# Chemical composition and antifungal activity for lion's foot (*Achemilla vulgaris L.*) flavonoids against soil born pathogenic fungi

**Rania A. A. Hussien**

Fungicides, Bactericides and Nematicides Department, Central Agricultural Pesticides Lab (CAPL), Agriculture Research Center (ARC), Giza, Egypt

**Abstract:** The current study was designed to evaluate antifungal capability of flavonoids isolated from lion's foot (*Alchemilla vulgaris* L.) and determination of extracellular enzymes activity secreted by soil born pathogenic fungi (*Rhizoctonia solani, Sclerotium rolfsii* and *Fusarium oxysporium*), along side the qualitative and quantitative analysis of flavonoid compounds was made using TLC and HPLC. The obtained results showed the flavonoids content was spectrophotometrically determined equal to 76.9mg RUE/g d.w. The antifungal activity of extracted flavonoids (50, 100, 150, 200 and 250 mg/l) assessed by the food poison technique compared to Vitavax-t fungicide. The $EC_{50}$ values of flavonoids were 90.87, 138.15 and 149.76mg/l against *F.oxysporum, S.rolfsii* and *R.solani* ,respectively. The treatment liquid PD media with flavonoids at $EC_{50}$ had effect on significantly reduction of peroxidase, protease, polyphenol oxidase, cellulase and polygalacturonase activities secreted by tested fungi. The received TLC and HPLC information were collectively supportive which displayed three different flavonoid components{rutin (*Rf* =0.40); 39.6%, quercetin (*Rf* =0.56); 41.7% and isoquercetin (*Rf* =0.70); 18.7%}, respectively. These findings provide clear evidence approximately the promising antifungal effect of *A. vulgaris* flavonoids, this helped lay the foundation for larger-scale efficacy investigations under greenhouse and field conditions in the future.

**Keywords:** flavonoids, *Alchemilla vulgaris* L., *Rhizoctonia solani, Sclerotium rolfsii, Fusarium oxysporium*, extracellular enzymes, TLC and HPLC.

## 1. Introduction:

*Alchemilla vulgaris* L., commonly known as lion's foot and member of the Rosaceae family, is a perennial herbaceous plant that grows widely in Asia and Europe, particularly in mountain meadows. It has small, yellow-green flowers(Sârbu*et al*., 2013; Tasic-Kostov *et al.,* 2019).The medicinal portions of the *Alchemilla vulgaris*is aerial parts and roots. It is a huge treatment in people medication, known particularly for the helpful impacts in numerous gynecology and digestive system diseases (Condrat *et al.,* 2010). *A. vulgaris* is a plant that is listed in the European Pharmacopeia. Its aerial and root sections have been shown to contain a variety of phytochemical components that have a range of biological activities. Phenolic compounds are the transcendent mixtures recognized in past examinations. Previous published research have shown that the aerial parts of *A. vulgaris* are wealthy in tannins, phenolcarboxylic acids, flavonoids (rutin, quercetin, kaempferol, quercetin 3-glucuronide, avicularin), as well as phytosterols (Duckstein *et al*., 2012, Kaya *et al*., 2012 and Mandrone *et al.,* 2018). These bioactive components applied calming, wound-recuperating, antihemorrhagic, antispastic, antifungal, astringent, antiviral, antibacterial, cancer prevention agent properties (Boroja *et al*., 2018, Choi *et al.,* 2018, Filippova, 2017 and Ibrahim *et al*.,2022).

Various fungi were found to be associated with tomato showing root decay, damping off, and shrivel signs tested from impacted fields inside the of late recovered land in El-Behera governorate. *Fusarium* spp., *Rhizoctonia solani*, *Sclerotium rolfsii* have been regular over the amassed tests and recuperated in frequencies of 86.9% , 67.1% and 50.0%, respectively (Atta-Alla *et al*., 2004). *Sclerotium rolfsii* is a dirt possessing omnivorous fungal pathogen contaminating a gigantic scope of veggies, including tomato. The fungus live on diseased crop particles or within the soil for decades as sclerotia. The dirt borne inoculum of the fungus influences seed germination and reasons damping off. Additionally, The fungus contaminates the stem at or near the dirt line incurring necrotic decay. The out of the blue creating injuries support the stem, fundamental to withering of the plant unexpectedly and totally (Mullen, 2001). Fungal plant illnesses may also result in heavy yield losses. Phytopathogenic fungi by myself purpose almost 20% reductions inside the yield of important food and cash plants (Agrios, 2000). The disorder causing organisms enter the host tissue through mechanical pressure exerted by the developing germ tube or dissolving the host cell wall via secretion of pollutants or enzymes. The enzymes produced through pathogens have an effect on chemistry of mobile wall that is accompanied by cellular wall breakdown (Albersheim and Jones, 1969). *R.solani*, *S.rolfsii* and *F.oxysporum* secretes cellulase and polygalacturonase (PG) adaptively. A excessive relationship exists between the capacity of *R.solani* to create huge amounts of PG in vitro and their pathogenicity. PG was many times liable for tissue maceration. Though, cellulase respected to play an optional situation in the strategy (Bateman, 1993).

Chemical fungicides might be accessible to successfully and widely decrease the unsafe impacts of microbes however inordinate and ill-advised utilization of these fungicide causes a risk on plants,

soil, animals, human health and environment. In order to manage plant diseases, current efforts have concentrated on creating environmentally safe and efficient biocontrol strategies (El Mohamedy *et al*., 2014). According to Chaachouay and Zidane, (2024) natural plant products represent a significant source of novel agrochemicals for the management of plant diseases. phenolic components are becoming more and more important nowadays through their significant function in oxidation processes. They exhibit a number of advantageous antimicrobial and anticancer properties (Kova *et al*., 2022). Flavonoids are abundant in nature and one of the most significant classes of phenolic components. According to studies on these compounds, they provide protection against UV rays, plant diseases (bacteria and fungi), and substances that can interfere with biochemical processes including enzyme inhibitors and antioxidants (Harborne and Herbert, 1993). Furthermore, flavonoids show many natural properties, for example, antifungal, antiviral, mitigating, and against allergenic properties (Agrawal, 2001). Flavonoids are among the main kinds of optional metabolites from regular items through their few applications in the food, diet and clinical ventures. Despite the fact that an enormous number has been accounted for from normal and manufactured sources, researchers are as yet keen on flavonoids and subsidiaries (Awouafack *et al*.,2017). There no publications is available in previous literature approximately extraction and biological activity of *A. vulgaris* flavonoids. Thus, the present study was focused on assessment of chemical composition and antifungal effect of total flavonoids extracted from *A. vulgaris* herb and determination of extracellular enzymes activity secreted by soil born phyto-pathogenic fungi.

# 2.Material and Methods:

## 2.1. Plant material:

*Alchemilla vulgaris* herb applied in this study was acquired from herbal shops. The plant material was finely smashed to coarse powder and kept in opaque screw tight plastic sacks at ordinary room temperature until use.

## 2.2.Preparation of crude methanolic extract:

About 100g of *Alchemilla vulgaris* powdered have been extracted the use of 300 ml methanol 50% by means of soaking for 48 hrs. The extract changed into filtered by Whatman No. 1 filter paper. The plant buildup was re-extricated with the expansion of new methanol for another twice. Joined filtrates have been concentrated utilizing Revolving evaporator at 40°C underneath vacuum. The subsequent dry concentrate was re-broken down in clean methanol to make (10µg/µl) stock arrangement and kept at 4°C for additional examination. The percentage of extraction yield of unrefined methanolic extract became determined with the equation:-

Extraction yield (%)= dry weight of extract (g)/ weight of plant (g) × 100

## 2.3.Determination of total flavonoids content:

The content of total flavonoids compounds was estimated through a colorimetric method described by (Jiao and Wang, 2000) .0.5 ml of crude methanolic extract (1mg/ml) have been made up to 2 ml with methanol then combined with 0.1 ml of 10% hydrated aluminum chloride and 0.1 ml of 1M sodium acetate. The methanol was added to the mixture to bring the final volume to 5 ml. The absorbance changed into measured at 415 nm after 30 min of incubation at room temperature. The amount of extract was substituted by means of the same amount of methanol in blank. The overall flavonoids content was communicated as milligram of rutin equivalent (RUE) per gram of dry extract (mg RUE/g d.w.).

## 2.4. Extraction of flavonoids:

Flavonoids extracted with the aid of immersing 100g of *A. vulgaris* in 300ml methanol 50% for 24hrs at room temperature using magnetic stirrer and filtered the usage of Whatman No.1 filter paper (Jain *et al*., 2007). The filtrate changed into dealt with 100ml lead acetate (1%) for 4 hrs for precipitation. The mixture was filtered, then a mixture of 250ml acetone and 30ml of HCl was added to the precipitate. The resulting changed into focused the use of Rotary evaporator at 40°C below vacuum and kept at 4°C for further analysis. The percent of overall flavonoids prompted yield became calculated with the formula:-

Yield (%)= dry weight of precipitate(g)/ weight of plant (g)×100

## 2.5. Antifungal Activity of flavonoids:

The activity of flavonoids extracted from *A. vulgaris* herb was evaluated *in vitro* against *Rhizoctonia solani*, *Sclerotium rolfsii* and *Fusarium oxysporium* using the poisoned food technique. The PDA (Potato Dextrose Agar) medium was prepared so that it contained various concentrations of flavonoids (50, 100, 150, 200 and 250ppm) in comparison with Vitavax-t (Carboxin 75% WP, England) (5,10,15, 20and 25ppm)as a positive control. Then seven-day old fungal culture disk of 9mm diameter was taken and inoculated to the center of petri dishes containing

tested materials. Rather than PDA medium without flavonoids filled in as negative control. All dishes were incubated at 25±2°C and radial growth of colony was estimated when the mycelia of control had nearly stuffed the petri dishes. Each test was acted in three-fold. The fungal development restraint which was determined because of treatments against control utilizing the accompanying equation : (Satya *et al*., 2014).

$$\% \text{ inhibition} = C - T \ / \ C \times 100$$

Where C is the average of three replicates of hyphal extension (mm) of negative control and T is the common of three replicates of hyphal extension (mm) of plates treated with examined substances. $EC_{50}$ and $EC_{90}$ values had been decided by using the linear regression (LPD) line computer software) of the probit of the examined fungus percent inhibition vs. Logs the concentrations (mg/l) of flavonoids. The $EC_{50}$ and $EC_{90}$ notation used to signify the effective concentrations (mg/l) that purpose 50% and 90%, respectively, growth inhibition.

## 2.6. Assessment of enzymes created by soil born pathogenic fungi

For this study, 2ml of flavonoids or Vitavax-t at $EC_{50}$ was added to 100ml of sterilized PD medium inoculated with a 5 discs (9mm) of tested fungi and incubated at 25±2°C. For every treatment, four flasks were used as replicated. The mycelia matrix was removed by filtering and overnight drying at room temperature once the mycelia growth had covered the surface media in the negative control. After homogenizing the dry mycelia mates, an enzymatic determination was made.

### 2.6.1. Assessment of peroxidase activity

One gram of every fungus was removed using 0.1M potassium phosphate buffer pH 4.7 with 0.25M sucrose. The total peroxidase in extract colorimetric assay was done by Sreenivasulu *et al*., (1999). The peroxidase activity assigned as Δ O.D.470nm/5min/g fungus.

### 2.6.2. Assessment of polyphenol oxidase (PPO) activity

Citric-phosphate buffer pH 6.5 was utilized to extract one gram of each fungus in a 1:2 (w/v) ratio. The enzyme activity was performed out using the Dewez *et al*., (2005) technique. PPO activity characterized as an increment of 0.1 unit O.D. / min at 420nm.

### 2.6.3. Assessment of polygalacturonase (PG) activity

PG action became examined by utilizing assessing the loss in viscosity of 1.2% fluid methoxy pectin at 37°C through the technique depicted by Echandi *et al*., (1957).

### 2.6.4. Assessment of protease activity

Protease activity was decided by means of the technique depicted by Dewez *et al*., (2005). The blue color evolved become estimated after 5min. at 625nm.

### 2.6.5. Assessment of cellulase activity

Cellulase enzyme became estimated by way of assaying the loss in viscosity of carboxy methyl cellulose at 37°C ( Matta and Dimond, 1963).

## 2.7.Idenification of flavonoids:

The identity of character flavonoids extracted from *A. vulgaris* herb turned into done the use of thin layer chromatography (TLC) system. The methanolic solutions of rutin, quercetin and isoquercetin had been used as reference standards for the identification of flavonoid compounds in *A. vulgaris*, which was accomplished with the aid of evaluating retention coefficients (*Rf*) and their colors. The mobile segment turned into: ethyl acetate : acetic acid : formic acid : water (100:10:10:25(v/v). The plate became sprayed with the PEG (5% ethanol polyethylene glycol 4000) detection reagent. The plates have been found the use of an ultraviolet chamber underneath the UV mild at λ=365 nm (Kaya *et al*., 2012).

## 2.8.Qualitative and quantitative estimation of flavonoids compounds by High Performance Liquid Chromatography (HPLC):

Identification of flavonoids compounds in *A. vulgaris* herb turned into carried out using JASCO HPLC (Agilent technology 1260 infinity), with a hypersil C18 reversed-segment column Eclipse plus (250x4.6mm) and 5µm particle length. HPLC evaluation of flavonoids extract changed into carried out via re-dissolving 100mg of extract in 1ml of methanol and filtered thru a 0.2 µm filter out sterilized membrane prior to HPLC evaluation. Injection by using a Rheodyne injection price (Model 7125) with 50 pJ constant loop changed into used. A constant drift fee of one ml/min became used with cellular phases: (A) 0.5% acetic acid in distilled water at pH 2.7; and solvent (B) 0.5% acetic acid in 99.5% acetonitrile. The elution gradient become linear beginning with (A) and ending with (B) over 50 min, the use of an UV detector set at wavelength 235nm. The concentration of flavonoids compounds was calculated on the idea of peak location measurements (Mattila *et al*., 2000).

## 2.9.Statistical analysis:

All experiments were performed in a very randomized layout (CRD) with 3 replications for each treatment. The statistical analysis of all records became carried out utilizing the principles of analysis of variance (ANOVA) and Duncan's multiple-range take a look at (Duncan, 1955).

# 3.Results and Discussions:

## 3.1. Total flavonoids content in *A. vulgaris* herb:

The yield of *A. vulgaris* herb methanolic extract was recorded 13.4% of dry plant. Total flavonoids content in crude methanolic extract was spectrophotometrically determined equal to 76.9mg RUE/g d.w.). Previous research has found that the crude methanolic extracts *A. vulgaris* were 12% for aerial parts and 16.13% for roots. Total flavonoids content in roots (19.8mgRUEs/g) was higher amount in comparison with aerial parts (13.3mgRUEs/g) (Boroja *et al*.,2018). While, Abdel Gawad *et al*., (2023) reported that flavonoids content in *A. vulgaris* herb was recorded 26.1mgQE/g D.W. Whereas, EL-Hadidy *et al*., (2018) found that flavonoids content in *A. vulgaris* leaves was 183.10mg /100g D.W. The differences of amount flavonoids content in *A. vulgaris* could be due to part used of plant, geographical areas, dry, storage, extraction methods and different experimental protocols etc (Benedec *et al*., 2021).

## 3.2.Isolation of total flavonoids:

The precipitated total flavonoids fraction from *A. vulgaris* herb is an amorphous, light brown in colour. It is freely soluble in methanol, ethanol, ethylacetate and DMSO, while slightly soluble in water. The yield of total flavonoids precipitated was recorded 5.5% of dry plant. There no guides is to be had in preceding literature about isolation and extraction of *A. Vulgaris* flavonoids.

## 3.3. Antifungal activity of *A. vulgaris* flavonoids:

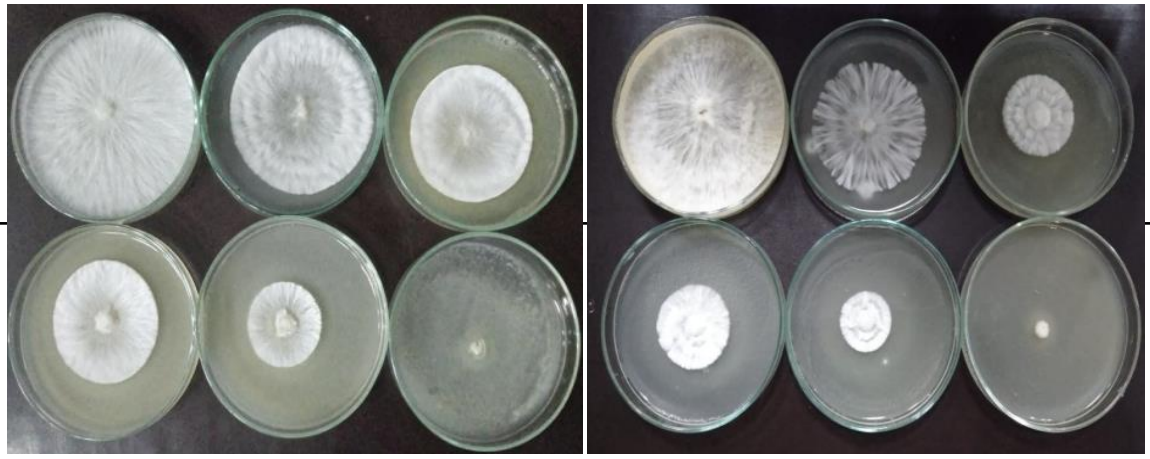
This study showed effect of *A. vulgaris* flavonoids and Vitavax-t fungicide at different concentrations on the linear growth of *Rizoctonia solani*, *Sclerolium rolfsii* and *Fusarium oxysporum*. Results in Table (1) and Fig.(1) illustrated that, the treatment with flavonoids or Vitavax-t to PDA medium, decreased the straight development of three fungi. The highest concentration of both of tested materials showed the most noteworthy reduction of the fungal growth. *S.rolfsii* was delicate to flavonoids at all concentrations under study. The development of *S.rolfsii* was totally inhibition at 250mg/l. Though, the reduction rate was 81.2 and 77.8% for *R.solani* and *F.oxysporum*, respectively, at a similar concentration. The outcomes demonstrated that *F.oxysporum* seem to be more tolerant to high concentration of flavonoids than *S.rolfsii* and *R.solani*. The $EC_{50}$ values of flavonoids were 90.87, 138.15 and 149.76mg/l against *F.oxysporum*, *S.rolfsii* and *R.solani*, respectively. Furthermore, *S.rolfsii* and *R.solani* was delicate to fungicide Vitavax-t compared to *F.oxysporum*. The growth of *S.rolfsii* and *R.solani* were completely inhibited but *F.oxysporum* was still growing even at 100mg/l of fungicide. The $EC_{50}$ values of Vitavax-t were 11.40, 12.55 and 16.43mg/l against *S.rolfsii*, *R.solani* and *F.oxysporum*, respectively. Our effects are according with the results posted with the aid of Boroja *et al*.,(2018) showed that methanolic extract of *A. vulgaris* roots and aerial parts exerted moderate antifungal activity against *F.oxysporum* with MICs from 2.5 to above 20mg/ml. While, Ibrahim *et al*., (2022) studies that antifungal performance of *A. vulgaris* root extract towards *R. solani*, *F. oxysporum* and *Penicillium italicum*. They illustrated that the most powerful inhibition impact of the extract become discovered in opposition to *F. oxysporum* which recorded 45.56% at 1000µg/ml. This effect may be due to polyphenol components in extract. Mode of action for flavonoids as antifungal activity suggested by Dey and Harborne, (1997) and Feeny, (1998) mentioned that the presence of alcoholic agencies inside the structure of flavonoids boom the interest of the plant extract to inhibit the fungal increase. So, the flavonoid components are taken into consideration as antiseptic dealers that are converting the cellular protein nature and increase the permeability of the cell membranes. Taghva *et al*., (2016) confirmed that flowers boom and improvement, in conjunction with protection against contamination and harm, depend on flavonoids.

**Table(1):In vitro antifungal activity of (A) *A.vulgaris* flavonoids and (B)Vitavax-t at different concentrations on soil born pathogenic fungi**

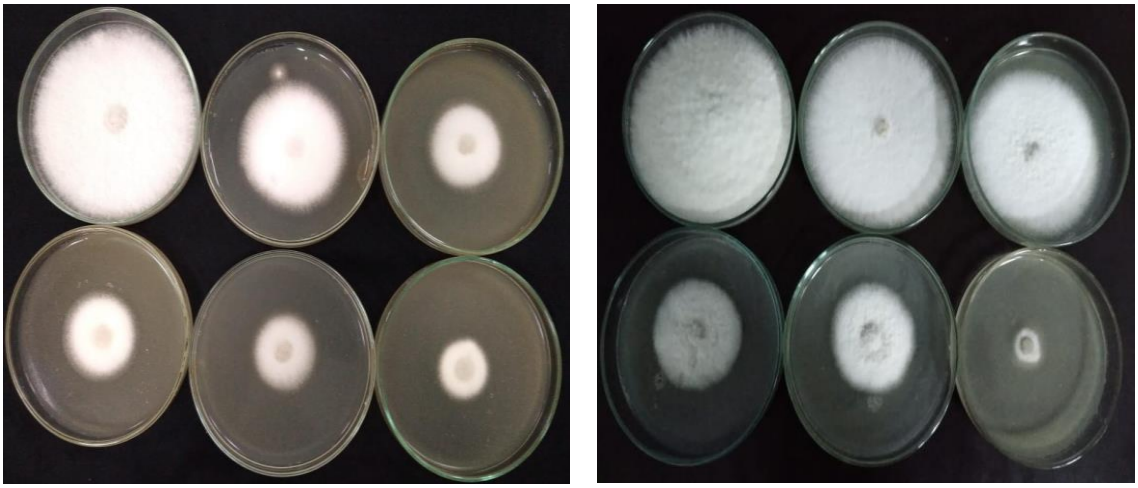
| Concentration (ppm) | % inhibition | $EC_{50}$ (ppm) | $EC_{90}$ (ppm) | Slope value |
|---|---|---|---|---|
| (A) | | ***R.solani*** | | |
| 50 | 16.7 | | | |
| 100 | 23.8 | | | |
| 150 | 44.2 | 149.76 | 465.86 | 2.6003±0.2723 |
| 200 | 60.6 | | | |

| | | | | |
|---|---|---|---|---|
| 250 | 81.2 | | | |
| | | ***S.rolfsii*** | | |
| 50 | 10.3 | | | |
| 100 | 29.7 | | | |
| 150 | 42.2 | 138.15 | 313.25 | 3.6047±0.3109 |
| 200 | 60.4 | | | |
| 250 | 100 | | | |
| | | ***F.oxysporum*** | | |
| 50 | 32.6 | | | |
| 100 | 53.3 | | | |
| 150 | 63.0 | 90.87 | 488.59 | 1.7543±0.2408 |
| 200 | 74.1 | | | |
| 250 | 77.8 | | | |
| (B) | | ***R.solani*** | | |
| 5 | 33.0 | | | |
| 10 | 43.1 | | | |
| 15 | 51.0 | 12.55 | 24.19 | 4.4966±0.3519 |
| 20 | 74.8 | | | |
| 25 | 100 | | | |
| | | ***S.rolfsii*** | | |
| 5 | 16.3 | | | |
| 10 | 40.8 | | | |
| 15 | 53.3 | 11.40 | 27.44 | 3.3593±0.2854 |
| 20 | 73.6 | | | |
| 25 | 100 | | | |
| | | ***F.oxysporum*** | | |
| 5 | 0 | | | |
| 10 | 20.3 | | | |
| 15 | 44.4 | 16.43 | 32.62 | 4.3026±0.3864 |
| 20 | 52.2 | | | |
| 25 | 86.3 | | | |

**(A)** ***Rizoctonia solani*** **(B)**



***Scleroliumrolfsii***



***Fusarium oxysporum***

**Fig.1.Antifungal activity of (A) *A.vulgaris* flavonoids and (B)Vitavax-T at different concentrations on soil born pathogenic fungi**

## 3.4. Effect of flavonoids and fungicide Vitavax-t on enzymes created by soil born pathogenic fungi:

Tables (2), (3) and (4)showed that impact of the treatment liquid PD media with flavonoids or fungicide Vitavax-t at $EC_{50}$ on enzymes activities created through *R. solani, S. rolfsii* and *F. oxysporum*. Data indicated that, protease activity for *F. oxysporum* and *R. solani* was enormously inhibited resulting to treatment with tested materials, this reduction was significant and articulated in case of treatment with fungicide which reached 72.5 and 59.6%, respectively. While, protease activity for *S. rolfsii* was not detected. The treated media with flavonoids induced low decrease in peroxidase activity of the tested fungi compared to control negative. While, the treatment with fungicide expressed highly significant reduction in this enzyme produced through three fungi. Such effect become substantially major in case of *S.rolfsii* which kept completely inhibition in peroxidase activity. The reduction of this enzyme for *R.solani* and *F.oxysporum* dealt with fungicide become recorded 35.3 and 26.6%, respectively, of that obtained in control negative.

**Table(2): Effect of (A) *A.vulgaris* flavonoids and (B)Vitavax-t at $EC_{50}$ on enzymes activities created by *R.solani***

| Enzymes | Control negative | (A) | (B) |
|---|---|---|---|
| Peroxidase activity(O.D./5min) | $0.451\pm0.02^{a}$ | $0.380\pm0.01^{bc}$ | $0.292\pm0.00^{bc}$ |
| % | 0.0 | 15.7 | 35.3 |
| PPO activity(O.D./5min.) | $0.717\pm0.01^{a}$ | $0.610\pm0.00^{bc}$ | $0.550\pm0.01^{bc}$ |
| % | 0.0 | 15.0 | 23.3 |

| | | | |
|---|---|---|---|
| PG activity (RA units/1ml) | 43.3±0.93[a] | 30.3±0.46[b] | 35.2±0.55[b] |
| % | 0.0 | 30.0 | 18.7 |
| Protease activity(O.D./5min.) | 0.706±0.01[a] | 0.365±0.01[bc] | 0.285±0.01[bc] |
| % | 0.0 | 48.3 | 59.6 |
| Cellulase activity (RAunits/1ml) | 53.0±1.40[a] | 32.6±1.34[ba] | 40.3±0.38[ba] |
| % | 0.0 | 38.5 | 24.0 |

**Table(3)Effect of (A) *A.vulgaris* flavonoids and (B)Vitavax-t at $EC_{50}$ on enzymes activities created by *S.rolfsii***

| Enzymes | Control negative | (A) | (B) |
|---|---|---|---|
| Peroxidase activity(O.D./5min) | 0.714±0.01[a] | 0.541±0.00[b] | n.d. |
| % | 0.0 | 24.2 | 100 |
| PPOactivity(O.D./5min.) | 0.649±0.01[a] | 0.524±0.00[b] | 0.414±0.01[b] |
| % | 0.0 | 19.3 | 36.2 |
| PG activity (RA units/1ml) | 11.1±0.23[a] | 7.5±0.23[ba] | 7.9±0.00[ba] |
| % | 0.0 | 32.4 | 28.8 |
| Protease activity(O.D./5min.) | n.d. | n.d. | n.d. |
| % | --- | --- | --- |
| Cellulase activity (RAunits/1ml) | 15.4±0.31[a] | 6.3±0.54[ba] | 8.1±0.23[ba] |
| % | 0.0 | 59.1 | 47.4 |

The reduction in polyphenol oxidase (PPO) activity turned into significantly low in *S.rolfsii* and *R.solani* when media handled with flavonoids. PPO activity for *S.rolfsii* and *R.solani* handled with fungicide was significant and pronounced lower which reached 36.2 and 23.3%, respectively, compared to control negative. While, PPO activity was not recognized in *F.oxysporum*. On settlement of that, the remedy with flavonoids to three fungi turned into more effected than fungicide on cellulase and polygalacturonase (PG) activities. The reduction of cellulase and PG activities have been highly significant and noticeable in case of *S.rolfsii* (59.1 and 32.4%) and *R.solani* (38.5 and 30.0%), respectively, compared to control negative. as observed in Table (2), (3) and (4).

Previous studies confirmed the presence of cellulases and pectic enzymes in plant tissues infected by *R. solani* (Gawade *et al*., 2017). Cell wall corrupting enzymes such as proteases , cellulases, hemicellulases and pectic enzymes are viewed as fundamental for plant cell wall harm during pathogenesis (Gvodeva *et al*., 2006). Phytopathogenic fungi express high measures of hydrolytic and oxidative compounds (Suryanarayanan *et al*., 2012). Abd-Elaziz *et al*., (2022) illustrated that black cumin seed methanolic extract at 2000ppm inhibited 55%, 38% and 74.8% of the activity of pectinase, PG and protease, respectively, secreted by *R. solani* compared to negative control. Amentoflavone, the major component in the extract could be due to responsible to the effect. El-Sharkawy and El-Shora, (2020) Verified that performance of Ammi leaf extracts in suppression of *Fusarium oxysporum* pathogenicity through inhibited the fungal growth and the activity of enzymes responsible for pathogenicity including pectinase, protease and cellulase. It has been said that flavonoids, phenolics and different compounds in plant leaf extracts can be the motive for the inhibition of pectinase, proteases, xylanase and cellulase activities (Nayebi *et al*.,2013). Furthermore, it was suggested that diverse phenolics inhibited the activity of cell wall degrading enzymes of fungi (Modafar and Boustani, 2001). Flavonoids are essential for the proper growth, improvement and protection of plants against infection and damage (Taghva *et al*., 2016).

**Table(4 ): Effect of (A) *A.vulgaris* flavonoids and (B)Vitavax-t at $EC_{50}$ on enzymes activities created by *F. oxysporum***

| Enzymes | Control negative | (A) | (B) |
|---|---|---|---|
| Peroxidase activity(O.D./5min) | 0.331±0.01[a] | 0.317±0.01bc | 0.243±0.00[c] |
| % | 0.0 | 4.2 | 26.6 |
| PPO activity(O.D./5min.) | n.d. | n.d. | n.d. |
| % | --- | --- | --- |
| PG activity (RA units/1ml) | 35.6±1.34[a] | 28.5±0.38[ba] | 31.6±0.81[ca] |
| % | 0.0 | 19.9 | 11.2 |
| Protease activity(O.D./5min.) | 0.411±0.00[a] | 0.233±0.01[bc] | 0.113±0.00[c] |
| % | 0.0 | 45.7 | 72.5 |
| Cellulase activity (RAunits/1ml) | 30.7±1.33a | 24.8±0.57[b] | 28.1±0.64[cb] |
| % | 0.0 | 19.2 | 8.5 |

**n.d. : not detected**

**3.5. I**

**3.5.Identification of major Flavonoid in *A.vulgaris*:**

Identification and qualitative analysis of *A.vulgaris* flavonoids components were conducted using thin layer chromatography (TLC) by standard silica layers. As a result of the comparison of values *Rf* and the colour with PEG under UV light, the flavonoids compounds were identified. On chromatogram, flavonoids appeared as orange-yellow band or spots. Flavonoids compounds found in *A.vulgaris* estimated are rutin (*Rf* = 0.40), quercetin(*Rf* = 0.56) and isoquercetin (*Rf* =0.65), as shown in Table (5). Our findings were supported by other researchers who reported that TLC analysis showed the presence of three flavonoids components in *A.vulgaris* such as rutin, hyperoside and isoquercetin (Benedec *et al*., 2021). Flavonoids components of Alchemilla species are generally quercetin derivatives and very few in numbers (Fraisse *et al*., 2000, D̀ Agostino *et al*., 1998 and Kaya *et al*., 2012).

**Table(5): *Rf* values of *A. vulgaris* flavonoids compounds identified on TLC**

| Flavonoids compounds | *Rf* values | Colour under UV 365nm |
|---|---|---|
| Rutin | 0.40 | Orange-yellow |
| Quercetin | 0.56 | yellow |
| Isoquercetin | 0.65 | Orange-yellow |

**3.6. Qualitative andquantitive of flavonoid components using HPLC device .**

qualitative and quantitive of *A. vulgaris* flavonoid components using HPLC device expressed the predominant flavonoids were rutin (39.6%) and quercetin (41.7%) followed by isoquercetin (18.7%), as shown in Table(6) and fig.(2&3). Analogous results were reported by Kaya *et al*., (2012) and Fraisse *et al*., (2000) which reported that flavonoids components of the genus Alchemilla are generally quercetin derivatives and few in number. While, Ibrahim *et al*., (2022) found that the common flavonoid components in methanolic *A. vulgaris* root extract were hisperdin and quercetin (16.5 and 14.7µg/g root powder), respectively. Additionally, Trendafilova *et al*.,(2011) reported that methanolic extract of *A. mollis* was characterized by the presence of three different types of flavonoids (quercetin, kaempherol and gossypetin). It is noticeable, that quercetin and kaempherol have been already found in *A. xanthochlora* (Lamaison *et al*.,1991), *A.speciosa* (Felser and Scimmer, 1999) and *A.vulgaris* (D̀ Agostino *et al*.,1998).

**Table(6): *Rt* values and percentage of *A. vulgaris* flavonoids compounds identified on HPLC**

| Peak | Flavonoids compounds | *Rt* min. | Area% |
|---|---|---|---|
| 1 | Rutin | 2.484 | 39.59 |
| 2 | Quercetin | 2.714 | 41.73 |
| 3 | Isoquercetin | 3.107 | 18.66 |

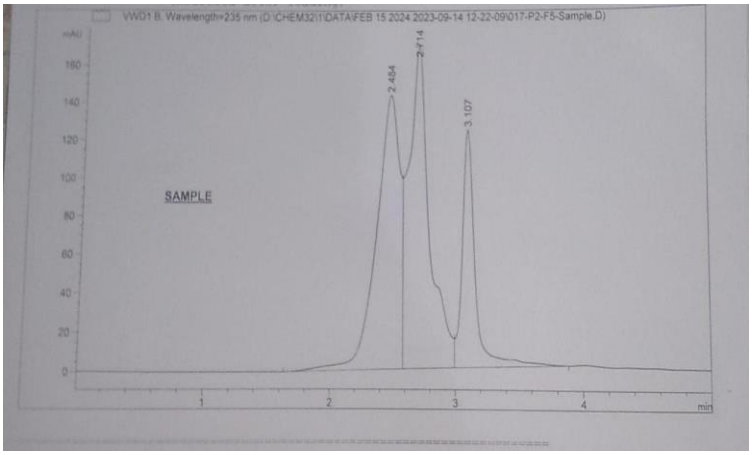



**Fig.2.**HPLC chromatogram of *A. vulgaris* flavonoids

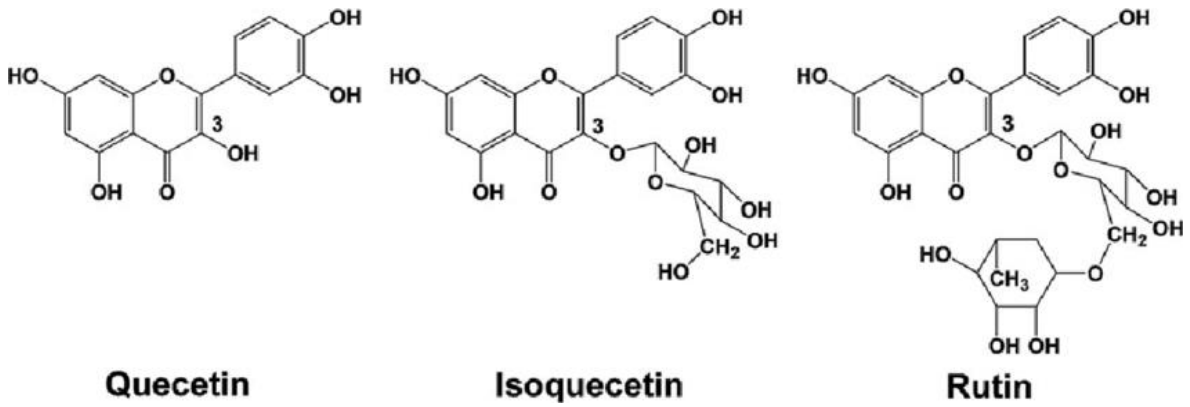



**Fig.3.**Chemical structures of rutin, isoquercetin and quercetin

## Conclusion:

Finally concluded of this study that, plant secondary metabolites (polyphenol compounds especially flavonoids)are characterized by a wide variety of chemical structures, high availability, low price, no side effects and high antifungal activity. Therefore, plant secondary metabolites may be important resources for the isolation, identification and development of natural antifungal drugs alternative of chemical fungicides after carried out on greenhouse and field conditions.

## References:

**Abd-Elaziz, SMS, Abo-Shady A, Ibrahim MAR, Helmy M (2022).**Inhibition of *Rhizoctonia solani* growth and its extracellular hydrolytic enzymes by different extracts of cinnamon(*Cinnamomum cassia*) and black cumin seeds(*Nigella sativa*).Arab Universities Journal of Agricultural Sciences 30(1):1-18

**Abdel-Gawad, S, Baz MM, Taie, HAA, Selim A, Moustafa S, Khater HF (2023).** Novel acaricidal efficacy of nine Egyptian plants against the camel tick, *Hyalommadro medarii* (Ixodida: Ixodidae).Persian Journal of Acarology12(1):121-136.

**Agrawal, AD (2001).** Pharmacological activities of flavonoids: A review. International Journal of Pharmaceutical Sciences and Nanotechnology 4(2):1394-1398.

**Agrios, GN(2000).** Significance of plant disease. In: Plant Pathology, Academic Press, London, pp. 25–37.

**Albersheim, P, Jones TM (1969).** Biochemistry of cell wall in relation to infective process. Annu. Rev. Phytopathol. 7:171:194.

**Atta-Alla, SI, El-Korany AE, Mahros MM, El-Sheikh MA, Abd El-Whab GM (2004).** Efficacy of botanical extracts for controlling the soil borne fungi of tomato. J. Agric. &Env. Sci. Alex. Univ., Egypt.3(1)

**Awouafack, MD, Tane P, Morita H (2017).** Isolation and Structure Characterization of Flavonoids .Flavonoids - From Biosynthesis to Human Health chapter 3 pp45-58

**Bateman, DF (1993).** The "Macerating Enzyme" of *Rhizoctonia solani*. Phytopathol. 14:1179-1185

**Benedec, D, Hanganu D, Osan DE, Oniga I (2021).** Preliminary research on some *Achemilla vulgaris* L. medicinal teas. Hop and medicinal plants 1-2:245-257.

**Boroja, T, Mihailovic V, Katanic J, Pan SP, Nikles S, Imbimbo P, Monti D, Stankovic N, Stankovic M, Bauer R(2018).** The biological activities of roots and aerial parts of *Alchemilla vulgaris* L., South African Journal of Botany 116: 1750-184.

**Chaachouay, N, Zidane L (2024).**Plant-Derived Natural Products: A Source for Drug Discovery and Development. Drugs and Drug Candidates 3:184–207

**Choi, J, Park YG, Yun MS, Seol JW (2018).** Effect of herbal mixture composed of *Alchemilla vulgaris* and *Mimosa* on wound healing process, Biomedicine & Pharmacotherapy 106: 326-332.

**Condrat, D, Mosoarca C, Zamfir A, Crişan F, Szabo M, Lupea A (2010).** Qualitative and quantitative analysis of gallic acid in *Alchemilla vulgaris, Allium ursinum, Acorus calamus*and *Solidago virga-aurea*by chip-electrospray ionization mass spectrometry and high performance liquid chromatography, **C**ent. Eur. J. Chem. 8(3): 530-535.

**D'Agostino, M, Dini I, Ramundo E, SenatoreF(1998).** Flavonoid glycosides of *Alchemilla vulgaris* L., phytother. Res. 12:162-163.

**Dewez, D, Geoffroy L, Vernet G, PopovicR(2005).** Determination of phosynthetic and enzymatic biomarkers sensitivity used to evaluate toxic effects of copper and fludioxonil in alga *Scenedesmus obliquus*. Aquatic Toxicol.74:150-159.

**Dey, PM, Harborne JB (1997).** Plant biochemistry. Academic press. London.p.554.

**Duckstein, SM, Lotter EM, Meyer U, Lindequist U, Stintzing FC (2012).** Phenolic constituents from *Alchemilla vulgaris* L. and *Alchemilla mollis*(Buser) Rothm. at different dates of harvest, Zeitschriftfür Naturforschung C 67(11-12): 529-540.

**Duncan, DB (1955).** Multiple ranges and multiple F. Test. Biomet. J. 11, 1–42.

**Echandi, ES, Van-Gundy D, Walker JC (1957).** Pectolytic enzymes secreted by soft rot bacteria. Phytopathol.47:549-552.

**El-Hadidy EM, Refat OG, Halaby MS, El-Metwaly EM, Omar AA (2018).** Effect of Lions Foot (*Alchemilla vulgaris*) on liver and renal functions in rats induced by CCl4. Food and nutrition sciences 9:46-62.

**El-Mohamedy, RSR, Abdel-Kareem F, Jabnoun-Khiareddine H, Daami-Remadi M (2014)**. Chitosan and *Trichoderma harzianum* as fungicide alternatives for controlling Fusarium crown and root rot of tomato. Tunisian Journal of Plant Protection 9(1): 31-43.

**El-Sharkawy, RM, El-Shora HM (2020).** Biocontrol of wilt-inducing *Fusarium oxysporum* by aqueous leaf extract from Egyptian *Ammi majus* and *Ammi visnaga*. Egyptian Journal of Botany 60(2): 423-435.

**Feeny, P (1998).** Inhibitory effect of oak leaf tannins and the hydrolysis of proteins by tyrosine. J. Photochemistry 8:2116-2129.

**Felser, C, Schimmer O (1999).** Flavonoid glycosides from *Alchemillaspeciosa*. Planta medica 65:668-670.

**Filippova, EI (2017).** Antiviral activity of Lady's Mantle *(Alchemilla vulgaris* L.) extracts against orthopox viruses, Bull Exp Biol Med. 163(3):374-7.

**Fraisse, D, Heitz A, Carnat A, Carnat AP, Lamaison JL (2000).** Quercetin 3-arabino-pyranoside, a major flavonoid compound from *Alchemilla xanthochlora*, Fitoterapia. 72:463-464.

**Gawade, DB, Perane RR, Suryawanshi AP, Deokar CD (2017).** Extracellular enzymes activity determining the virulence of *Rhizoctonia bataticola*, causing root rot in soybean. Physiological and Molecular Plant Pathology 100, 49-56.

**Gvozdeva, EL, Volotskaya AV, Sof'in AV, Kudryavtsevaa NN, Revinaa TA, Valuevaa TA (2006).** Interaction of proteinases secreted by the fungal plant pathogen *Rhizoctonia solani* with natural proteinase inhibitors produced by plants. Applied Biochemistry and Microbiology 42, 502–507.

**Harborne, JB, Herbert H (1993).** Phytochemical Dictionary, A Handbook of Bioactive Compounds from Plants. Taylor & Francis Ltd., London, UK, 791p.

**Ibrahim, OHM, Abo-Elyousr AMK, Asiry AK, Alhakamy AN, Mousa AAM (2022).** Phytochemical characterization, antimicrobial activity and In vitro antiproliferative potential of *Alchemilla vulgaris* Auct root extract against prostate (PC-3), breast (MCF-7) and colorectal adenocarcinoma (Caco-2) cancer cell lines. Plants11(2140):1-14.

**Jain, R, Patni V, Arora DK (2007).** Isolation and identification of flavonoid "quercitin" from Acasia catechu (L.F,) willd-Akatha yielding plant. J.Phytol.Res. 20:43-45.

**Jiao, H, Wang SY(2000).** Correlation of antioxidant capacities to oxygen radical scavenging enzyme activities in blackberry. J. Agric. Food Chem. 48(11):5672–5676.

**Kaya, B, Menemen Y, Saltan ZF (2012).** Flavonoid compounds identified in *Alchemilla* L. species collected in the North-Eastern Black Sea region of Turkey, Afr J Tradit Complement Altern Med. 9(3): 418-425.

**Kovaˇc, MJ, Joki´c S, Jerkovi´c I, Molnar M (2022).** Optimization of deep eutectic solvent extraction of phenolic acids and tannins from *Alchemilla vulgaris* L. Plants 11, 474.

**Lamaison, JL, Carnat A, Petitjean-Freytet C, Carnet AP (1991).** Quercetin-3-glucuronide, the main flavonoid of Ladyṡ Mantle, *Alchemilla xanthochlora* Rothm .(Rosaceae). Annals pharmaceutiques francaises 49: 186-189.

**Mandrone, M, Coqueiro A, Poli F, Antognoni F, Choi Y (2018).** Identification of a collagenase-inhibiting flavonoid from *Alchemilla vulgaris* using NMR-based metabolomics, Planta Med. 84(12/13):941-946.

**Matta, WF, Dimond AF (1963).** Symptoms of *Fusarium* sp. in relation to quantity of fungus and enzyme activity in tomato stems. Phytopathol.53:574-578.

**Mattila, P, Astola J, Kumpulainen J (2000).** Determination of flavonoids inplant material by HPLC with diode-array and electro-array detections. Journal of Agriculture and Food Chemistry 48, 5834-5841.

**Modafar, CE, Boustani EE (2001).**Cell wall bound phenolic acid and lignin contents in date palm as related to its resistance to *Fusarium oxysporum*. Biologia Plantarum 44: 125-130.

**Mullen, J (2001).** Southern blight, Southern stem blight, White mold. The Plant Health Instructor. https://doi.org/ 10.1094/PHI-I-2001-0104-01

**Nayebi, S, Kakeshpour T, Hasanvand A, Nadri M, Monfare SR (2013).**Composition of volatile compounds of extract of *Ammi majus* from Iran

by GC-MS. Journal of Science, Islamic Republic of Iran 24(4), 335-338.

**Sârbu, I, Ștefan N, Oprea A (2013).** Plante vasculare din România. Determinato rilustrat de teren, București: Victor B Victor 252-260.

**Satya, PR, Manisha SP, Khushboo S (2014).** Evaluation of the antifungal activity of the potent fraction of hexane extract obtained from the bark of *Acacia nilotica*. Int. J. Sci. Res.3, 730–738.

**Sreenivasulu, N, Ramanjulu S, K. Ramachandra-Kini K, Prakash HS, Sudhakar C (1999).** Total peroxidase activity and peroxidase isoforms as modified by salt stress in two cultivars of fox-tail millet differential salt tolerance. Plant Sci.141:1-9.

**Suryanarayanan, TS, Thirunavukkarasu N, Govindarajulu MB, Gopalan V (2012).** Fungal endophytes: an untapped source of biocatalysts. Fungal Divers. 54(1):19–30.

**Tasic-Kostov, M, Arsic I, Pavlovic D, Stojanovic S, Najman S, Naumovic S, Tadic V (2019).** Towards a modern approach to traditional use: *in vitro* and *in vivo* evaluation of *Alchemilla vulgaris* L. gel wound healing potential, Journal of Ethnopharmacology238: 111789.

**Trendafilova, A, Todorova M, Nikolova M, Gavrilova A, Vitkova A (2011).** Flavonoid constituents and free radical scavenging activity of *Achemilla mollis*. Natural product communications 6(12):1851-1854.

**Taghva, PH, Zamzad M, Khalafi L (2016).** Total flavonoids content and essential oil composition of *Chaenomeles japonica* (Thumb.) Lindl. exspach from North of Iran. Indian J Nat Prod Resour. 7:90–92.

# التركيب الكيميائي والنشاط المضاد للفطريات لفلافونيدات نبات رجل الأسد ضد فطريات التربه الممرضه للنبات

**رانيــا عبـده عبـده حسـين**

قسم بحوث المبيدات الفطريه والبكتيريه والنيماتوديه – المعمل المركزي للمبيدات- مركز البحوث الزرعيه –الدقي-الجيزة

## الملخص العربى:

تم تصميم الدراسة الحالية لتقييم القدرة المضادة للفطريات للفلافونويدات المستخلصه من نبات رجل الاسد وتقدير نشاط الإنزيمات خارج الخلية التي تفرزها فطريات التربه الممرضه للنبات (ريزواكتونيا سولاني ، اسكليروشيم روسفياي و فيوزاريم اوكسيسبورم)، إلى جانب التحليل النوعي والكمي لمركبات الفلافونويد باستخدام TLC, HPLC. أظهرت النتائج التي تم الحصول عليها أن محتوى الفلافونويدات تم قياسه طيفيًا بما يعادل ٧٦,٩ ملجم روتين/جم وزن جاف .تم تقييم النشاط المضاد للفطريات للفلافونويدات المستخلصة بالتركيزات (٥٠،١٥٠،١٠٠ ،٢٠٠و٢٥٠ملجم/لتر) بتقنية السم الغذائي مقارنة بالمبيد الفطري فيتافاكس-ت وكانت قيم $EC_{50}$ للفلافونيدات هي٩٠,٨٧ ، ١٣٨,١٥ و١٤٩,٧٦ملجم/لتر ضد فيوزاريم اوكسيسبورم ،اسكليروشيم روسفياي وريزواكتونيا سولاني ،على التوالي . معامله البيئه السائله PD بالفلافونويدات لها تأثير كبير في تقليل أنشطة البيروكسيديز والبروتياز والبوليفينول أوكسيديز والسيلوليز والبولي جالاكتوروناز التي تفرزها الفطريات المختبرة. كانت بيانات TLC وHPLC التي تم الحصول عليها داعمة بشكل جماعي والتي بينت وجود ثلاثة مكونات مختلفة من الفلافونويد : روتين(٣٩,٦%، Rf = 0.40) ، كيرسيتين (٤١,٧%، Rf = 0.56)، وإيزوكيرسيتين (١٨,٧%، =0.65Rf ) على التوالي. توفرهذه النتائج دليلاً واضحًا على التأثير المضاد للفطريات الواعد للفلافونويدات نبات رجل الاسد ، مما ساعد هذا علي وضع الأساس لإجراء تحقيقات فعالية واسعة النطاق تحت ظروف الصوبه والحقل في المستقبل .

**الكلمات الداله** : الفلافونويدات ، نبات رجل الاسد ، ريزواكتونيا سولاني ، اسكليروشيم روسفياي ، فيوزاريم اوكسيسبورم ، الإنزيمات خارج الخلية TLC, HPLC