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Effect of extraction solvent of *Allium sativum* on *fasciola hepatica* in vitro Samia

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Abstract:

The current research to determine the anti-activity of *Allium sativum* (garlic) on the hepatic fascia parasite, the study included the effect of aqueous extract and ethanolic extract of *Allium sativum* on the growth and efficacy of *fasciola hepatica* cultivated in 50% of sheep red blood cells with 15% dilution agar technique. Halving was counted within 96 hours of growth when inhibiting concentrations (1,1.5,2,2.5) $\mu\text{mg} / \text{ml}$ were applied to the plant after drying and obtaining the crude, and then dissolving it in each extract after determining the concentrations separately by following scientific methods. The results revealed that the ethanolic extract had a higher effect than the aqueous extract. Where a high concentration (2.5) for both extracts was observed to inhibit) for ethanolic extract $8 \pm 10 81035.6991x$) for the aqueous extract and ($8 \pm 10 81046.511x$) during 96 hours of growth. $8 \pm 10 81060.000x$ when compared with the control group (We can conclude that medicinal plants can affect microorganisms effectively, even if the proportions differ without affecting the affected host, unlike chemical compounds, and this is what we observed during the research.

Keywords: aqueous and ethanolic extract, *Allium sativum*, *fasciola hepatica*

Introduction:

Fasciola hepatica (liver accident) is a parasite of extraordinary financial significance, with an overall appropriation. The parasite contaminates an assortment of mammalian hosts and is liable for noteworthy misfortunes in creature creation assessed at over US\$ 3.2 billion every year (Mi et al, 2016) *Fasciola* contaminates around 2,000,000 and half of the human populace around the globe, and a huge number are stay in high danger to disease (Beesley et al, 2017). Later and progressed atomic methodologies were used to the exact investigation of *Fasciola* as a parasite. Most ordinarily utilized strategies were; Random Amplified Polymorphic DNA (RAPD). (Aryaeipour et al, 2014), single nucleotide polymorphism (SNP) (Cwiklinski et al, 2015), PCR-confined section length polymorphism (PCRRFLP) (Simsek et al, 2011), and sequencing of the entire genome The accident arrives at the liver, where it develops and discharge eggs through bile, being wiped out to the climate through the dung. This accident was begun in the Eurasian close to east area, arriving at a practically overall circulation, being available in a few nations from Africa, Americas, Asia, Europe and Oceania (MAS-COMA et al., 2007). Ungulates and people become tainted through ingestion of polluted water or plants containing metacercariae, prompting unfavorable wellbeing consequences (Cwiklinski et al 2015) Disease of *Fasciola* in man originates from access through utilization of water or potentially water plants sullied with the metacercariae. In certain nations watercress establish

primary element of plates of mixed greens and human disease is more successive in such populace contrasted with those where it isn't devoured. Utilization of crude lettuce and other oceanic plants, and water from water system channels are likewise significant wellspring of contamination with fascioliasis in people. Drinks like horse feed have likewise been involved in endemic territories for sending fascioliasis in people. In India, however contamination of *F. gigantica* has not been accounted for up until now, yet thinking about the relationship of provincial individuals with creatures and its current circumstance, the likelihood of human disease ought to be high. Trapa, an oceanic organic product developed during storm season in India where *L. auricularia* positive for *F. gigantica* disease are additionally richly found, can be a significant wellspring of contamination to man *F. hepatica* disease has two unique stages with very various signs and side effects. (first, intense or intrusive) phase of the ailment happens when the creature punctures the liver and starts to relocate through the liver parenchyma towards the biliary radicles. The beginning of this stage happens 1–3 months following ingestion of metacercariae. Fever, urticaria, torment in the righthypochondrium, hepatomegaly, hypergamma-globulinaemia and stamped eosinophilia are the traditional signs and manifestations of this stage. Gentle hepatitis, extreme subcapsular discharge and blunt hepatic rot can likewise be watched. A mix of the side effects of outright eosinophilia, fever and right upper quadrant torment ought to infer the chance of *F. hepatica* disease. The second or biliary stage generally gives discontinuous right upper quadrant torment with or without cholangitis or cholestasis. Eosinophilia can likewise be identified (Schiappacasse et al, 2005).

Allium sativum:

Allium sativum (*A. sativum*) or garlic has been utilized as both food and medication in numerous societies for a huge number of years, Garlic is a lasting bulb, thought to be indigenous to Central Siberia, Asia and west of the Himalayas and has been developed in England from before 1540. It is currently broadly developed everywhere on the world (Tattelman, 2005))

Avicennas, law book, has referenced garlic as a valuable plant in the therapy of joint ailments, mark torments, ongoing hack, parasitic contaminations, snake nibbles, ladies' and sicknesses, diseases and cerebral pains, particularly one way migraines (Avecina, 1991) in light of the fact that the broad conviction of the remedial properties of garlic among the individuals, it has been particularly focused on in the advanced medication as a restorative plant, too Garlic has various mixes, for example, prostaglandins, gelatin, adenosine, nutrients E, C, B6, B2, B1, A, biotin, unsaturated fats and fundamental amino acids (McNulty et al, 2008). A few helpful impacts and various properties have been referenced for this plant with the goal that it has been considered as the disinfectant of stomach related plot, energizer plant, diuretic and tidbit (Jakubowski, 2003). It has been perceived as a substance which applies a control on microorganisms. 1–3 s surprising for various possibly dynamic synthetic constituents likewise as a flavor. It contains seventeen amino acids as arginine, in any event 33 organosulphate mixes as aliin and allicin, eight minerals (germanium, calcium, copper, iron, potassium, magnesium, selenium and zinc), proteins as allinase, and the nutrients A, B1 and C. The physiological development of dietary *A. sativum* is ascribed to allicin (diallyl thiosulphinat), which is one of the organosulphate mixes found in the bulb. It is answerable for the counter microbial properties and the trademark kind of new garlic. To control of this ailment has been founded on the use of anthelmintics, however because of the improvement of opposition it appears to be that the viability of some synthetic medications has diminished (Ceballos et al, 2010) The utilization of plants with anthelmintic movement might be an option in contrast to accident control, given the incredible variety of biological systems. The chance of finding bioactive mixes with hostile to accident properties altogether increments on the grounds that recent considers have announced the anthelmintic

impact of plants, for example, *Artemisia Mexicana*, *Mentha piperita*, *Achillea millefolium*, *Allium sativum*, *Piper nigrum*, and *Carica papaya* with parasiticidal impacts against *F. hepaticus*. This examination meant to evaluate how the ability of *Allium sativum* L concentrates can be repress development.

Materials and methods:

This investigation was begun between Jan–Sep 2016. Where is taken Five ml of blood tests from various country region in Nasiriya (thi_qar) the jugular veins of 100 sheep, which were chosen from different areas of by arbitrary examining technique. Fasciolosis seroprevalence in sheep was examined by the ELISA strategy, utilizing business packs (ELISA *F. hepaticus* test) and put away at 25°C in Eppendorf tubes for ensuing serological and Laboratory tests. Complete blood picture was directed utilizing automated hematology analyzer (Genex) while leukogram was performed by the technique recently depicted (Barger and MacNeil, 2015). was chosen *F. hepaticus* from every creature and set among slides and were fixed in Bouin answer for 24 hours. After this period, the examples were histologically handled after the strategy (Toner et al, 2011)

Cultivation of *Fasciola hepatica*:

The way of life medium NCTC 135 included with half sheep red platelets have been discovered to be best of many culture mediums tried for the development and advancement of excysted *F. hepatica* metacercariae. grown-up accidents gathered in chilled ordinary saline from liver tissue and bile channels, to eliminate hints of host tissue and bile material it must washed altogether with typical salt arrangement (0.15M NaCl). *F. hepatica* is likewise brooded in 6 hours at 37 °C, RPMI 1640 medium containing 15 ml⁻¹ 25 mM HEPES support, 7 ml penicillin 100U/ml, streptomycin 7.5% sodium bicarbonate, 10 g/ml hatched at 37 °C for 24 hours. To cleanse characterized accident grown-up *Fasciola* are brooded in RPMI 1640 containing 2% glucose, 30 mM HEPES and gentamycin 25 g/ml at 37 °C for 3 hours. The parasite is as yet dynamic toward the finish of all these hatching.

Bioassays:

To decide the antihelmintic impact of the plant removes on the mortality of excysted accidents a progression of in vitro tests were embraced. Recently excysted flukes were gotten by the counterfeit excysment of *F. hepatica* metacercariae following the strategy described by Ibarra and Jenkins (Ibarra and Jenkins, 1984) This investigation was directed in the Environmental Health Department in Thi-Qar utilizing by agar weakening strategy. The base inhibitory focus (MIC) and least parasitological fixation (MBC) of garlic to control clinical segregates of *f. hepatica* were resolved utilizing agar weakening strategy

Plant material collection:

Aqueous extract:

Assortment of plant material *Allium sativum* inspected in bought from Attar natural market and logical name was resolved in the herbarium It was mixed new garlic Lobes (80 g) in 30 ml sterile refined water and centrifuged at 5000 rpm and cleaned by filtration (0.45µm). By taking away the heaviness of the insoluble material from the heaviness of the first Lobes, the last centralization of the garlic separate in arrangement was resolved to be half (w/v). Aliquots were put away at – 25°C until required. (Miron et al., 2002) .

Ethanollic extract:

They were ground to fine powder in fluid nitrogen utilizing mortar and pestle and put away cautiously at 30 –20°C. 5mg of powdered material from unrefined was absorbed 50 ml of 70% ethanol weakening with 20% refined water and was kept at room temperature for 24 hrs; and afterward, they were sifted utilizing Whatman No. 1 channel paper. The filtrate was warmed at 30-40°C utilizing water shower, to totally eliminate any remaining parts of ethanol and get dried powder It was then gauged and disintegrated in equivalent measure of refined water to acquire 2g/ml convergence of ethanol separates. These concentrates were put away as aliquots at 5°C for additional testing (Lee et al, 2004).

Determination of concentrations:

All techniques were performed under aseptic conditions using a laminar stream hood. At the beginning before decided a concentration of Plant separates it must have added to ascertain the deadly focus to slaughter (1, 2, 3, 4, 4.5, 5, 5.5) µmg of the accidents (LC50) and afterward it has seen the executed concentrations was between (3mg, 4mg) Accordingly, the fixations utilized for the examination were determined (1 µmg, 1.5 µmg, 2 µmg, 2.5 µmg).

Apply extract of plant on sample of f. hepatica:

put f. hepatica in NUNC culture dishes All mixes were defined as follows: 600 mg of the compound were set in a screw-covered 20 ml Eppendorf tube to which 70% ml of ethanol in 20% refined water were added to disintegrate the concentrate. At that point two crease dilutions using refined water were made to get ready centralizations of 400, 200 and 100 mg/L. Each very much contained 1.5 mL of RPMI-1640 of the way of life medium, 0.3 mL of solubilized remove and 0.3 ml containing 10 liver accidents. five wells were utilized as untreated controls, four containing just a complete medium (RPMI-1640), the last one containing a culture medium (control) and 0.2 ml of ethanol CO₂ air; each examination was replicated three times. The plant extricates with in vitro anthelmintic efficacy higher than 80% were reevaluated twice all together to confirm the outcomes, and a grouping of 375 mg/L was

Calculated of f. hepatica:

For 96 h every day of hatching worms were fixed with 3% (v/v) glutaraldehyde in PBS cushion (pH 7.4) for 10 second for The examples are not harmed at room temperature. Washing, drying out and fixation of slides was conveyed out according to the strategies portrayed by Keiser et al. (2006b). Slides were inspected by high goal

SEM The adequacy of the plant separates was surveyed with the following recipe

Efficacy % = $\frac{\text{No: of flukes alive in control group} - \text{No: of flukes alive in treated group}}{\text{No: of flukes alive in control group}} \times 100$

No: of flukes alive in control group x100

Statistical analyses:

The test was performed with POLO PLUS (LeOra et al, 2003) to decide the LC50 of the concentrates that appeared in vitro fascioliscide adequacy A Kruskal-Wallis test, P < 0.05 was utilized to determine significant contrasts and a PROBIT (Statgraphics, 2011).

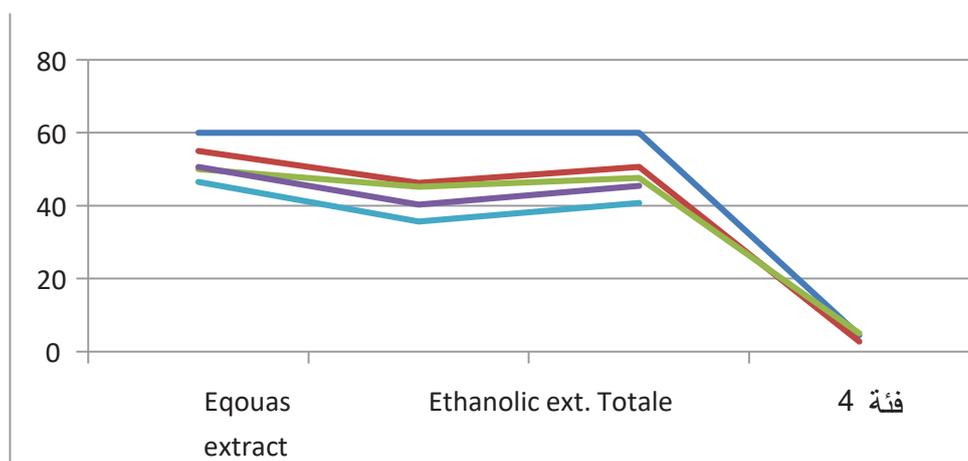
Results:

the old various human advancements, there were various plants have been utilized as medications and so as to treat and prevent from various infections (Jiang et al, 2010). Allium sativum L is a palatable and restorative plant from Liliaceae family which was being utilized in old Iraqi medication in treating diverse diseases. Garlic (Allium sativum) removes had antimicrobial action against the two tried life forms at the base inhibitory focuses (1.1, 5, 2, 2.5) mg/ml. Results indicated antibacterial action of garlic (Allium sativum) against F. hepatica. Tables see the impact of Garlic separate on the development and practicality of Parasite F. hepatica

Table (1) effect of mixit between concentration and treatment on growth and active of parasite.

ml / mg concentration					Treatment
10 ⁸ ± 10 ⁸ X number of parasite					
2.5	2	1	1		
		.5			
46.511	50.671	50.070	55.021	60.000	Eqouas extract
35.699	40.348	45.231	46.302	60.000	Ethanollic ext.
40.796	45.509	47.650	50.661	60.000	Totale

D 0.05= 1.52



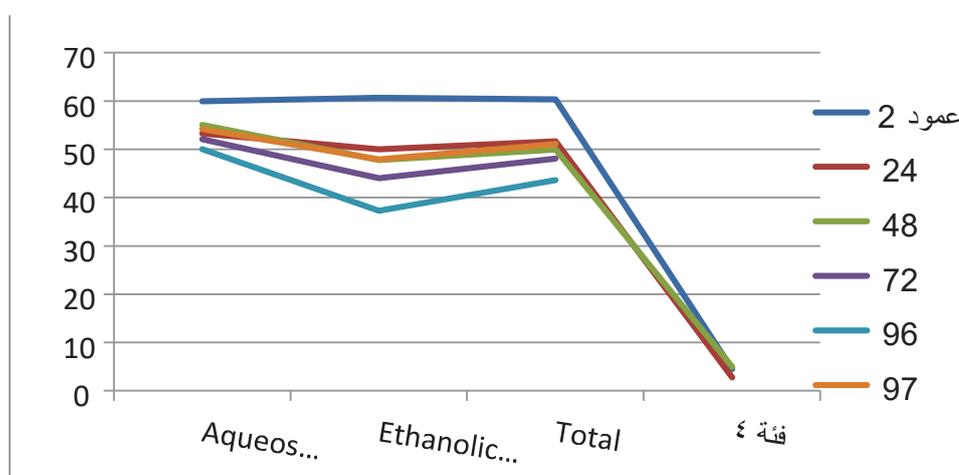
4.1 Effect of extaction on active and growth of fasciolla h

while we look at this table we can see the ethanollic extract killed more than aqueous extract by inhibited many number of parasite. we can limited the aqueos extract inhibits approximately (46.511x10⁸ ±10⁸) in (2.5) concentration but the inhibition of ethanollic extract arrived to (35.699x10⁸ ±10⁸) in the same cocentratin when we comparad with control group.

4.2 Table (2) effect of mixit between time and treatment on growth and active of parasite

Total	Time (hour)					Treatment
	96	72	48	24		
54.238	50.072	52.111	55.100	53.261	60.000	Aqueos extrat
47.944	37.300	44.000	47.820	50.000	60.698	Ethanolic extract
51.091	43.686	48.055	49.965	51.630	60.349	Total

R.L.S.D 0.05= 1.52



4.3 Effect of time and extract on active and growth of fasciolla h:

The result in table (2) show significant different , see the inhibition of parasite increases with time that mean whenever increase of time ,there are more killed of parasite population and we Can be seen vary a number Simple proportions of warme through treated the sample by equeous extract but the ethanolic extract Continuously inhibit a number of parasite Significantly more than aqueose extract it reached to $(37.300 \times 10^8 \pm 10^8)$ while the aqueos extract in the same time reaching to $(50.072 \times 10^8 \pm 10^8)$.

4.4 Discussion:

The right selection of the solvent for extraction helps to obtain the desired bioactive compounds from plants: solvent of similar polarity will suitably dissolve the solute. The examples of solvents with different polarity are hexane, chloroform, ethyl acetate, acetone, methanol and water. Microwave-assisted extraction is especially used in extracting antioxidants. In this process, the required amount of solvent and the time of extraction is reduced due to participation of electromagnetic waves which heat up the solvent. our study was used two type extracts (equeous extract and ethanolic extract) diluted on it crude of *Allium sativium*L with concentration used this extracts on *f.hipatica* parasite to signification how can be this extract to controlling on disease and wich different between them when we use same concentration in the same time

..looking in this study the alcohol extract more active and speed with inhibit the parasite than equeous extract.

our study agree with (Chehregani *et al*, 2007) Results viewed that the highest antibacterial activity of *Allium sativum* was against *Staphylococcus aureus*. *S. aureus* was extensively studied and its sensitivity to plant extract is reported widely . and deal with investigation of essential oils' effect on fungi, isolated from mint, nettle, marigold, horsetail and corn silk, the most oregano and thyme oils (Stevic *et al.*, 2014). The antifungal properties of the oils tested could be assigned to the activity of phenol compounds carvacrol and thymol, since these compounds are marked as having the highest antifungal potential. Therefore, the combination of carvacrol and thymol is significant in the overall effectiveness of essential oils (Stevic *et al.*, 2014). And deal with(Ross *et al*, 2001) when he used Garlic extract caused death of microorganisms through oxidative stress as was demonstrated in *Candida albicans* with concomitant inhibition of both growth and respiration of the yeast .And there is same result we can see in(Asdaq *et al*, 2010) where has been prover ethanolic extract more active than aqueous extract ,he given due to allicin product is isolated in alcohol ,this material more effect on microbiological.

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