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RESEARCH ARTICLE

Analysis of Phytochemical Compounds from the Leaves of *Kitaibelia Balansae* Plant and Their Biological Activity on Some Fungi

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ABSTRACT

In this work, the preliminary phytochemical screening of the compounds found in the leaf extracts of *Kitaibelia balansae* Boiss. plant, obtained from solvents of different polarities, was investigated by LC/ESI/QTOF in the negative ion mode, and their biological activity on fungal species was studied. More than sixty compounds were observed, most of which were recognized and identified as flavonoids of all classes. Acids such as shikimic, chlorogenic, jasmonic, and its ester, methyl jasmonate, were also detected. The activity of these extracts on fungi was investigated and the results showed a dual biological effect on fungi *Fusarium solani* and *F. equiseti,* while inhibiting *F. redolens* and *Beauveria bassiana*. MIC values were also determined. However, to date, no detailed research has been conducted on the molecular constituents of the leaves of *K. balansae* and their antifungal activity.

Keywords: Antifungal activity, Flavonoids, LC/ESI/QTOF, Plant extract

Introduction

Plants having medicinal properties have been widely investigated since they compose several complex mixtures of various concentrations of many compounds, of which an important proportion is unknown.[1–3] Traditional Turkish medicine includes many compounds with variations in chemical and physical properties. *Kitaibelia balansae* Boiss. is the only Turkish representative of the genus *Kitaibelia,* which is traditionally used in the form of a cataplasm in the Anatolian part of Turkey.[4] However, in our previous work, we have reported the antimicrobial activity of the essential oil and extracts of the *K. balansea* Boiss species.[5] Since the literature lacks information about the flavonoid constituents of the total extracts of the leaves of this plant, and since, up to date, there is no work conducted on its activity against fungi, LC/ESI/QTOF in the negative ion mode was used to identify the molecular content of this plant extracts and test their biological activity against fungi. There are few studies on *Kitaibelia vitifolia* content[6] but not in detail on *K. balansea* Boiss., that was identified by the botanist Pierre Edmont Boissier from Anatolia, Turkiye (1867). Some studies on the flavonoids present in the flowers of *K. vitifolia* such as quercetin, luteolin, kaempferol, and their glycosides have been reported.[7] Various activities of the extract of this species were also reported.[8] However, flavonoids are naturally occurring polyphenolic groups and are found in plants as secondary metabolites.[9] They are synthesized by plants to prevent

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microbial infections and play an important role as defense and signaling compounds in plants.[10,11] They are also the active components of many medicinal plants that exist in natural products, and most of them are conjugated to a carbohydrate moiety, they occur in plants as O-, C- glycosides and as free aglycones. They are responsible for various biological activities that are structure-dependent.[12]

At present, medicinal plants have proved to be an alternative to synthetic drugs in preventing and treating some diseases[13] for example, the flavonone eriodictyol exerts potent anticancer activity against the A549 human lung cancer cell line by inducing mitochondrial-mediated apoptosis, while luteolin induces apoptotic cell death via antioxidant activity in human colon cancer cells.[14] However, the antifungal efficacy and mechanisms of many classes of known flavonoids have been well studied, for example, the flavonone eriodictyol inhibits each of the following: *A. parasiticus, A. flavus, F. semitectum* and *P. expansum* fungal strains, while the isoflavone genistein inhibits the *T. rubrum* strain, and the flavanols gallic acid inhibits *Candida albicans* strains.[15–17] The flavonol isoquercitrin shows activity on *C. albicans* and *C. parapsilosis* strains. The anthocyanidin peonidin has exhibited activity on each of the following: *C. albicans, C. tropicalis, C. parapsilosis, C. glabrata, C. krusei and C. dubliniensis, C. albicans, C. parapsilosis, C. krusei and Candida* strains.[18] The potential of quercetin and its glycoside derivatives as an antidepressant, as well as other activities, has been reported.[19] The association between dietary flavonoid consumption and the prevalence of chronic respiratory diseases has been also reported.[20] Flavonoids have potent anti-tumor effects and considerable preventive and therapeutic effects on numerous types of gastrointestinal cancers.[21] There is increasing evidence for their effects on hypertension, atherosclerosis, and other diseases, which have been widely explored.[22] Therefore, many efforts have been made to synthesize active molecules and investigate their anticancer and antitumor activities.[23] Other researchers have synthesized new molecules and studied their antimicrobial efficiency and cytotoxic effects.[24] However, the excessive use of synthetic drugs has led to the emergence of multidrug-resistant isolates in pathogens such as *Fusarium*, making it difficult to treat infections in humans.[25]

Pathogenic fungi species, such as Fusarium, are known to cause various diseases in plants and humans, as well as being responsible for significant economic losses worldwide in the agricultural field. Various Fusarium species have been identified to cause local or widespread infections in both immunocompromised and healthy individuals.[26] Moreover, strains of this species isolated from the environment acquire resistance due to their frequent exposure to fungicides, making it difficult to resist infections in agricultural areas. Consequently, fungi used for biological control, such as *Beauveria bassiana* and some *Trichoderma* species, have been observed to cause various infections, especially skin diseases including corneal keratitis, in people working in the agricultural field.[27–30] Therefore, alternative methods have been searched to fight against fungal infections in plants,[30] humans, and animals.[29–31] These alternative methods have emerged as biological control methods and are sought in nature.[30,32]

In this study, the molecular constituents, and the biological activity of *K. balansae* leaf extracts against *Fusarium* species and the entomopathogenic filamentous fungi *B. bassiana* were determined. The effectiveness of the extracts obtained from different solvents against the mycelial growth of fungi was investigated.

Materials and methods

Plant collection

The aerial parts of *K. balansae* Boiss. were collected (100 g) during the flowering stage on the 20th of July 2015 from Konya, Turkiye. Collection locations were at an altitude of 1400 m, and the plant material was dried and identified according to the classical Herbarium rules (Niğde Ömer Halisdemir University Herbarium stock no. 4700). The leaves were collected separately and were finely ground before extraction.

Experimental

Extraction of the leaves of K.balansae

12.00 Grams of the ground leaves of *K. balansae* plant were extracted in a Soxhlet for five hours using 500 mL from each of: dichloromethane, methanol, and water, respectively. The solvents were evaporated under reduced pressure to give colored, gum-like extracts. The content of these extracts was examined by LC/ESI/MS. A yellow-colored extract (1.05 g) was obtained from the extraction by dichloromethane, while the methanol extract was dark green and the yield was 0.85 g. Water extraction was carried out for three hours to yield 1.17 g of an orange-colored, gum-like compound.

LC/ESI/MS acquisition

In this work, the G6530B component model MS Q-TOF dual ESI ion source in negative ionization Mode was used. The LC column was Poroshell 3 ×

50 × 2.70 EC, C-18. The temperature was 30°C, and the analysis temperature was 0.80°C. MS threshold (abs) 200, MS/MS abs. the threshold was 5, and the minimum (m/z) range was 100, and the maximum (m/z) range threshold was 1000. The qualitative analysis of the total extracts was performed in the auto MS/MS mode with preferred mass ions m/z at 121.0508 and excluded mass ions at m/z 922.0097. Isolation width MS/MS: (∼4 amu), Fixed collision energy:15.00, the mass ranges from 100 to 1000 m/z. The total time of analysis was 90 min, the UV detection wavelength was 190–600 nm, the scan rate (spec/sec): 2, Nitrogen gas temperature was 350°C, and the N2 flow rate was 10 L/min. Nebulizer (psig) 35, V Cap: 3500, Fragmentor voltage: 175, Skimmer1: 65, Octopole RF Peak: 750, Ref Nebulizer (psig): 5, Average Scans: 1, Detection Window (ppm):100, Minimum Height (counts):1000 HIP auto sampler Model, G1367E, Draw Speed 200 μL/min., Eject Speed 200 μL/min., Injection volume 20 μL/min. LC pump; Binary Pump Model G1312B, Flow 0.400 mL/min. Solvent system; gradient; (A) H2O + 0.1% Trifluoroacetic acid (TFA): (0–20 min, 100–80%; 20–30 min, 80–65%; 30–50 min, 65–30%; 50–70 min, 30–20%; 70–80 min, 20–0%). Gradient; (B) Methanol: (0–20 min, 0–20%; 20–30 min, 20–35%; 30–50 min, 35–70%; 50–70 min, 70–80%; 70–80 min, 80–100%). Samples were dissolved in methanol and 10 ppm solutions were prepared and injected.

Biological activity studies

In this study, fungi were isolated within the framework of a project in Niğde, Ömer Halisdemir University, Biotechnology Department. For the growth of *Fusarium* species (*F.solani, F. redolens, F.equiseti, and B. bassiana*) and antifungal studies, PDA (potato dextrose agar, Condalab) was used after sterilization.

The biological activity of each extract obtained from different solvents against fungi species was investigated by dissolving three different concentrations (10 ppm, 20 ppm, and 40 ppm) of each extract in a 10% DMSO (dimethyl sulfoxide) solution. Sterilization by filtering through a 0.22-micrometer filter. Each extract was stored at +4°C.

Bioassay

Seven-days-old pure cultures of the fungi were used in this study. Fungal disks with a 5 mm diameter were taken from these cultures and placed on a petri dish containing different concentrations of plant extract (10, 20, 40 ppm). The results obtained from the three parallels were given as the average of mycelial diameter (mm) and calculated as a percentage of mycelial inhibition using the formula reported by[32] as shown below:

$$\%MI = \left[\left(dc - dt\right)/dc\right] \times 100$$

Where, dc is the colony diameter developed in the extract-free medium as a control.

Dt is the colony diameter of the fungi colony developed in the medium containing extract in the test medium.

Determination of minimal inhibition concentration (MIC)

The minimum inhibitory concentration is the lowest concentration of an antimicrobial agent that provides complete inhibition (100%) of visual growth. The protocol of CLSI (Clinical Laboratory and Standards Institute Methods, the "Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi, Approved Standard) was used to determine MIC values of the extracts.[32] Itraconazole was used in its commercial formulation (Funit 100 mg, Nobel) as a reference drug. However, following the protocol of CLSI, this drug was dissolved in 100% dimethyl sulfoxide (Merck) and was prepared as a stock solution of 2,000 μg/ ml. From this stock solution, serial two-fold dilutions were prepared. The concentrations of itraconazole ranged from 0.1 to 60 μg/mL, and each plant extract ranged from 0.05 to 60 μg/mL.

As described by CLSI, the antifungal reference drug and three plant extracts were placed in a potato dextrose broth (PDB) medium in microfuge tubes with concentrations ranging from (0.05–60 μg/mL). The final concentrations of the inoculated fungal spore suspensions for *B. bassiana* were adjusted to 3,1 × 10^4 cells/ mL and 3,8 × 10^4 cells/ mL for *F. redolens*. For the MIC tests, triplicate parallel inoculations were made, and the tubes were incubated at 28 ± 2°C. After seven days, the tubes with growing fungi were considered positive, and the ones that inhibited the growth were recorded as negative. The individual tubes containing itraconazole and plant extract that showed minimum inhibition were regarded as MIC. The tubes containing only fungal spores inoculated in the PDB medium were used for comparison as the positive control.

Results and discussion

The mass spectrometric data chromatograms

The mass spectrometric data chromatograms obtained from the extracts of the leaves of *K. balansae* plant by each of dichloromethane, methanol, and water are given in Figs. 1 to 3. The compounds in each extract shown in the mass data chromatograms in Figs. 1 to 3 were tentatively identified by comparing their masses with those in the Main, Wiley, Mass Bank Data Base, and the Mass Bank of North America (MoNA) Libraries. However, the compounds that were tentatively identified belonged mostly to the known classes of flavonoids Fig. 4 and acids such as Shikimic, Jasmonic, Chlorogenic and the fatty acid eicosenoic, in addition to some Glycerophospholipids, as is shown in Tables S1–S3.

From the mass data chromatogram of the leaves of *K.balansea* Boiss. extracted by dichloromethane, given in Fig. 1 and Table S1, many flavonoids having various biological activities have been detected.[10,33–35] Catechin/ Epicatechin and (-)- Epigallocatechin, which possess a variety of antimicrobial properties were present.[36] The anthocyanidin, Cyanidin-3-O-alpha-arabinopyranoside and iridin that exhibits anti-inflammatory and antioxidant activity,[37] were all observed in the early elution range of (0.653–1.679). Kaempferol, which is known to have many pharmacological effects,[38] and luteolin, that possesses many activities[14] and inhibits fungal strains such as *C. albicans, C. glabrata, C. krusei, C. parapsilosis, C. tropicalis, A. fumigatus,* and *T. rubrum,* were also present. Some flavones, for example, the isoflavone Equol that inhibits the *C. albicans* fungal strain.[18] The glucosides, kaempferol-7-O-glucoside that possesses antiviral activity and is considered as a new potential drug candidate for the treatment of infections, as well as 8-Prenylnaringenin[39] were all observed in the region (46.137–64.4). Whereas the range (65.926–71.128) included mostly water-soluble flavonoid derivatives, anthocyanidins, and anthocyanins, which are the universal plants pigments responsible for the colors of flowers and fruits.[37] They have been considered as active principles of many medicinal plants and as an important part of the human diet. These phytochemicals have been reported to have many bioactive properties, most notably their significant abilities, in the suppression of tumor cells, for example, the ability of the plant pigment, Cyanidin-3, 5-di-O-glycoside in suppressing prostate cancer has been reported.[37,39–41] Some anthocyanidines such as peonidin and other cyanidines have exhibited antifungal activities against *C. parapsilosis*, *C. glabrata, C. krusei* and *C. dubliniensis* strains[16] However, in addition to these, Cyanidin-3-O-glucoside, Cyanidin-3-O-sambubioside and digalacturonic acid, were all observed in this region. The biflavonoid amentoflavone that displays multifunctional biological activities has a role as antiviral, antimicrobial antifungal and anti-cancer activity. It is widely used in traditional Chinese medicine.[41,42]

The region from (70.514–84.081) included mainly the major flavonol, flavonone, isoflavanoids, flovones, flavan and anthocyanidin type flavonoids, and organic acids in addition to flavonoids having one or more carbohydrate moieties in their structure. 2'.4'-Dimethoxy-3-hydroxy-6-methyl flavone had antioxidant, anti-inflammatory activities and the ability to scavenge reactive oxygen species was detected in this region. It is worth mentioning here that most of the flavonoids present in these extracts in Tables S1–S3 show many pharmacological and antifungal activities,[34,40] for example methoxyflavone (Wogonin) has exhibited antifungal activity against *A. fumigatus* and *T. rubrum*.[16,18] Table S2 included many other flavonoids or their derivatives, in the region (70.514–76.737).

Fig. 2 represents the mass data of the extract by methanol which shows many components that were tentatively identified and listed in Table S2. The components present in this chromatogram include D-Glucose, L-Histidine, 4,6-Dimethyl-3(4'-Hydroxyl phenyl) coumarin, Dihydroxyflavanone, Anthocyanidins, Flavonol O-glycosides, Flavone C, C glycosides, Prenylated flavanones, and isoflavones. Many

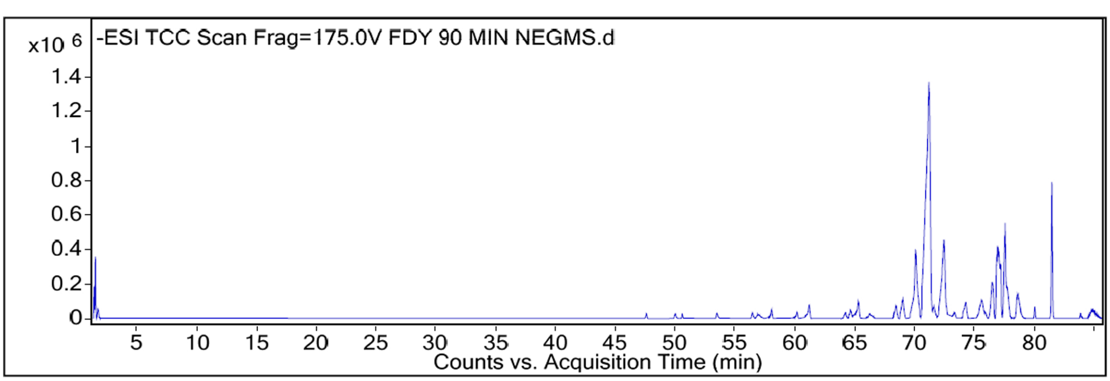

Fig. 1. LC/MS/QTOF Mass data chromatogram of the dichloromethane extract of *K. balansae* leaves.

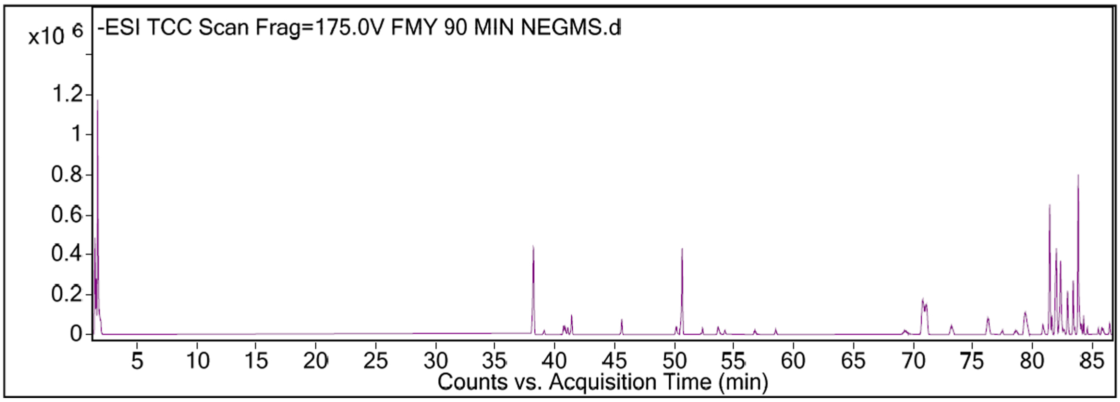

Fig. 2. LC/MS/QTOF Mass data chromatogram of the methanol extract of *K.balansae* leaves.

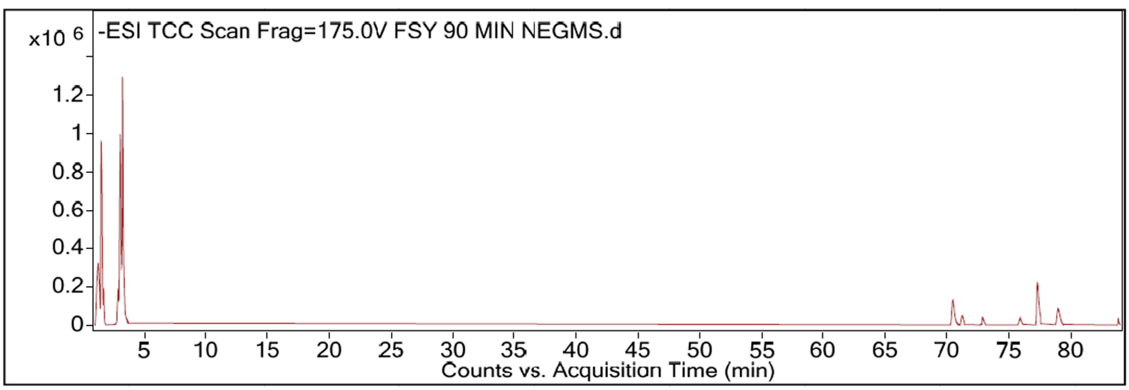

Fig. 3. LC/MS/QTOF Mass data chromatogram of the water extract of *K.balansae* leaves.

more flavonoids were also present in this extract as is shown in Table S2. However, the flavonoids such as Catechin, Iridin, epigallocatechin, and cirsiliol and isoschaftosides, that were observed in the dichloromethane extract, were observed in the methanol extract too, while many others were not detected in the dichloromethane extract as expected Table 1. This indicates that the polarity of the solvent does play an important role in the extraction process of a mixture of compounds with a wide range of polarities.

The water extract of the leaves of *K. balansae* plant is given in Fig. 3 and the compounds present are listed in Table S3. The most interesting components that were present in this extract were methyl dihydroxy jasmonate, jasmonic acid [43] and methyl jasmonate [44] in addition to prenylated flavonoids [34,45] such as 8-prenylnaringenin and 6-prenylnaringenin. The jasmonic acid and its derivatives (jasmonates), are of particular importance as they are involved in diverse signal transduction pathways to regulate various physiological and molecular processes in plants, thus protecting plants from the lethal impacts of abiotic and biotic stressors,[43,44] While Prenylated flavonoids are a novel type of nutraceuticals with great health benefits, possessing a wide variety of bioactivities, such as estrogenic activity, antioxidant activity, immunomodulatory activity. They have a low abundance in nature and are complicated to be synthesized chemically. Prenylation significantly enhances some bioactivities of flavonoids, especially estrogenic activity and anticancer activity.[46] This extract has also contained acids such as shikimic, quinic, eicosenoic, 4-acetylbutyric acid, and 2, 3-dihydro-p-coumaric acid as well as the alcohol tryptophol. 4, 6-dimethyl-3 (4'-hydroxy phenyl) coumarin, and catechin, genistein, naringenin, myricetin and many other flavonoids that possess antifungal activities against many fungal strains[18] such as 2'.3'.6- trimethoxy flavone and wogonin, as is shown in Table S3. However, the common compounds present in each of the dichloromethane, methanol and water extracts of the leaves of *K.balansae* plant are given in Table 1.

Determination of biological activity on Fungi

The mycelium growth of fungal species in cultures containing plant extract was compared to the control, i.e. cultures grown in extract-free media in Fig. 5 shows that *K. balansea* extracts have promoted the

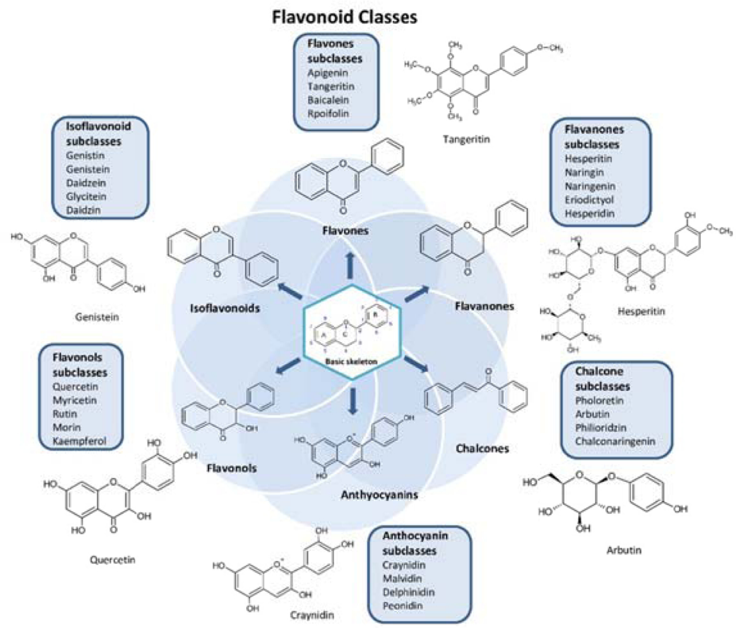

Fig. 4. Basic skeleton structure of flavonoids and their classes.

growth of *F. solani* and *F. equiseti* species and have inhibited *F. rodelens* (effective against agricultural pests). While *B. bassiana* species (entomopathogenic) did not grow at any concentration of the extracts. Fig. 5 also showed that the extracts have stimulated the two fungi (*F. solani* and *F. equiseti*) while inhibiting the other two fungi (*F. redolens* and *B. bassiana*). However, in Fig. 5B, the water extract of the plant has exhibited significant activity on *F.equiseti* at 40 ppm concentration when compared to the dichloromethane extract in Fig. 5A and methanol extract in Fig. 5C. This may indicate that the compounds extracted by water which include certain flavonoids, fatty acids, and some organic acids in Table S3 had affected the growth of *F. equiseti* fungi more than the others in Table 2. The mycelial inhibition percentage for the inhibited two fungal strains was also calculated and is shown in Fig. 6.

The inhibition rates of *F. redolens* and *B. bassiana* species were determined at all concentrations and were found to be similar in extract types. *B. bassiana* was inhibited at the three concentrations of all extracts by 100%. The inhibition of *F. redolens* was (80%) in the methanol extract (10 ppm C extract) and 93% in the other extracts. As a result, the compounds present in all extracts have inhibited the growth of *F. redolens* and *B. bassiana* fungi. Therefore, we can conclude that the fungi to be used in biological control are affected by the chemical content of the plant.

Similar studies have been carried out in the literature and the antifungal activity of flavonoids isolated from mango (*Mangifera indica* L.) leaves has been reported.[47] *In vitro,* antifungal activity of phenolic content obtained from grapes was also studied.[48] However, the effects of yarrow (*Achillea millefolium* L.), tansy (*Tanacetum vulgare* L.), sage (*Salvia officinalis* L.) and wormwood (*Artemisia absinthium* L.) leaf extracts on various phytopathogenic *Fusarium* species were investigated. In the study, 5%, 10%, and 20% concentrations of plant extracts were used and their effects on the mycelial growth of *Fusarium avenaceum, F. culmorum, F. graminearum, F. sporotrichioides* were determined. Plant extracts inhibited the mycelial growth of phytopathogens depending on the fungal species, extract type, and concentration. At 20% concentration, extracts of sage and tansy plants showed a strong inhibitory effect of 83.53% and 72.58% against the tested fungi, respectively. Yarrow and wormwood extracts were found to have low fungistatic effects (63.82% and 67.57%), respectively, indicating that low concentrations of plant extracts stimulate the growth of *Fusarium* species.[49] *In vitro* and greenhouse studies conducted by Ain et al.,[50] showed that *Pinus wallachiana* leaf extracts showed an inhibitory effect when used to combat the damage caused by *Fusarium oxysporum* species to banana plants. The extracted content was investigated by HPLC and, showed that the phytopathogenic activity was due to polyphenols such as quercetin, myricetin, kaempferol, rutin, gallic acid, trans-ferulic acid, coumaric acid, epicatechin, and catechin.

Other researchers have studied the effect of aqueous extracts of 22 plant species on *Fusarium solani* and *Rhizoctonia solani* under *in vitro* conditions and

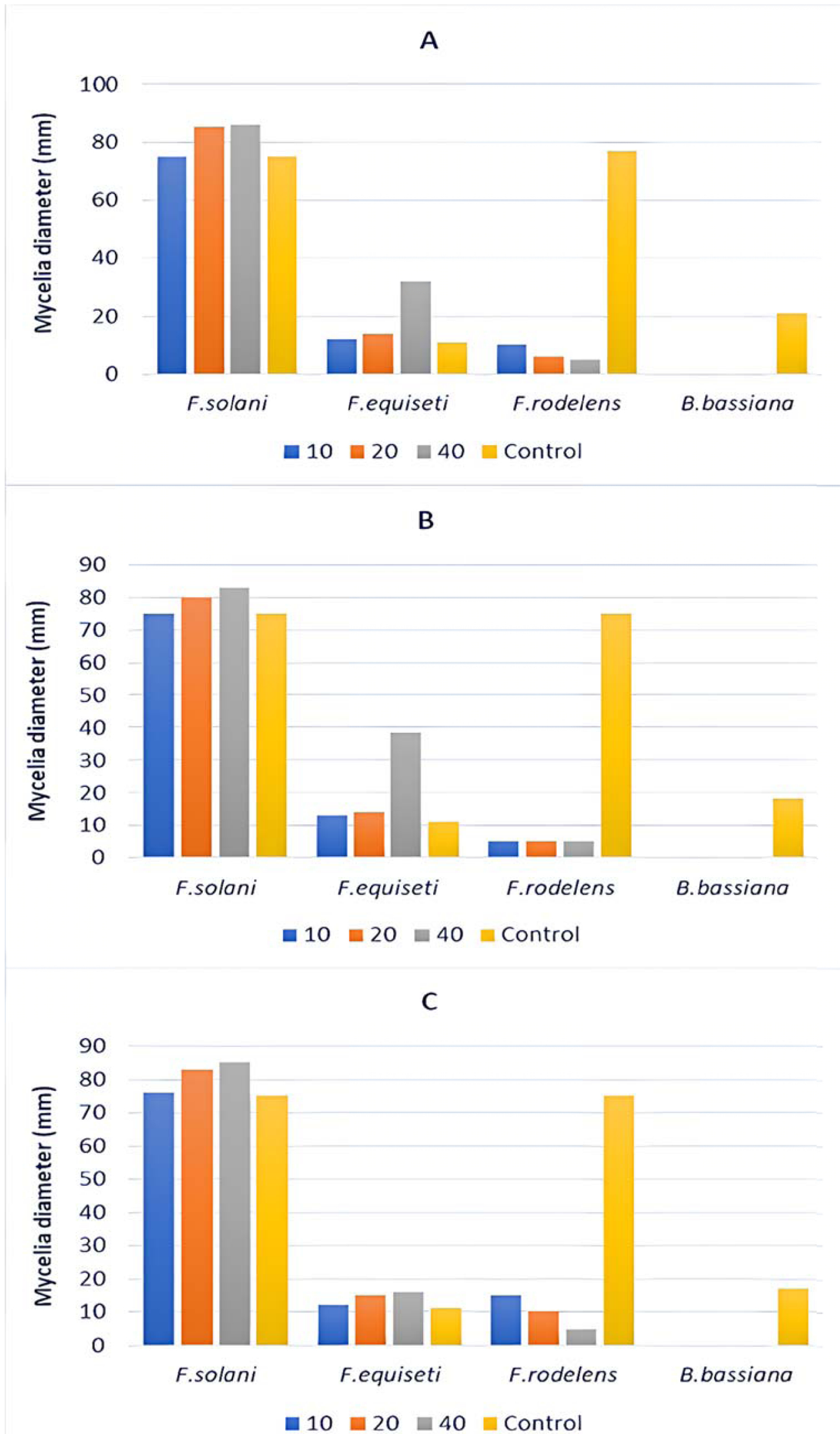

Fig. 5. Biological activity of the leaf extracts with each (A: dichloromethane; B: water; and C: methanol) at (10, 20, and 40 ppm) concentrations on fungi. For control, mycelial growth was followed on PDA media in the absence of extracts.

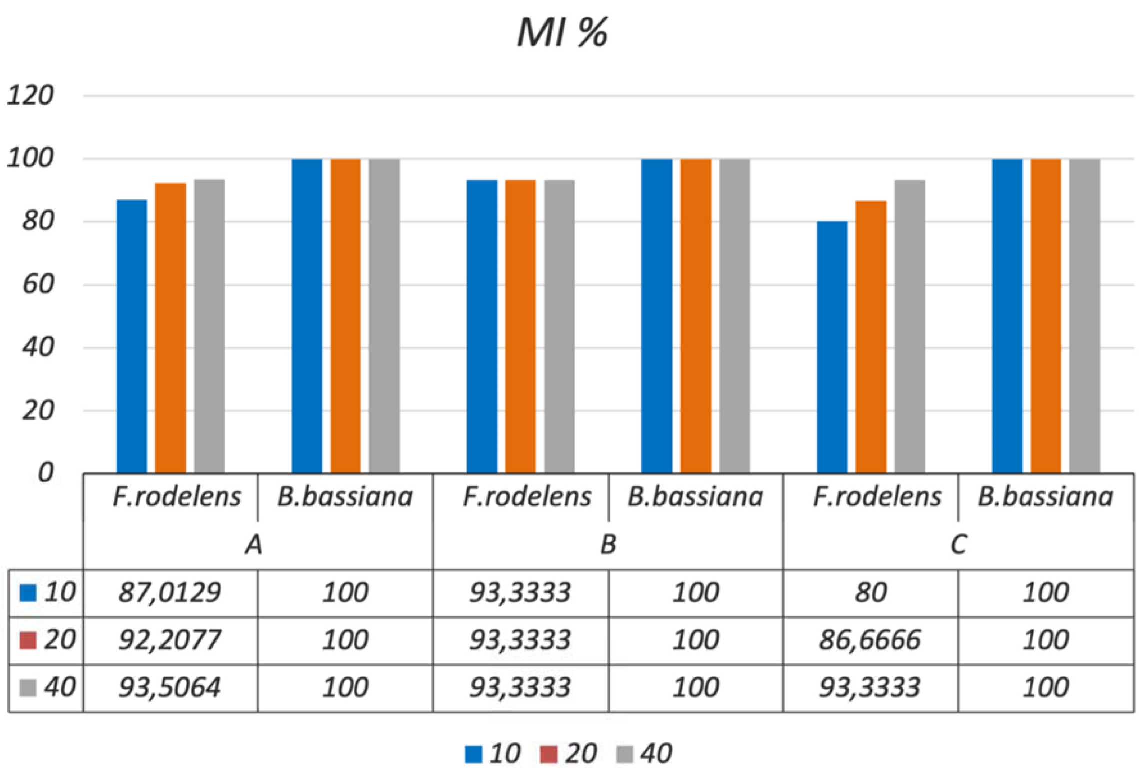

Fig. 6. Mycelia inhibition by *K. balansae* extracts with each (A: dichloromethane; B: water; C: methanol).

Table 1. Common compounds present in all of the leaf extracts of *K.balansae* by the three different solvents; dichloromethane (DCM), methanol (MeOH) and water (H2O).

NO	Compound	*m/z* [M-H]$^-$	DCM	MeOH	H_2O
1	(+/−) Catechin/epicatechin	290.9363	+	+	+
2	Cyanidin-3-O-alpha-arabinopyranoside	419.8978	+	+	–
3	Iridin	521.9139	+	+	–
4	Cirsiliol	330.9791	+	+	–
5	(R,S)-Equol	243.179	+	+	+
6	Hispidulin acetate	426.9677	+	+	–
7	Sambicyanin	581.2953	+	+	–
8	Jasmonic acid	209.9517	+	–	+
9	Eicosenoic acid	310.1774	+	+	+
10	Chlorogenic acid	355.2008	+	–	+
11	Shikimic acid	174.0528	+	+	+
12	Flavanone + 4O, 1Prenyl	563.2849	+	+	–
13	3-Hydroxy-3’.4’-dimethoxyflavone	298.1563	+	+	–
14	8-Prenylnaringenin	340.1992	+	+	+
15	2’.3’.6-Trimethoxyflavone	312.1721	+	+	–
16	Naringenin 7-O-glucoside (Prunin)	434.2998	+	+	–
17	5.7-Dihydroxy-8-methoxyflavone (Wogonin)	284.2678	+	+	+
18	7,4’-Dimethoxy-3-hydroxyflavone	298.156	+	–	+
19	3’.7-Dimethoxy-3-hydroxyflavone	298.156	+	–	+
20	4.6-Dimethyl-3(4’-hydroxyphenyl) coumarin	266.1511	+	–	+
21	3’.4’-Dimethoxy-3-hydroxy-6-methylflavone	312.1721	+	–	+

reported that except for Basil, Castor bean, Chamomile, and Mint. All plant extracts were shown to have antifungal activity against the two fungi. Aqueous extracts of chili, lantana, lemon grass, and Onion seeds were reported to significantly reduce the mycelial growth of *F. solani* from 60.0% to 74.4% and the mycelial growth of *R. solani* from 42.2% to 71.1%. Field experiments of these extracts

Table 2. Comparison of MIC values for *B. bassiana* and *F. redolens*.

Fungal species	Itraconazole*	A*	B*	C*	Control (Fungal growth)
F. redolens	10.0	60.0	60.0	60.0	+
B. bassiana	2.0	2.0	2.0	2.0	+

*: MIC values (μg/mL) of the leaf extracts with each (A: dichloromethane; B: water; and C: methanol, and Itraconazole drug; (+): positive fungal growth).

were also conducted and showed that the plant extracts have reduced the activity of phytopathogens increased the survival percentage of the plant, and improved the yield parameters.[51] In another study, pea plant root extract stimulated the germination of *F. solani* spores, due to the flavonoids present in the extract.[52] However, plant extracts can have different effects on fungi depending on their content, and therefore, the *in vitro* effectiveness of plant extracts must be determined before using them against phytopathogens in agricultural fields. From the human health point of view, it is necessary to know how infectious fungi respond to plant extracts in vitro under similar conditions, and that random plant extracts should not be used for alternative treatment purposes. In this study, antifungal studies showed that the polyphenolic content of the extracts of plants such as *K. balansae*, is responsible for the various effects on fungi as it is reported in the literature.[6,7,18,48] This requires further research to isolate and identify the polyphenolic compounds responsible for the antifungal activity.

The minimum inhibitory concentrations (MIC_{100}) of plant extracts are compared in Table 2. According to Fothergill,[32] MIC research has not been enough to allow the classification of pathogenic molds for their antifungal susceptibility. However, general guidelines have been established to assist in analyzing mold data. Based on large amounts of data, some fungi are regarded as susceptible to antifungal compounds such as itraconazole, when MIC is ≤ 1.0 μg/mL, intermediate when MIC is 2.0 μg/mL, and resistant when the MIC is ≥ 4.0 μg/mL according to Fothergill.[32]

The MIC_{100} ranges for the two fungi (*F.redolens* and *B. bassiana*) that have been investigated for their antifungal susceptibility indicated that the reference drug itraconazole and the plant extracts had the lowest MIC values (2.0 μg/ml) for *B. bassiana* and showed the highest MIC values, (10 μg/ml) for the plant extract and (60μg/ml) for *F.redolens*. The scientists have considered that this variation might be due to species-specific susceptibility to the drug and plant extracts.

As reported in the literature, the phytochemical composition of the extracts, particularly polyphenols such as quercetin, kaempferol, and gallic acid and coumarin derivatives have been reported to significantly contribute to antifungal activity, correlating these compounds with the observed inhibition has provided insights into the mechanisms of action[53,54] Comparative literature analysis has highlighted the importance of combination with other compounds and concentration dependence of extract effects,[55,56] showing that bioactive polyphenol compounds, singly or in combination with organic acids, interfere with the life processes of fungi by binding to their protein molecules, therefore acting as chelating agents, altering structural component synthesis. this results in the weakening or destroying of the permeability barrier of the cell membrane and changing the physiological status of the cells, which has been shown to be active against *F. oxysporum*.[57] Ruan et al.[52] proposed that flavonoids could stimulate spore germination while other authors,[18,51,56] have found that extracts of some plant species were able to inhibit fungi and bacteria and that their ability was due to the presence of flavonoids. These findings have practical implications for biological control strategies and demonstrate that careful selection of extract type and concentration is critical to minimize undesirable stimulation of target pathogens while maximizing antifungal efficacy.

Conclusion

This work is the first phytochemical and antifungal study of the leaf extracts of *K. balansae* plants, where the extracts have shown dual biological effects on fungi by stimulating some species, while inhibiting others. The leaf extracts and the reference antifungal drug itraconazole had different effects on the fungal species. However, the selection of an appropriate concentration and species is critical for the effective use of the extracts against target pathogens. The antifungal agents of plant origin have arisen as safer alternatives, since fungal pathogens cause significant losses in agriculture, threatening food security and human health through mycotoxin contamination. *K. balansae* species contained more than 60 compounds, which were identified, mainly as flavonoids of all classes and organic acids, which are known to possess biological and pharmacological activities. The extracts of this plant have also been traditionally used by people in the Anatolian part of Türkiye as a painkiller in the form of a cataplasm and the roots were used for kidney and other infections. Therefore, this research will continue to isolate prominent compounds, test their activity and make use of their antifungal activity against the growth of various phytopathogenic fungi.

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Author's declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images that are not ours have been included with the necessary permission for republication, which is attached to the manuscript.
- No animal studies are present in the manuscript.
- No human studies are present in the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at Niğde Ömer Halisdemir University.

Authors' contributions statement

M.I.A. and A.O. designed the study, M.I.A. and F.Y.D. extracted the plant, M.I.A. and Z.Z.C. analyzed and identified the LC/ESI/MS data, A.O. carried out the anti-fungal studies, M.I.A. and A.O. wrote the paper with input from all authors.

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Supplementary materials

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تحليل المركبات الكيميائية النباتية في أوراق نبات (Kitaibelia balansae) ونشاطها البيولوجي على بعض الفطريات

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الخلاصة

في هذا العمل، تم إجراء الفحص الكيميائي النباتي الأولي للمركبات الموجودة في مستخلصات أوراق نبات Kitaibelia balansae Boiss.، والتي تم الحصول عليها باستخدام مذيبات ذات قطبية مختلفة، وذلك باستخدام تقنية LC/ESI/QTOF في نمط الأيونات السالبة، كما تمت دراسة نشاطها البيولوجي على الأنواع الفطرية. تم رصد أكثر من ستين مركبًا، تم التعرف على معظمها وتحديدها على أنها فلافونويدات من جميع الفئات. كما تم الكشف عن أحماض مثل الشيكيميك والكلوروجينيك والجاسمونيك وإستره ميثيل جاسمونات. تمت دراسة نشاط هذه المستخلصات على الفطريات، وأظهرت النتائج تأثيرًا بيولوجيًا مزدوجًا على فطريات Fusarium solani و F. equiseti، في حين تم تثبيط F. rodelens و Beauveria bassiana. كما تم تحديد قيم التركيز المثبط الأدنى (MIC). ومع ذلك، حتى الآن، لم يتم إجراء أي دراسة مفصلة حول المكونات الجزيئية لأوراق نبات K. balansae ونشاطها المضاد للفطريات.

الكلمات المفتاحية: النشاط المضاد للفطريات، الفلافونويدات، LC/ESI/QTOF، مستخلص نباتي.