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Article

The Impact of Qurecus Persica Stem Peel Secondary Compound Extracts on Enterobius Vermicularis Parasite in Vitro

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Abstract: Q. persica plant stem peel found in north region of Iraq and has active compound and also used in traditional medicine in ancient time. the present analysis was done to assess the impacts of cold as well as hot water extracts of Q. persica stem peel in the viability of E. vermicularis in vitro as well as detect the poignant plant for extraction secondary compound including alkaloids and phenolics to test in paralyze and killing of worms. The plant was purchased from Al-Hikma Herbarium, then the all extracts were prepared and experimented on this parasite, the results displayed that cold water extract of Q. persica stem peel have powerful impact especially the concentration of 60 mg/ml, in an average time (presented as mean± standared devision) for pinworms paralyzis and die equal to 143.66 ± 0.33 and 195.00 ± 0.57 min, respectively, with a significant differences. Concentration 60 mg/ml of praziquantil drug was succeeded against the pinworms; it led to paralyze it in 180.00 ± 0.57 mins, while the average time for causing death was 240.00 ± 0.57 i8 mins.In context of secondary compounds of Q. persica stem peel, a higher impact of phenolics on pinworms, it led to paralyzes and die at 80.00 ± 0.57 and 111.66 ± 0.88 min, respectively, especially in 60 mg/ml concentration; the alkaloids come behind phenolics, it was leading to paralyzes and die with 60 mg/ml concentration at 211.0 ± 0.57 and 203.0 ± 0.57 min, respectively.

Keywords: Q. Persica Plant, Q. Persica Stem, E. Vermicularis in Vitro

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1. Introduction

E. vermicularis (pinworm or threadworm) is considered as the most common intestinal parasitic worm infection of humans [1], it is a usual parasitic infection widespread all over the world and commonly leading to an infection in school-aged children, especially in rural and remote regions as well as poor urban regions, due to the phenomenon of fingers sucking [2]. One of the most important manifestations of infection is anal itching, which occurs as a result of mucous secretions of eggs around the skin. Infection with this type of pinworm is acquired as a result of close contact between infected and uninfected individuals, swallowing the egg or inhaling it [3]. As well, reinfections one of the foremost reasons of expansion of pinworms infection, also it could lay out by fecal-oral mean [4]. However, the entire life cycle of this worms from egg to an adult worm, ordinarily takes place in nearly 2-4 weeks [5].

Generally, disorders associated with pinworm infection include abdominal pain, urinary tract ulceration, eosinophilic ileocolitis, abscess of pelvic ,inflammations of anal or vaginal regions, intestinal infuriation and difficulty falling asleep.

The exclusive natural host for pinworms is human, as they reside in the large intestine, which residing in the large intestine [6], E. vermicularis are usually diagnosed by microscopic examination to look for their distinctively shaped eggs [7] As for collecting

samples for research purposes, it is done in the morning before defecation and before washing the anal area by taking swabs of the anal area using commercial adhesive cellulose tapes [8].

Praziquantel(PZQ), Albendazole and mebendazole are byproduct of Benzimidazole carbamate, which are consider as the predilection drug for E. vermicularis curing [9], (PZQ) is a pyrazinoisoquinoline derivative [10], It is the first pharmaceutical drug prescribed for deworming, which meets the requirements of the World Health Organization and is described against a wide-range of helminthes [11].

Despite the comprehensive and exciting effects of this treatment, the exact mechanism by which it affects adult worms is still unknown, the majority of evidence indicates that tegument and muscles of the sensitive parasites are inevitable targets of PZQs' action within seconds to exposure [12], where the adult worms undergone to a quick and persist musculature's contraction [13], along with vacuolization and disrupting of the parasite's tegument [14]. What happens in both responses may be related to the disrupting of calcium homeostasis dependent on the praziquantel [15]. The global protocol for treating E. vermicularis involves anthelmintic medication like mebendazole. Treatment with mebendazole has shown efficacy in eradicating pinworms, as seen in cases of liver infection due to E. vermicularis. Iraqi protocol to treat E. vermicularis parasite: Albendazol cap 400 mg single dose Repeated after 14 days.

Recently, the use of medicinal plants has spread to treat many diseases and to strengthen public health in general (10), where the people have been consuming various plants for their necessary demands such as food and medication, Therefore, plants have been utilized in alternative medication to treat or prevent various diseases that affect humans [16]. Notable benefits of the therapeutic uses of these plants include their safety, accessibility, effectiveness, and economic importance [17].

Medicinal plants can be mixed with drugs in order to improve the quality of these drugs and for economic purposes, as the drugs have high prices and are not free of side effects. Therefore, these herbs are used to reduce their side effects [18]. Many of the natural components within these plants are considered important antioxidants in various ways to reduce the manifestations of oxidative stress, whether in terms of eliminating free radicals or stimulating the effectiveness and gene expression of antioxidant enzymes [19].

Quercus spp. are medicinally significant plant and have been used since ancient times to treat large number of diseases [20]. Quercus spp. well-known as oak as well, deems as an essential genus of the Fagaceae family, it is exceedingly present in moderate forests of northern-hemisphere and tropical climatic regions [21]. A wide-range of its species had used to treat various human diseases and disorders, such as hemorrhoids, wound treatment, diarrhea of various causes, asthma, and stomach ulcers. Due to their possession of a wide range of biologically active compounds like: flavonoids, triterpenoids, and phenolic acids, these plants have a variety of health and biological importance. They act as preventive agents against diabetes, liver disorders, and various types of cancer. In addition to being important antioxidants, they have anti-bacterial and anti-inflammatory effects as well [22].

Kurdistan of Iraq contains Q. persica oak which is widely distributed in it, also present in Turkey, Syria, Persia, Cyprus and Greece. Herbal plants are unique in that they have high potential efficacy and low cytotoxicity as a result of their possession of phenolic compounds, especially tannins. Therefore, they are considered promising future therapeutic alternatives with almost non-existent or low toxic effects. Alkaloids, are substance of a extremely varied class of the secondary metabolites of plants possess a biological efficacy such as anticholinergic, anticancer, diuretic agent, anti-viral, Hypotensive action, Pain reliever, anti-ulcer and anti-inflammatory. Alkaloids are organic compounds characterized by the presence of cyclic nitrogen and include atropine, scopolamine and hyocsyamine, which are utilized in medical aspect, Alkaloids are also used in the field of controlling insects and herbivores to reduce their harmful economic effects [23], they act as a trigger to the nervous transport systems [24] In his daily life, humans consume different types of these compounds, such as caffeine, nicotine, opiates and cocaine.

As for phenols, they are characterized by the presence of an aromatic hydrocarbon ring with one or more (OH) groups, precursors phenylpropanoid pathway were attached. They are graduated in complexity, from simple compounds such as phenylpropanoids, coumarin and benzoic acid to more complex compounds such as tannins, stilbenes, as well as flavanoids [25], phenolics are of particular importance because they have toxic effects on insects, as they are used as pesticides due to their toxic effects on them [26]. It also has antibacterial and antifungal properties beside to its importance in absorbing UV-rays.

The goal of present work is to evaluates the impacts of active materials in Q. persica plant stem peel extracts on E. vermicularis parasite in vitro

2. Materials and Methods.

2.1 Plants Collection

Q. persica stem peels were purchased from Al-Hikma herbarium located in the center of Hilla city/Iraq during the month of September-2023. the plant was identified by a specialist in the Botany Branch/College of Science, University of Babylon. The plant was ground to obtain a fine powder and kept in special containers and kept in the Herbarium laboratory /Pharmacognosydepartment/ College of Pharmacy/University of Babylon.

2.1.1 Preparation of extracts of Qurecus persica:

The current study included the preparation of cold and hot water extracts for the purpose of conducting the experiment in vitro. The method for preparing the cold water extract from this plant included dissolving 10g of the powder in 400ml of distilled water using a blender for 30 mins, after which it was placed in test tubes and centrifuged for ten minutes at 3000 rolls/mins. Then it was dried in an oven at 45°C, and the dry extract was kept in the refrigerator until the experiment was conducted. As for the hot water extract, it was prepared in the same manner except for using boiled water instead of cold [27].

2.1.2 Extraction of secondary Plant component Crude1 alkaloids

10 g of dried powder was extracted by placing it in filter paper fixed with special thimbles, and then ethanol (99%) was added to it for 24 hours. After this step, the product was concentrated using a rotary evaporator. Then dissolve in 5ml ethanol, and added 30ml Sulfuric acid 2% for removing any remaining ethanol. Mayer test gave white color to confirm the presence of alkaloids. Hydroxide ammonium %10 was placing in the separation funnel. And when added 10ml of chloroform, mixture of product had separating into (2) layers, bottom layer was taken as it include alkaloids (that also concentrated with rotary evaporator), formed dehydrated product save in refrigerator [28].



Figure 1. Separation funnel instrument for alkaloid.

Crude phenolic

Procedure referenced by [29] was applied to extract phenolics, 20g of dried extract placed in a glazier flask has 400ml acetic acid(%2) by utilizing a reflex condenser in 70C water bath for 8 h. This suspension filtered and putted it with N-propanol, sodium chloride in the funnel of separation; the top layer containing phenolics was taken (also concentrated by evaporator rotory) and finally saved it in refrigerator.



Figure 2. Separation funnel instrument for phenolic.

2.2 Parasites Collection assay

Adult pinworms were collected from children from the city of Hilla, the center of Babil Governorate in the State of Iraq. The method referred to in the study [30) was followed to collect and preserve E. vermicularis samples.

2.2.1 Microscopic assay

Helminth samples collected from children were diagnosed employing a light microscope applying the wet drop procedure for adult worms or eggs observation at microbiology Laboratory/laboratory and clinical department/pharmacy college/University of Babylon.

2.3 The Impact of Qurecus persica extracts on E. vermicularis

From both extracts, a stock solution was prepared at a concentration of 60 mg/m by dissolving six grams of the previously prepared dried extract in 100 ml of distilled water, which was used to prepare the other concentrations of 20, 40 and 60 mg/ml. As for the control, it was prepared only from phosphate buffer saline. The study protocol included comparing the effectiveness of cold and hot aqueous extracts versus the drug praziquantel at the same concentration. The worms were divided into four groups, each group containing five worms, one of which represented a control group, and the other groups were treated with the specified concentration of both the extract and the drug. The time required for the worms to reach the stage of complete paralysis and death was documented, and the time of paralysis was confirmed using external stimuli. The time required for complete paralysis to occur is called the paralysis time, while the time of death was recorded after the immobile worm died and the color of its body faded [31].

2.3.1 Testing the extracts effectiveness

To examine the effectiveness of the extracts under study, the pinworms were collected in a Petri dish containing phosphate, then these dishes were placed in the incubator, and after approximately an hour, different concentrations of the extracts and the drug were placed in the Petri dishes containing the groups of worms individually [32] .

2.3.2 Testing the Praziquantel drug effectiveness

From praziquantel drug the stock solution has been also prepared by mean of placed 600 mg (or one tablet) of praziquantel drug subsequent to grind into powder in 10ml of distilled water to get the of concentrations of 60 mg/ml and the 20,40,60 mg/ml concentrations have prepared besides control group, the time required to get paralysis and die have been recorded with 3 replicates, then about 1ml from these concentrations had added to a petri dishes that contain pinworm (each on its own) .

2.3.3 In Vitro Evaluation of E. vermicularis Viability

The motion and dying of E. vermicularis has been observed by looking, Immobile or dead worm was recognized [33] .

2.4 Statistically analysis

The current data were analyses by using factorial experiment with completely randomized and performing the least significance differences at level (P<0.05) by employing SPSS statistics system.

3. Results



Figure(1): Adult females of Evermicularis(10X).



Figure (2): Adult females of E.vermicularis(10X).





Figure (3) Ova with larvae of Evermicularis (400X). Figure (4) Adult females of Evermicularis (10X).



Figure 4. Adult females of E.vermicularis(10X).

To solve the problem, it was necessary to develop a composition that, combining minimal concentrations of active substances, would provide results superior to known analogues, including by enhancing the adaptive capabilities of the body, accelerating recovery processes and reducing the degree of fatigue after intense physical activity.

Table 1. Impact of concentrations overlapping for cold and hot water extracts of Qurecus persica on worm paralysis and dying In Vitro.

Quiecus persicu on worm pararysis and aying in vitro.				
	Extract type	Extract	Time of paralysis/ mins	Time of dying/
ıţ		concentrations		mins
a lant		mg/ml	Means ±S.D.	Means ±S.D.
sic		60	143.66 ± 0.33	195.00 ± 0.57
Qurecus persica	Cold extract	40	202.00 ± 0.57	243.00 ± 0.57
		20	220.00 ± 0.57	295.00 ± 0.57
		Control	1218.33 ± 32.37	1584.66 ± 2.84
Ō		60	159.00 ± 0.57	213.00 ± 0.57
	Hot extract	40	190.00 ± 0.57	253.00 ± 0.57

20	226.00 ± 0.57	282.66 ± 0.88
Control	1239.33 ± 64.59	1592.00 ± 3.51
LSD at probality level 0.05	76.6	5.1

Table 2. The Interference impacts of Secondary Compound Concentrations for Qurecus persica plant on Paralysis and dying of E.vermicularis In vitro.

Secondary	Extract concentrations mg/ml	Time of paralysis/ mins	Time of dying/ mins
compound		Means ±S.D.	Means ±S.D.
	60	80.00 ± 0.57	111.66 ± 0.88
Phenolics compoun ds	40	100.00 ± 0.57	146.00 ± 0.57
hen Jimig d	20	131.00 ± 0.57	189.00 ± 0.57
⊡ υ	Control	1252.66 ± 32.91	1563.66 ± 1.85
ls n	60	211.00 ± 0.57	203.00 ± 0.57
aloid 1pou ds	40	331.00 ± 0.57	412.00 ± 0.57
Alkaloids compoun ds	20	511.00 ± 0.57	641.00 ± 0.57
Ψ ö	Control	1259.00 ± 30.00	1577.33 ± 6.06
LSD at prol	LSD at probality level 0.05		7

Table 3. The impacts of Praziquantel drug on Paralysis and dying of E.vermicularis In vitro.

deve consententions (ma/m1)	Time of paralysis/ mins	Time of dying/ mins
drug concentrations (mg/ml)	Means ±S.D.	Means ±S.D.
60	180.00±0.57	240.00± 0.57
40	201.00±0.88	275.66±0.33
20	328.00±2.51	442.00±0.57
Control	1252.66±40.95	1562.00±2.30
LSD at probality level 0.05	67	4

4. Discussion

Pinworms (E. vermicularis) are parasitic worms that are parasitic on humans, with a global distribution that is widespread in temperate regions. The use of medicinal plants represents the oldest form of treatment and medication, as it has been used for very many years as one of the traditional medicine options in treating and preventing various types of diseases and disorders [34], [35].

The outcomes of current work displayed a great effectiveness for a cold water Q. persica extract versus to a boiling extract on the paralysis and dying of E. vermicularis parasite. The ability of cold water extract to demolish or killed a pinworm could be justified on the basis that this extract contains active substances (coumarins, flavonoids, phenolic acids, carboxylic acids, amino acids along with vitamins) that influence the neuro-muscular system and caused the death of adult pinworms [36]. For example, as a result of it containing phenols (acids ferulic acid and synapic acid), it leads to alter a PH environment of pinworms and killing it. Also, phenols, due to their solubility in water, can stick with tegument cells of worm and block it's action [37].

The high potential of Q. persica cold water extract compared to the extract prepared from boiling water can be attributed to the fact that boiling water can cause the destruction

or damage of the active substances in the plant, which negatively affects its effect on worms.

Therapeutic effects of Q persica plant can be attributed to consume the bioactive elements including control the cell metabolism, regulation of immunological system, antioxidant capacity, anticoagulation [38], differential plant types of Q. persica having a contrasting impact on body metabolism and overall health [39].

Q. persica plant possess considerable activity versus other simple disinfectant substances [40]. This plant has important therapeutic and medical potential as an antitumor, anti-diabetic, anti-various cardiovascular disease, in addition to being an effective anti-microbial and antioxidant. [41].

This part of the ongoing investigation aims to reveal the effectiveness of secondary compounds in causing paralysis, its worsening, and the worm reaching the stage of death. The current study proved that phenolic compounds have greater effectiveness compared to alkaloids, where it causing paralysis and dying of pinworms in a relatively short time, the time for paralysis pinworm worm was 80.00 ± 0.57 mins while for dying was 111.66 ± 0.88 mins.

This finding appears that phenolics compound of Q. persica plant may be effect as a result to their ability to mitochondrial disrupt respiratory process, inhibition of carbohydrates and fats as well as mediates protein metabolism, which are crucial for pinworms viability Or it may be attributed to the ability of these chemical compounds to destroy the cell membrane containing proteins and fats. Therefore, treating pinworms with these extracts leads to the dying of pinworms in a relatively short time. A previous investigations revealed that extracts containing phenolics inhibit the enzymes of respiratory chain that involve thiol group which is substituted by carbonyl group present in phenolics following to oxidation of hydroxyl group by molecular O along with elimination of the hydrogen molecule [42].

Phenols are biologically active compounds and possess many therapeutic properties, as they have anti-inflammatory, anti-tumor, anti-bacterial and anti-viral activity, as well as being known antioxidants [43]. The reason for its effectiveness may be due to its abundant tannin content [44] tannin influence protein constitution in pinworms exposed to raised concentrations and thus they can affect the nerve receptors, and leading to paralysis of the pinworms and then their death[45]. Tannins polyphenols bind with protein via H-bonds and forming a tannin-protein complex [46], or tannins could demolish cell membrane of an organism's by influence the fats and proteins in it, so this organism loss the ability for growing or penetrates the cell membranes and affect the active sites for some enzymes necessary for growth [47]. In general, phenols are characterized by their high therapeutic potential, as they are known antioxidants and anthelmintic [48]. As for the results of alkaloid compounds, the current results showed that these compounds are effective against worms, as they led to the paralysis of the worm in 211.00 ± 0.57 mins, while the worms died in 203.00 ± 0.57 mins. These output could be justified on the basis that alkaloids may be affect enzyme action, block the receptors and proteins via formation of H-bonds with them where they possess functional groups, a proton accept nitrogen atom, and one or even more proton giving amine hydrogen [49] such as pergularinine and tylophorinidine alkaloids; both of them block the vigor's of dihydrofolate-reductase, which are accountable for synthesis of nucleic acid [50], or the alkaloids could be attached to a key protein in cell division with highest affinity leading to inhibit its enzyme activity and as a result inhibition of cell cycle [51], In addition, alkaloids can act through a mechanism of action similar to detergents, which work to destroy the outer membrane of microbes, and their effect may be due to their effect on virulence factors of microorganisms [52], [53].

Alkaloids have extremely important pharmaceutical importance, as they act as stimulants of the central nervous system, excellent anticholinergics, oxytocic activity, and vasoconstrictors, as well as being anti-inflammatory and antimalarial agents [54] Its anti-inflammatory effect includes controlling or inhibiting a key immune mediators [55].

The findings of praziquantil drug experiment concentrations give a demonstration that praziquantil drug has a altitude activity on the palsy and death of E. vermicularis exceptionally with 60 mg\ml, the time for getting paralysis and deathe were 180.00 ± 0.57 and 240.00 ± 0.57 mins, respectively. These results are consistent with experiments from previous studies, which demonstrated the high efficacy of this drug against parasitic worms. Where the investigation of [56] evaluated the impact of praziquantil on Schistosoma haematobium (S. haematobium), repression concentration (IC50) values on adult S. haematobium were detected in vitro, their findings revealed a highest activity against adult pinworms, revealing that IC50 of $0.007~\mu g/ml$ at 4h and $0.01~\mu g/ml$ at 72h. The study conducted by [57] accomplished an experiments to compare the efficacy of a herbal medication as Schitozim versus to praziquantel drug in administration the infection with Schistosoma mansoni in BALB/c mice and their experiment included three groups, two of whom were treated with the drug praziquantel (25 mg/kg, or 50 mg/kg), and the other group was a control group, and their results showed that the drug led to a noticeable reduce in the number of adult worms.

The effective effect of this drug is attributed to its ability to damage the beta subunits of voltage-gated Ca2+ channels [58], The prompt influx of calcium ion leading to morphological alteration ofpinworm that including muscular quick contraction, tegumental bubble, along with vacuole emergence in tegument and tegumental destruction [59], also it could block worm's adenosine receptor, leading to calcium rush as a result of drug's efficancy [60].

Praziquantel is a drug that is highly effective against a wide range of parasitic worms that infect humans and animals, as well as being safe for use. Praziquantel (PZQ) is the mainstay and responsible for the activity of parasitic worm control and has been successfully used for decades. Praziquantel is an essential drug for treating parasitic infections like schistosomiasis, [61]. It activates a transit receptor potential elastatin ion-channel in helminthes, leading to Ca+ entry and palsy.

5. Conclusion

The cold extract of Q persica exhibits a high efficacy on paralysis and mortality of E. vermicularis versus to boiling extract In Vitro. Phenolic compounds at a concentration of 60mg/ml exhibit greater efficacy in causing paralysis and dying of E. vermicularis pinworms followed by alkaloid compounds.

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