Determine kind and concentration of heliotropiumsuaveolens, Plantagomajorand Silybllummarianum plants ingredients and its effect on some plant pathogenic fungi

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Abstract. A study was conducted on the biology laboratories-Tikrit University to determine the ingredients of three local plants Heliotropiumsuaveolens, Plantagomajor and Silybllummarianum and effect its extracts on the growth of fungus Fusariumsolani, Fusariumoxysporum and Alternariaalternate. Results analysis by High performance Liquid Chromatographyte (HPLC) technique showed H. suaveolensplant contain alkaioidic compounds indicine 9.52%, supinine 3.95%, indicine-N-oxide 14.66%, heliotine 33.0%. heliotrine 31.88% and lidelofidine 6.95% and plantago major plant contain salysilic acid34.93%, kampferol 4.55%,gentisic acid 2.72%,vanilic acid 1.67%,chlorogenetic acid 0.70%,ferulic acid 21.42% and aucubin 9.12% While S. marianum contain salichristinA 42.24%,salichristin B 14.89%, salidianin 30.23%, silybins A3.30%,silybins B 2.74%, isosilybins A 4.86% and isosilybins B 1.71% Extract 20 concentration of H.suavelones and P.major showed high inhibition reached100% While S. mariumshowed no effect on fungus growth.

Keywords. Medicinal Plants, Aqueous Extract, Fungi Inhibitory Effect.

1. Introduction
Medicinal plant has big important in agriculture production, it contain active natural ingredients \cite{1} this natural products has effect on other plants and on its environment beside its effect on organisms growth \cite{2} such as using extracts of medicinal and aromatic plants which has one or more materials in its chemical composition able to inhibition fungus and insects growth \cite{3, 4}, There is a wide variety of funga genera causing diseases for human, animals and plants \cite{5, 6, 7} The intensive and indiscriminate use of pesticide in agriculture has caused many problems to the environment such as water, soil, animal and food contamination, poisoning of farmers \cite{8} besides its harmful effect on human health \cite{9, 10} and appearance resistant strain from fungi \cite{11}. The genus Heliotropium is bigger genuses of Boraginaceae family include 220-300 species in Iraq specialize by scorpoid inflorescence shape \cite{12} andplantago majorbelong to the Plantaginaceae family its high reach about 0.5 m give many leaves over the earth directly had about 5-7 lines with small black or yellow or weight, flowers on the head of long stem distrib around roads and in the gardens \cite{13} ,while marianumis one of the important species belong to the silybum genuisits annual or biannualplantwith 1-2 m high and simple or branched cavey stem and leaves spiny on the borders has white veins, the flowers is violet or pinky or white \cite{14}, distribution in the south of Aljazeera and Sedimentary plain of Iraq and commonly find around the fields and roads \cite{15, 16}. In recent years, antimicrobial properties of plant extracts have been reported with increasing frequency from different parts of the world because plant provide compounds such as alkaloids saponins, volataloi, coumarins, saponins, flavonoids and glycosides \cite{17}, extracts of Zingiberofficinale and Xylopiaethiopiaciplants inhibited
growth of Fusarium oxysporum [18], a study by [19] find activity extracts of Cymbopogon martini on inhibition growth of Fusarium solani. P. pinellianum extract with 50 and 75% concentration lead to full growth inhibition of F. solani, R. solani and A. alternata fungus [20] and sort rot diseases on potato affected by garlic extract [21], extracts of Salvia officinalis, Rosmarinus officinalis and seed of Cynara scolymus were quite comparable to values obtained with the conventional fungicide captain [22], turmeric rhizomes inhibited the mycelia growth of A. solani by 38.2% [23] and aqueous extracts from Eucalyptus citriodora leaves in 20% concentration were efficient to inhibited in 100% the mycelia growth of Phytophthora capsici and Sclerotium rolfsii and 75% in R. solani and in 45% to A. alternata under in vitro assays [24], while aqueous extract of wild basil at 5% concentration was enough to provide inhibition at 100% mycelia growth of A. alternata [25]. Study by [26] appeared that crude extract of Lippia alba and R. officinalis has bigger inhibition for A. alternata mycelia growth and the two plants mixture extracts produced better values than isolated extracts with 60% of growth inhibition of A. alternata [27]. Active inhibition appeared by extract of Nerium oleander leaves against R. solani and F. solani [28].

2. Materials and Methods

2.1. Determining plants ingredients

The plants collected from fields and around of the roads (leaves and stems) of three plants were washed by water. The plant dried at the shade and milled then 10gm from the sample put in 50 ml boiled water (90-100°C) for 3 hours then extracted whatman papers no.1 the extraction collected and put in closed glass tube in order measuring the concentration of active ingredients by High Performance Liquid Chromatography (HPLC) apparatus which supplied by Shimadzu company (Japan) type, LC-10A 2000 supplied with spectrum scale (Spectrophoto meter – spd – 10A – UV). A sample size 20µl injected on Fast liquid chromatographic column (LC) with dimension (50×4.6mm I.D) by the injector type (Rheodyne-712) at condition show in (table 1) and the data recorded by calculator which drawed the pick area and retention time. A standard solution of Heliotropium lasiocarpium plant used and sperated py HPLC apparatus and identification the pick area and retention time of studied plant sample at the same condition. Concentration of compounds in the sample calculated by the aquation [36]:

\[
\text{Conc. Compound in the plant} = \frac{\text{Pick area of compound}}{\text{Pick area of standard pattern}} \times \text{standard pattern conc.} \times \text{delution factor}
\]

\[
\text{Pick area of standard pattern}
\]

Table 1. Chromatographics separate condition.

| Column          | Mobile Phase | Following rate | Type of detector | Temperature | Fast of recorder paper | Size of injected sample |
|-----------------|--------------|----------------|-----------------|-------------|------------------------|------------------------|
| Reverse Phase   | Distill water| 10 ml/min.     | Ultra violate ray| 30°C/10     | mml/min                | 20mg/ml                |
| Reverse Column  | Ethanol 70% | 2: 80v/v       | 254 nm          |             |                        |                        |
| (50×4.6 mm I.D) |               |                |                 |             |                        |                        |

2.2. Preparing plants extracts

Leaves and stems of three plants were washed by water and soaked in 2% of sodium hypochloride solution for 15 minute and washed with sterilized water and air dried at room temperature, 100gm of each plant milled and used for extraction in 100ml of hot water and the extraction dried by using water path at 60°C in order obtain 8.2gm dried extraction then different concentration 5, 10, 15, 20% prepared from the dried extraction in addition to control 0% [20].

2.3. Isolation and identification of fungi.

Infected parts from potato and Solanum plants were collected, the pathogen was isolated on potato dextrose agar (PDA) medium. Infected piece of plant washed and sterilized by sodium hypochloride
2% then washed and transferred to (PDA) plate were incubated at 25 ± 2°C for 10 days. The fungus identification was done with using a key of [30].

2.4. interaction between fungi and plant extract

Three petridish (9 cm diameter) prepared for each fungi then disks with 0.5 cm transfer from each studied fungi taken from pure culture on age 7 days. Prepared different concentration plant extraction added in each treatment (three replication) without control treatment, the dishes incubated at 25 ± 2°C, then measured the growth of fungi in each treatment to edge of the dish then decided the percentage of inhibition by the equation:

\[ \text{Inhibition (\%) = \left(\frac{C - T}{C}\right) \times 100} \]  

(1)

Where, C and T represent the diameter of control and treated colony, respectively. Data on mycelial growth 9 days after incubation (DAI) when mycelial reach edge of petridish were recorded. Before addition the medium antibacterial Amoxicilin added to 1 L of PDA medium and mix well with the medium then added to the petridish.

3. Results and discussion

The analysis of three studied plants by HPLC apparatus appeared verily H. suaveolens plant contain several compounds Indicine, Supinine, Indicine-N-oxide, Heleurine, Heliotrine and Linelofidine Figure 1 and table 2, and P. major plant contain the compounds Salysilic acid, Kaempferol, Gentisic acid, Vanillic acid, Chlorogenic acid, Coumaric acid, Ferulic acid and Aucubin Figure 2 and table 3, while S. marinum plant contain, Silychristin A, Silychristin B, Silydianin, Silybins A, Silybins B, Isosilybin s A and Isosilybin A Figure 3 and table 4.

![Figure 1. Chromatogram HPLC analysis of studied H. suaveolens plant.](image)

**Table 2.** Compounds, Pick area and the concentration studied of H. suaveolens plant.

| Compounds             | Pick area of plant | Pick area of standard | standard Conc. mg/ml | Compound Conc. | Percentage (%) |
|-----------------------|--------------------|-----------------------|----------------------|----------------|----------------|
| Indicine              | 14222              | 39148                 | 50                   | 90.82          | 9.52           |
| Supinine              | 49530              | 32824                 | 50                   | 37.72          | 3.95           |
| Indicine-N-oxide      | 20682              | 36977                 | 50                   | 139.83         | 14.66          |
| Heleurine             | 46263              | 36748                 | 50                   | 314.73         | 33.00          |
| Heliotrine            | 33423              | 27478                 | 50                   | 304.08         | 31.88          |
| Linelofidine          | 92870              | 34983                 | 50                   | 66.36          | 6.95           |
**Figure 2.** Chromatogram HPLC analysis of studied Plantago major plant.

**Table 3.** Compounds, Pick area and the concentration of studied P. major plant.

| Compounds        | Pick area of standard | Pick area mg/ml | standard Conc. mg/ml | Compound Conc. mg/ml | Percentage(%) |
|------------------|-----------------------|-----------------|----------------------|----------------------|---------------|
| Salysilic acid   | 37357                 | 3606            | 25                   | 90.82                | 34.93         |
| Kaempferol       | 133752                | 3732            | 25                   | 37.72                | 4.55          |
| Gentisic acid    | 13164                 | 3864            | 25                   | 139.83               | 2.72          |
| Vanilic acid     | 3806                  | 18348           | 25                   | 314.73               | 1.67          |
| Chlorogentic acid| 3510                  | 40005           | 25                   | 304.08               | 0.70          |
| Coumaric acid    | 31321                 | 29463           | 25                   | 66.36                | 8.59          |
| Ferulic acid     | 6315                  | 23819           | 25                   | 29.13                | 21.42         |
| Aucubin          | 31890                 | 28229           | 25                   | 64.22                | 9.12          |

*Figure 3.* Chromatogram HPLC analysis of Studied S. marainum plant.
Table 4. Compounds, Pick area and Concentration of studied S. marinum plant.

| Compounds      | Pick area of plant | Pick area of standard | standard Conc. mg/ml | Compound Conc. mg/ml | Percentage (%) |
|----------------|--------------------|-----------------------|----------------------|----------------------|----------------|
| Silychristin A | 416853             | 2583                  | 25                   | 31.98                | 42.24          |
| Silychristin B | 15645              | 34646                 | 25                   | 11.28                | 14.89          |
| Silydianin     | 20637              | 22533                 | 25                   | 22.89                | 30.23          |
| Silybins A     | 3425               | 34445                 | 25                   | 2.50                 | 3.30           |
| Silybins B     | 2831               | 433993                | 25                   | 2.08                 | 2.74           |
| Isosilybins A  | 3091               | 20989                 | 25                   | 3.68                 | 4.86           |
| Isosilybin A   | 2000               | 38168                 | 25                   | 1.30                 | 1.71           |

Plant extracts differ in its effect on fungi growth, the concentration by 15% and 20% of P. major plant completely inhibit fungi growth and H. suaveolens plant completely inhibit the fungi growth by 20% concentration, while S. marinum doesn’t show any effect on fungi growth Table 5 and 6.

Table 5. Effect of various concentration of plant extracts on the radial mycelia growth of Fusarium solani, Fusarium oxysporum and Alternaria alternate (average mm).

| Concentration% |
|----------------|
|                |
|                |
|                |
|                |
|                |

* The same letter in the line means no significant difference between the concentration.

Table 6. Inhibition concentration of Fusarium solani, Fusarium oxysporum and Alternaria alternate growth at various concentration of plant extracts.

| Concentration% |
|----------------|
|                |
|                |
|                |
|                |
|                |

Analysis by (HPLC) technique is characterized by in procedure quantitative and qualitative estimation of plant ingredients by its ability to calculate curves and its high and determine this ingredients on one operation [31], the author [32] and [33] said verily separation and diagnosis of ingredients extracted from plants by (HPLC) apparatus gave fast results and high accuracy in comparative with other chromatographic methods and using (HPLC) technique showed high speed and
accuracy on appreciation quantity and quality of volatil oil [34] and [35]. Using this technique appeared existence 15-20 compounds on the volatil oil of Cuminum cuminum[36] and discovery several glycosidic compounds on the Heliotropium sp plant such as Quercite, 4-isorahamanine, 4-Heliotrope, 4-isopyrolidine, Narengnine and Triterpene [37]. The inhibition on fungi growth due to the alkaloidal compounds in plant extract which prevent fungus growth on severl plant kinds [3], this compounds has high treatment efficiency and its toxic [21] exist in the plant and mature seeds contain it more than immature seeds[38] also alkaloidal compound N-oxide is toxic [39] and the inhibition ability of this species due to its high content from alkaloid reach (233.71)mg/ml in comparative with low content of another compounds[37], our study agree with [40] whom proved that aquoses and alcoholic extract of Heliotropium genus inhibit growth of types of bacteria and fungi and with [41] who showed difference on inhibition activity of Artemisia sp. Achillespand Saliva officinalis plant extracts against F. oxysporum fungi also agree with study appeared completely inhibition on F. solani and A. alternata growth when use Pimpinella anism seed extracts [42]. The analysis appeared several acidic compound in the content of S. marinus and this may lead to inhibition of fungi growth because acidic materials or acid medium affect on enzymes production [43] Organic acids have been used to prevent the growth reproduction of harmful fungi and secreting of aflatoxins. The effect of eight organic acids as antifungal agents on the growth of four fungi were studied. Acetic acid (10%) showed the highest inhibition effect on A. flavus growth being 45.21% while tartaric acid (5%) and citric acid (5%) gave the lowest inhibition effect of 0.42%. Formic, acetic and propionic acids had the highest inhibition effect on A. flavus growth [44].

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