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Evaluating the efficacy of *Capparis spinosa* Total flavonoids to control Bird Lice (*Menacanthus stramineus*)

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ABSTRACT:

The present study included an *in-vitro* and *in-vivo* insecticidal investigation for one of wild Iraqi plant *Capparis spinosa* total flavonids on locally chicken lice (Menacanthus *stramineus)*. Extraction of total flavonoids from 115g of fresh plant samples by reflex extraction with 600ml distilled water with (10% v/ v) HCl, for 8 hours continuously and the aglycon part was obtained with ethyl acetate. The acetate layer was dried and the residue was weighted and subjected for qualitative and quantitative analysis. Three flavonoids concentrations of *Capparis spinosa* total Flavonoid (5,3 and 1) mg/ml in 100ml distilled water to treat a collected sample of Bird Lice (*Menacanthus stramineus*) *in vitro* and *in vivo* at different life stages in comparison to a traditional anti lice drug treatment permethrin (0.5mg/ml) as positive control. Distilled water application was considered as negative control. Results of present study showed that the plant was rich with different types of flavonoids and the effect of flavonoid extracts of the *Capparis spinosa* plant in the decimation of the different stage of chicken body lice, as the concentration factor had non-significant effect on the killing of adult and a significant effect on the killing of eggs/nymph, and this effect increased with the increase in the concentrations of the extract.

KEYWORDS: *Capparis spinosa*, insecticidal, chicken lice, total flavonoid.

1. INTRODUCTION:

Lice are a widespread and economically significant ectoparasites of domestic animals, including poultry[1]. Herbal medicine is the oldest type of medicine known to our planet, and it is regarded as a gift from nature to humans, assisting them in living a disease-free and stable existence. Plants produce a wide variety of secondary metabolites as part of their daily metabolic activities, which are used as pharmaceuticals, agrochemicals, flavors, fragrances, colors, biopesticides, and food additives[2].

Flavonoids are a type of Polyphenolic secondary metabolite that can be found in plants. It's the most common compound in nature, and it's what gives plants their colors, such as flowers and fruits, which humans consume as vegetables and berries[3]. Flavonoid compounds such as rutin and quercetin have been found in the *C. spinosa*[4]. Because of their anti-oxidant properties, flavonoids have gotten a lot of coverage for their health benefits[5]. Permethrin, an artificial pyrethroid, is extensively utilized for lice control, and to time there is no indication of dur ability. Because of the absence ovicidal activity and the probabilities that around fowl were lost in the preliminary sprinkle of huge herds, re-sprinkle is desirable after 7-10 day[6] and because wide variety of insects have developed resistance to permethrin ; the present study was aimed to extract natural secondary compounds (flavonoids) from plant *Capparis spinosa* and tested the effectiveness of these compounds in some aspects of the life performance of *Menacanthus stramineus*, including the effect on eggs, nymph and adults. Flavonoids are present in fruits, vegetables, nuts, stems, flowers, tea and honey and are responsible for the colours and flavours in them [7].

2. MATERIALS AND METHODS:

2.1 Plant Collection and Classification:

The aerial parts of the plant were collected from the garden of Baghdad university at Al-Jadrihya. The plant was classified as *Capparis spinosa L*. The caper was harvested during 1 - 30 October 2020. The aerial part was dried at room temperature in the dark covered for 10 days, then finely ground by using an electric grinder.

2.2 Total Flavonoids Extraction from the plant *Capparis spinosa*[8]:

About (115) g of fresh plant samples was placed in a 1-liter glass flask and then added 600ml distilled water with (10% v/v) HCl, then reflex extraction was performed for 8 hours continuously to ensure that the cleavage and broken of glycoside linkage of the flavonoids and the aglycon part was obtained, plant extracts were filtered and cooled. The aglycon portions that

represent the biologic active part of flavonoids, were extracted by an organic solvent such as ethyl acetate by adding 50ml per each 50ml extract and repeated three times using a separating funnel. The acetate layers of each plant were collected in the separating funnel again and an equal amount of distilled water was added to remove HCl residues used in extraction. The acetate layer was dried using rotary evaporator at 45°C. The plant's output were weighted and saved to complete the rest of the analysis.

2.3 Qualitative assay (Thin layer chromatography (TLC) [9]:

A stock solution from the extracted total flavonoids was prepared by dissolving (5) mg residue in ml of 50% ethanol to get a stock solution 5mg/ml. A standard Rutin, Quercetin, Kaempferol, and luteolin solutions were prepared in 50% ethanol also. Thin layer chromatography (TLC) was carried out using a silica coated silica 60 plate with a thickness of 0.1mm which represents the stationary phase in the chromatography separation process and for the mobile phase: n – hexane (15ml): Ethyl acetate (10ml): Acetic acid (AA) (0.7ml) was used. The type of flavonoids separated can be detected in corresponding to standard flavonoids spots in their distance that called RF value. This value is derived from dividing the distance travelled by each flavonoid in each model phase to the distance traveled by the solvent:

$$\text{Rf value} = \frac{\text{The distance traveled by each flavonoid}}{\text{Distance traveled by the mobile phase}}$$

Each Flavonoids can be detected separately by the exposure of the silica plate to the UV light as a colored spot. The silica plate is covered with Fluorescent material, which flashes when it binds to the active groups of flavonoids under UV at a wavelength of 254 nm. The result is shown as bright spots under the UV light.

2.4 Quantitative Assay for Total Flavonoids[10]:

Several Querecetin standard flavonoids solutions were prepared with concentration of (1, 0.75, 0.5, 0.25, 0.1, 0) mg/ml in 50% ethanol solution. The following interaction was performed. Aliquot of 1ml of stock total flavonoids extract solution (5mg/ml) was transferred to a glass tube and 1ml Querecetin standard solutions of each concentration was placed in separated glass tube, then 1 ml of 5% sodium nitrite solution was added to all tubes, to be stirred and left in room temperature for 5 minutes, then 1.5ml of aluminum chloride 10% were added to all tubes and mixed well and left for another 5 minutes at room temperature. Finally, 5ml of 1N NaOH solution was added to the mixture and the resulting color was read with spectrophotometer at 510nm wavelength. A standard curve is then performed between absorbance reading of each standard solution verses their concentration to get the straight-line equation and then calculating the concentration of the total amount of flavonoids in extracted plant.

2.5 Collection of Chicken:

Chickens (*Gallus gallus domestics*) were collected from Al-Kadhimiya/Al-Mehani Market, with a white and red color, and a weight ranging between 2 - 2.5kg, and a life of between 1 - 1.5 years. Several wooden cages were prepared, with a dimension of 1m x 1m x 2m, and 3 chickens were placed in each cage randomly. Each cage represented a replicate. Water and food were provided daily, and the cages were cleaned daily, and the distance between cages were 2 meters.

2.6 Collection of Chicken lice (*Menacanthus stramineus*):

The specimens of lice taken from poultry consist of adult and nymphal third instar. Chickens were selected randomly, depending upon their activity. Lice were collected from the feathers of ventral and perineal region from the body by the hand manipulation or with aid of blunt pointed forceps and with magnifying glass to distinguish the adult and nymph to avoid any harm to lice and host. These collected lice were put in vials and labeled with all details of lice such as sex, breed, age and date of collection. The vials with lice adults and nymphal instar were wrapped in cotton net cloth for oxygen supply and transported to laboratory where they were identified up to species level.

2.7 Preparation the samples for Identification *Menacanthus stramineus*:

The samples *from* 2.6 directly transport to ethyl alcohol 70% to kill adults and nymph and then placed in cold KOH solution 10% for 24 hours then washed with distilled water and placed them in Xylol for 1-2 minutes and have been mount on a glass slide by Canada Balsam and covered with cover slide then left to dry for used in examination[11]. Isolated samples of lice were given to Iraq Natural History Research Center and Museum/ University of Baghdad.

2.8 preparation different concentrations of *Capparis spinosa* total flavonoids extract and the traditional drug:

Three solutions from each Capparis *spinosa* total Flavonoid residues to get 100ml in distilled water containing the following concentration (5,3 and 1) mg/ml. The traditional anti lice drug (permethrin in concentration of 0.5mg/ml was used in treatment as positive control. Distilled water application was considered as negative control.

2.9 Treatment Lice with different concentrations of *Capparis spinosa* extracts flavonoids *In vitro*[12]:

Thirty adult males and females were isolated from infected chicken with lice, 10 adults were placed in each plate consider a replicate for each concentration for each treatment, three replicates for each treatment and a filter paper Whatman no. 1 was

placed at the bottom of the plate with soft feathers and a little skin, the same method for the nymphal third instar.

In *the vitro* effect of adult and nymph, 5ml of each concentration of *C. spinosa* extracted flavonoids in small sprayer volume 50ml and sprayed with a distance of 10cm to each replicate, the same method for permethrin and distilled water (control) treatments. The dishes were placed in the incubator at a temperature of 35±2 and a humidity of 85±5 and a water dish was placed to maintain humidity with a thermo hygrometer to control the temperature and humidity. The mortality of lice in all groups was recorded after 30 min, 1 hr., 2 hr., respectively. The mortality was recorded for the treated Adults and nymphs and corrected the mortality based on an equation Abbott 1925[13].

2.10 Treatment Lice with different concentrations of *Capparis spinosa* extracted flavonoids *In vivo* (Kumar *et al*., 2011)
Fifteen chickens infected with lice were used and they were divided into five groups, each group contains 3 chickens represented 3 replicates, each chicken sprayed with 100ml of extract as following:

First group: three chickens were treated with concentration 5mg/ml of extract flavonoids.
Second group: three chickens were treated with concentration 3mg/ml of extract flavonoids.
Third group: three chickens were treated with concentration 1mg/ml of extract flavonoids.
Fourth group: three chickens were treated with the permethrin (0.5mg/ml).
Fifth group: three chickens were treated with distilled water (control). The mortality of lice was calculated by summation of the total lice counted by using the whole-body area. After spraying treatment of extracted total Flavonoids on the site (ventral and perineal region) for each treatment, mortality was recorded at 30 min, then 1, 2, 4 hours and lastly after 5 days. The mortality was recorded for the treated Adults and nymphs and corrected the mortality based on an equation[13].

$$\text{Corrected depreciation Percentage} = \frac{\%\text{ killing lice in treatment} - \%\text{killing lice in control}}{100 - \%\text{ killing lice in control}} * 100$$

2.11 Statistical Analysis:
The Statistical Analysis System- SAS[14] program was used to detect the effect of difference factors in study parameters. Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significant compare between means. in this study.

3 RESULTS AND DISCUSSION:

3.1 Qualitative Determination of Total Flavonoids:

Many flavonoids detected in the total Flavonoid residue were shown in figure (3.1). The chromatogram indicates the presence of Luteolin, Kaempferol, Quercetin, Rutin and other flavonoids in comparison with standard flavonoids. The figure shows that both Quercetin and Luteolin are more abundant in the plant extract[15]. The same result reported by[16] who found in aqueous extract of *C. spinosa* the Quercetin, Rutin and other flavonoids were dominant flavonoid in the plant. In the case of leaves maximum flavonoid was obtained in the methanolic extract of Syzygium samarangense (Blume) Merrill and Perry leaf (1.117±0.006%)[17].

Figure (3.1) Thin-layer chromatography for total flavonoids of *Capparis spinosa*

3.2 Quantitative Assay of total Flavonoid residue:

The number of total flavonoids found in the aerial part of the plant was estimated depending on absorption of the different concentrations for the standard Quercetin as shown as in table (3.2) where the straight-line equation was obtained as shown in figure (3.2).

Table (3.2) Absorption Values of Different Concentrations of Standard flavonoids Quercetin and Total Flavonoids of Plant Extract

Quercetin Standard Solution (mg/ml)	Absorption at (510 nm)
0	0
0.1	0.055
0.25	0.107
0.5	0.173
0.75	0.353
1	0.487
Total flavonoids extracted	0.861

Figure (3.2) Quercetin Standard Curve

From the equation of the straight line of the standard flavonoid curve of the Quercetin with different concentrations, the concentration of the total flavonoids for the extract was as follows:

$Y = 0.4618\ x$

Y=0.861
So: X= 0.861/0.4618 = 1.864 mg/ml total Flavonoid.

In 115g dried aerial part of the plant, the total flavonoids were 243.885mg (212%w/w) that means each 100 g dried aerial part of the plant should contain (212 mg) total flavonoids as Querecetin. Flavonoids and phenolic acids were the most common compounds contained in the bioactive fraction,[18] reported that total flavonoid contents ranged from 0 to 254mg/100g fresh weight, about 75% of samples were found to contain flavonoids > 0.5mg/100g with the group mean 33mg/100g. Also, [19] found that Caper fruits yielded 24% dried.[20] pointed out that quercetin, kaempferol, myricetin, and Isorhamnetin derivatives represented, 38%–67%, 15%–36%, 4%–7%, and 0.85–3% respectively of total flavanols in caper flowers, but their contents strongly depended on the growth stage and depended on a genotype and growing stage of caper flowers.

3.3 Effect of Capparis *spinosa* flavonoid extract on different stage of chicken body lice *Menacanthus stramineus* in vivo:

Table (1) shows the effect of flavonoid extracts of the *Capparis spinosa* plant in the decimation of the different stage of chicken body lice, as the concentration factor had non-significant effect on the killing of adult and a significant effect on the killing of eggs/nymph, and this effect increased with the increase in the concentrations of the extract, as the percentage of mortality in eggs/nymph (82.67, 88.67 and 97.33%) in concentration (1, 3 and 5mg/ml) respectively and 82.33% in positive control while 4.33% in the negative control treatment. The results obtained in the present study indicated that the highest concentration of flavonoids compounds extract was more effective in the mortality of lice/eggs compared with the rest of the other concentrations of the *Capparis spinosa* plant. Flavonoids are cytotoxic and interact with different enzymes via complexation, making them one of the chemicals that have been identified to control oviposition and feeding. Plants are protected from insect pests by flavonoids and Isoflavonoids, which influence their behavior, growth, and development[21]. In insects, proteases play vital role in the digestion of protein and generally, the Serine proteases of insects are inhibited by the plant proteinase inhibitors (PIs)[22].

The larvicidal activities of rotenone(flavonoid) were tested against the late third and early fourth instar larvae of *Anopheles gambiae*. Rotenone showed 100% mortality at 24 hours[23,24] found that *Syzygium malaccensis* (Myrtaceae) flower organic alcoholic extracts (2%) had the highest mortality (75 and 59.24%) against *Rhipicephalus* (*Boophilus) microplus*. Ascorbic acid, flavonoids and phenolics are the major contributors to antioxidant activity Ehretia acuminata[25]. Flavonoids were toxic to adult *Callosobruchus chinensis*, mortality percentage exposure of 48 hr. to 10mg mL^{-1} partially purified flavonoids of flavone and *Calotropis procera* caused (53,100%) mortality at 4 days after treatment for all flavonoids[26]. Minimum insecticidal activity was observed at (5mg/5ml) concentration killing only 5 insects after 24h[27], the methanol extract of both bark and core wood possessing high total phenolic and flavonoids showed better antioxidant activity than the other extracts [28].

Table 1: Effect of different concentrations of the *Capparis spinosa* flavonoid extract on different stage of chicken body lice *Menacanthus stramineus* in vivo

Flavonoids extract Concentration (mg/ml)	Mean ± SE of killing of chicken lice in all life stage within 7 days	
	Adult	Egg/ Nymph
0 Water (-ve Control)	0.00 ±0.00 b	4.33 ±0.63 c
5 mg/ml	100 ±0.00 a	97.33 ±0.32 a
3 mg/ml	100 ±0.00 a	88.67 ±0.16 b
1 mg/ml	100 ±0.00 a	82.67 ±0.16 b
Permethrin (+ve Control)	100 ±0.00 a	82.33 ±0.12 b
LSD value	10.00 **	6.961 **
Means having with the different letters in same column differed significantly. * (P≤0.05).		

3.4 Effect of *Capparis spinosa* flavonoid extract on different stage of chicken body lice *Menacanthus stramineus in vitro:*

The table (2) shows the percentage mortality in lice was 100 percent within 1 minutes in concentrations of 5,3 mg/ml and within 5 minutes in concentrations of 1 mg/ml, according to in vitro results of Licicidal activity of flavonoids extract of *Capparis spinosa* plant against lice. Water (negative control) does not destroy lice, while permethrin (positive control chemical pesticide) destroys lice**.** Flavonoid compound has the most significant contribution to radical scavenging[29]. Similarly, quercetin, myricetin, isoquercetin and rutin were found in crude flavonoid extract of *Annona squamosa* leaves which at 0.09mg/ml, caused 100% mortality of adult *Callosobruchus chinenesis*[30]. Lethal doses of *(Punica granatum)* peel and seed suggested these became more toxic to aphids (*Macrosiphum rosaeformis*) after 24 hr. of exposure. A significant difference was obtained in percentage aphids reached to untreated leaves over peel extract treated leaves (23:57 and 23:77) and seed extract treated leaves (7:80 and 17:80) at 90- and 180-min time intervals in food choice assays[31]. it is found that methanol extract of *Hibiscus syriacus L.* contains substantial amount of phenolics and flavonoids responsible for antioxidant activity[32], The higher radical scavenging efficacy of EFS may be due to retention of antioxidant phytochemicals in this extract[33]. However, in many studies, the phytochemicals like terpenoid, saponins, coumarins, and flavonoids are also

extract . However, in many studies, the phytochemicals like terpenoid, saponins, coumarins, and flavonoids, are also reported to have antioxidant activity[34].

Table 2: Effect of different concentrations of the *Capparis spinosa* flavonoid extract on different stage of chicken body lice *Menacanthus stramineus* *in vitro*

Extract Concentration (mg/ml)	No. of Lice	Percent mortality					
		5 min.	30 min.	1 hr.	2 hr.	4 hr.	6 hr.
5	10	100	100	100	100	100	100
3	10	100	100	100	100	100	100
1	10	50	100	100	100	100	100
0 water(-ve) control	10	0	0	0	0	0	0
Permethrin (+ve Control)	10	100	100	100	100	100	100

4. CONCLUSION:

The current study showed that the flavonoid extract of caper plant possesses strong activity which was comparable to that of the reference drug. There is a high possibility that these plant extracts provide effective ecofriendly herbal formulations for the control of lice infestation on animals.

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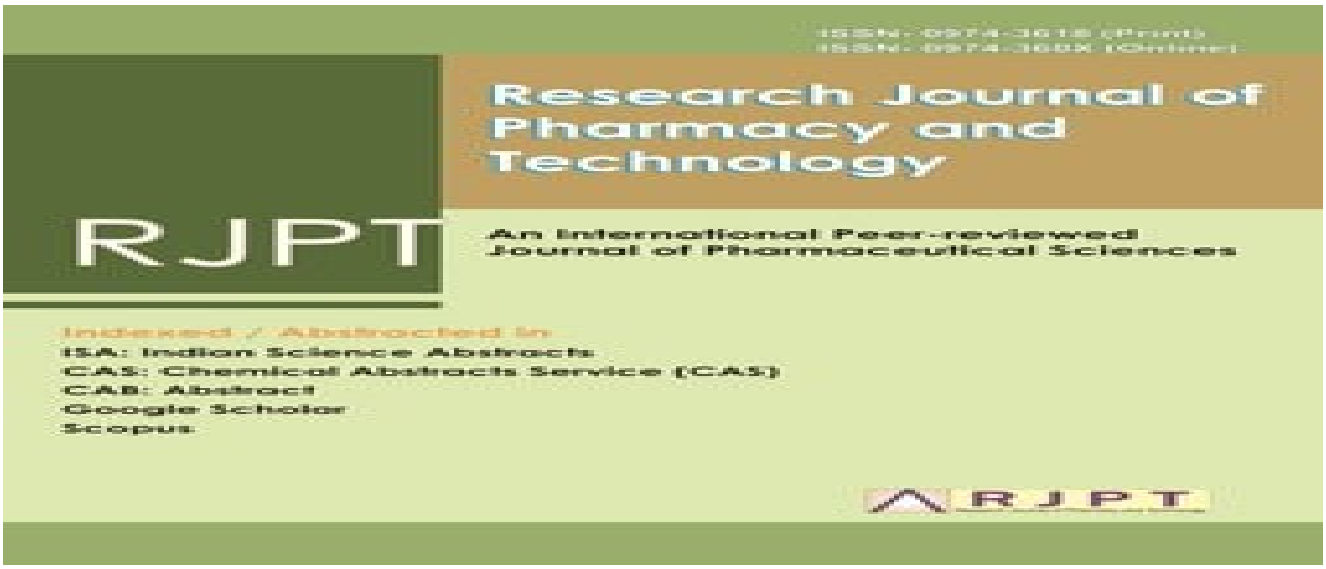

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