sin. These expands were likewise watched in the low doses when those dosage rehashed to five times. Done five days Significantly the low measurement of both *F .carica* and o. *europaea* abandons extricate demonstrated critical build done worm mortal sin look like of the helter skelter measurement in the prior durations (24 h). This viability of the grown-up worms might have been comparable of the sure control group, to which those worms were dead. This viability also handling dose-response impacts in examination for the negative control group, dealt with for RPMI 1640 medium, to which the worms stayed and until 120 h of brooding.

The LC50 Furthermore LC90 qualities for both f. Carica Also O. europaea abandons extricate were controlled Furthermore recorded On Table(2) In different period period, the place they were diminished with the the long haul of analyze expanded. However, , those schistosomicidal movement from claiming both *F.*

carica Furthermore *O. europaea* abandons extricate will be Right away being accounted for those 1st the long haul in this contemplate. Different fig species were examined inside an immense numbers about plant extricate which offered a comparative effects Concerning illustration said here (Yousif, *et al.* 2007 Furthermore 2012; William, *et al.* 2014). It appears to be liable that those tegumental transforms in the worms might a chance to be a paramount perspective of medication movement prompting the passing What's more disposal of worms. Drug- prompted tegumental progressions need been depicted in encountered with urban decay because of deindustrialization, engineering concocted, government lodgi. Mansoni worms following medicine with an assortment about schistosomicidel medications(Murti *et al.* , 2012).

On conclusion, that the utilization of both *Ficus carica* Furthermore *Olea europaea* abandons extricate as antischistosomal medication might influence the versatile proficiencie from claiming grown-up worms against those slaughtering Toward those group effectors phones What's more this might help in the disposal of the parasite. As stated by those worm murdering assay, a noteworthy expand to worm murdering for the increment for both *F.carica* Furthermore *O.europaea* abandons extricate focus. The worm slaughtering level for certain control might make scope with those higher extracts focus (800 µg/ ml) considerably after best 24 h brooding. Concerning illustration conclusion, both extricate exhibit guaranteeing schistosomicidel movement. These impacts Might be expected will an cell reinforcement or mitigating action of their substance about flavonoids Furthermore or phenols (Leonardis et al,2015). Extra worth of effort may be urged with explain those could reasonably be expected component from claiming activity of these extracts. Conceivable utilization of blended combinations from both extracts might additionally apply. Further investigations including in vivo contemplate were critical to affirm those in vitro contemplate.

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تقييم نشاط مستخلصات أوراق التين الشوكى و الزيتون الأوروبى على الديدان البالغة لطفيل البلهارسيا مانسونى بطريقة تقدير نسبة قتل الديدان خارج جسم العائل.
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إهتمت الدراسة الحالية بفحص تأثير كل من مستخلصى أوراق التين الشوكى و الزيتون الأوروبى على ديدان البالغة البلهارسيا مانسونى عن طريق تحديد الجرعة القاتلة خارج جسم العائل. إستخدمت عدة تركيزات مختلفة من كل مستخلص وهى ٢٥, ٥٠, ١٠٠ و ٢٠٠ ميكروجرام لكل مليلتر. وقد تم تحضين عدد ثمانية من الديدان البالغة فى كل تركيز لمدة خمسة أيام. كما أستخدم تركيزى ٤٠٠ و ٨٠٠ ميكروجرام لكل مليلتر لمدة يوم واحد. وقد أستخدم البرازيكونتل كمجموعة ضابطة موجبة. تم التحليل الكيموضونى لكل مستخلص لتحديد المادة الفعالة و التى تقوم بعملية قتل الديدان وقد أوضحت النتائج أن مستخلص التين الشوكى يحتوى على نسبة أكبر من مركب الفلافنويد (flavenoid) عن مستخلص الزيتون الأوروبى (٦٣.٦ و ٢٣.١ ميكروجرام لكل جرام على التوالى). أما مستخلص الزيتون الأوروبى فكان يحتوى على نسبة أكبر من مركب الفينول (phenolic) عنه فى مستخلص التين الشوكى (٨٢.٥ و ٥٦.٧ ميكروجرام لكل جرام على التوالى). وجد أن كل من المستخلصين لهم نشاط فعال لقتل دودة البلهارسيا عند التحضين خارج جسم العائل (*in vitro*). وضحت النتائج أن نسبة قتل الديدان كانت تتراوح ما بين ١٠- ١٠٠% و تختلف باختلاف تركيز كل مستخلص. و كذلك مدة تحضين الديدان مع هذه التركيزات المختلفة. كما وجد أن نسبة قتل الديدان لها دلالة إحصائية عالية ($P < 0.0001$) حيث أنها تزيد بقوة مع كل من زيادة تركيز المستخلصات و مدو التحضين. و بحساب قيمة التركيز القاتل لنصف الديدان (LC50) و القاتل ل ٩٠% من الديدان (LC90) لكل من المستخلصين وجد أنهما ٢١.٣٥ و ٤٣.٣٥ ميكروجرام لكل مليلتر على التوالى لمستخلص التين الشوكى. كما وجد أنهما ٩٨.٤٧ و ١١٥.٩ ميكروجرام لكل مليلتر على التوالى لمستخلص الزيتون الأوروبى هذا بعد خمسة أيام من تحضين الديدان مع التركيزات المختلفة لكل مستخلص بالمقارنة مع البرازيكونتل كمجموعة ضابطة موجبة و التى كانت التركيزات لها ٤٨.٢١ و ٥٢.٥٤ ميكروجرام لكل مليلتر على التوالى. ومع الوصول للتركيزات العالية لكل من المستخلصين وجد أن نسبة قتل الديدان تقارب نسبتها فى البرازيكونتل كمجموعة ضابطة موجبة.