International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Bioherbicidal potential of *Ficus carica* on *Phalaris canariensis* : detection of flavonoid allelochemicals by thin layer chromatography and infra-red spectroscopy

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Abstract

A potentially useful strategy for developing ecologically safe bioherbicides is to target weeds with allelopathic plant species. In vitro bioassays of the allelopathic effect of aqueous *Ficus carica* leaf extract were carried out on the germination of *Phalaris canariensis* and *Lactuca sativa*. While the aqueous extract had no discernible effect on lettuce, it significantly inhibited the germination of canary grass, and the impact became more pronounced as the concentration of the phyto-allochemical extract increased. The inhibitory concentration of 50% germination of the weed was 0.32 g/100 ml. The bioherbicidal properties of *Ficus carica* were attributed to the allelochemicals retained in the organic fraction of ethyl acetate, and their qualitative identification revealed the presence of flavonoids, notably flavones and flavonols.

Keywords: Allelopathy, Allelochemicals, Flavonoids, Biohericide, *Ficus carica*.

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1. Introduction

Unwanted plants called weeds impede the growth and development of crops, hence reducing their yield. Weeds reduce crops' access to space, light, and moisture in the agricultural system, which lowers crop quality [1]. Chemical herbicides are the main tool used by the agriculture industry to manage and control weeds. But when these synthetic products are used excessively and inappropriately, food, water, and soil are contaminated, which lowers human health. In fact, ingesting chemical residues that may be hazardous to humans can have a variety of negative health repercussions, such as cancer, genetic diseases, endocrine disruption, and neurological issues [2]. Allelopathy may be an advantageous approach in weed management programmes for reducing commercial reliance on herbicides [3]. It is an ecological phenomenon in which an organism creates one or more biochemical compounds that affect other organisms in the same community's germination, growth, and development as well as their survival and ability to reproduce asserts that allelopathy is one of the most successful weed management strategies since it may have a significant impact on weed growth inhibition [4-5].

Allelochemicals are often stored by allelopathic plants in their roots and leaves, whereupon they get released and affect neighbouring plants [6]. Allelopathic water extracts are water-soluble allelochemicals extracted from plants. Allelopathic water extract is used as natural herbicide because most of allelochemical compounds are water-soluble and are easy to apply without additional wetting agent, and they are more environmentally friendly than synthetic herbicide [7]. A member of the family Moraceae, the common fig tree (*Ficus carica*) is a deciduous fruit tree. The existence of a fig makes all the species of the genus ficus distinct, and its many parts, including leaves, latex, bark, roots, and fruits, offer medicinal benefits [8]. *Ficus carica*, commonly known as the common fig, is a fruit tree that belongs to the Moraceae family and is deciduous. The presence of figs distinguishes all ficus species, and its many parts—leaves, bark, roots, latex, and fruit— have medicinal qualities [8]. Figs have long been utilised in medicine for their antipyretic, purgative, and aphrodisiac qualities as well as for the treatment of inflammatory illnesses [9].

Various hydroxycinnamic, hydroxybenzoic, and chlorogenic acids, along with some flavonoids, have been

identified by analysing the phenolic composition of *F. carica* leaf and fruit extracts [10-11].

2. Materials and methods

2.1. Effect of aqueous extract of fig leaves on germination of Phalaris canariensis and Lactuca sativa

2.1.1. Preparation of the aqueous extract

Ficus carica leaves were collected between June and August. The plant material was dried for 72 hours at 50°C in an oven, ground into powder, and kept at room temperature in paper bags in the lab. 100 ml of distilled water and 4 grams of plant powder were mixed, then stirred magnetically for an hour. The mixture was filtered through muslin, and the filtrate was centrifuged at a speed of 3000 rotations per minute for 10 minutes [12]. Wathman n°1 filter paper was used to filter the recovered supernatant once more under pressure. The resulting mixture was the 4% crude extract, which was then diluted with distilled water to produce a further series of three concentrations (2%, 1%, and 0.5%).

2.1.2. Germination test

The effects of aqueous extract on germination of canary grass (*Phalaris canariensis*) and lettuce (*Lactuca sativa*) were tested using 30 petri dishes of 80 mm diameter. In each box, we placed ten seeds of canary grass or lettuce and added 5 ml of either the extract of a considered concentration (test) or distilled water (control). Petri dishes were closed with parafilm to prevent evaporation and incubated at 18°C and 12 h photoperiod for 10 days. Each treatment was repeated three times.

2.1.3. Observation and measurements

The percentage of germination inhibition was determined according to the following formula:

$$\% \text{ inhibition} = ((N-n) / N) * 100$$

N being the average number of germinated seeds in the control for 10 days and n being the average number of germinated seeds in the treatment for 10 days. The corresponding 'IC50' inhibitory concentration index values were calculated by plotting the extract concentration on a logarithmic scale (X) and the germination reduction response on the y-axis. The data appear linear in a semi-logarithmic graph paper. This method is widely used to study the phytotoxic effect or toxic efficiency of allelochemicals and herbicides [13-14].

2.2. Detection of Ficus carica flavonoids by Thin Layer Chromatography (TLC) and Infrared Spectroscopy (IR)

2.2.1. Preparation of the organic phase of ethyl acetate (OPEA) of Ficus carica

The aqueous extract was confronted with two solvents of increasing polarity. The crude extract (4%) was transferred to a separatory funnel and washed with three times 50 ml of Hexane to recover lipids thus recuperating the organic phase from the Hexane. The remaining solution at the bottom of the funnel was washed with three times 50 ml of ethyl acetate recovering the phase containing specific molecules [15]. The different phases recuperated are the organic phase of Hexane which contains only the fats, chlorophylls and impurities; the organic phase of Ethyl Acetate which allows the extraction of flavonoids by entraining the aglycones, the mono-glycosides and partially the di-o-glycosides and the residual water phase which contains the sugars and the inorganic compounds [16-17].

2.2.2. Effect of recovered fractions on germination of Phalaris canariensis

For fractions in the two organic solvents (hexane, ethyl acetate), 10 ml of aliquots are placed in Petri dishes that are left uncovered in the dark and under a laminar flow hood to evaporate the solvents. Then, 10 ml of distilled water is added and the bottom of each dish is scraped with a clean spatula to solubilize the organic deposit. In parallel, other Petri dishes were prepared in which 10 ml of aliquots of the aqueous fraction were deposited. Other Petri dishes containing 10 ml of distilled water were used as controls. 10 phalaris seeds were placed in each petri dish in the presence of plant extract or the control. Each treatment was repeated three times. Petri dishes were then sealed with parafilm to prevent water evaporation. Petri dishes were maintained for 10 days under laboratory conditions (room temperature of 18°C at noon with diffuse light during the day).

2.2.3. Detection of flavonoids in the organic phase of ethyl acetate by Thin Layer Chromatography (TLC)

Only the organic fraction of ethyl acetate (OPEA) with a total volume of 150 ml was then used after being concentrated using a rotary evaporator (Buchi) under pressure at a temperature of 40°C. In order to know the chemical composition of its flavonic fingerprints, the OPEA concentrated to 10 ml was subject of a qualitative analysis by TLC on aluminium plates (20*20 Cm) covered by a silica gel. The plate was developed with a set of solvent systems and the best separation was obtained with an eluant consisting of Chloroform/Methanol (18/2). The migration time was 2 hours. The plate was then dried under the host using a hair dryer. Flavonoid detection was performed using a UV lamp at 366 nm in a dark room [18]. A molecule's UV fluorescence and frontal ratio, or "Rf" which measures the distance a molecule has gone relative to the distance travelled by the mobile phase (a solvent), are indicators of how that molecule will behave in a particular system (table 2).

2.2.4. Infrared spectroscopy (IR) of the organic phase of ethyl acetate (OPEA)

Based on peak values in the 4000 to 500 cm^{-1} wavelength range, infrared (IR) spectra are used to categorize functional categories of secondary metabolites. An IR spectrum makes it possible to learn more about the researched molecule's distinctive group. Hence, each IR absorption band has a certain kind of bond that makes it possible to identify the characteristic group [19]. The IR analysis is performed at a temperature of 20°C by a mauque perkin Elmer ATRFTIR apparatus, model spectrum two. The analysis was performed without treatment (without prior use of KBr).

2.3. Statistical analysis

Statistical analyses were performed with the SAS program. An Arcsin transformation of the square root of the germination inhibition percentages and a conversion of the negative means to zero values (0) were adopted before submitting the data to statistical analysis. The student-

Newman and Keuls test at a probability of 5% was applied for comparison of means.

3. Results and discussion

3.1. Effect of aqueous extract at different concentrations on germination of Phalaris canariensis and Lactuca sativa

Canary grass germination was considerably suppressed by the aqueous extract of *Ficus carica*, starting from 51.9% at the lowest concentration (0.5%) and going up to 96.3% at the highest concentration (4%). Morever, the phytoextract had no noticeable negative effect on lettuce germination (no difference compared with the control) (Table 1). The inhibitory effect on canary grass seed germination was exacerbated by increasing the concentration of *Ficus carica* aqueous extract. In contrast, found that the germination rate of *Elymus nutans* seeds was significantly enhanced by *Medicago sativa* aqueous extracts at a concentration of 5.5%, whereas it was inhibited at a concentration of 14.5% [20]. Allelochemicals have been shown to inhibit seed germination by suppressing the mitotic activity of immature cells [4]. This has been linked to disturbances in mitochondrial respiration and in the activity of metabolic enzymes involved in glycolysis and the oxidative pentose phosphate pathway [21-22]. Certain allelopathic substances interact with the mitochondrial membrane and impede mitochondrial respiration directly, as demonstrated by [23].

3.2. IC50 calculation

The value of the inhibitory concentration index "IC50" was calculated by a simple extraction of the unknown X from the numerical equations noted in the graphs below (Figure 1), giving Y the value 50. A comparison of IC50 values shows that aqueous extract of *Ficus carica* is significantly more phytotoxic to Phalaris seed germination than to lettuce seed germination. In fact, the aqueous extract exerted a 50% inhibition (IC50) on *Phalaris canariensis* at a very low dose (0.32g/100ml), in contrast to the much higher dose (26.91g/100ml) required for *Lactuca sativa* also reported that weed seeds were more susceptible than those of cultivated plants, and that the effect of phytoextracts depended on concentration [24]. These authors found that the average inhibitory concentration (IC50) for weed seed germination was 15 times lower than that needed for cultivated plants in their study on aqueous extracts of *Adenosma buchneroides*. The outcome is comparable to the allelopathic action of *Sonchus oleraceus*: the weeds *Brassica nigra* and *Melilotus indicus* had their seed germination impeded, while the crop of *Trifolium alexandrinum* was unharmed [25].

3.3. Effect of Ficus carica extract fractions on germination of Phalaris canariensis

The only part of *Ficus carica* that still had a very strong (96.5%) inhibitory impact on *Phalaris canariensis* germination was the organic component in ethyl acetate (Figure 2). The quality and quantity of phytochemical substances might differ based on the extraction solvent, as demonstrated by [26]. Additionally, observed that variations in the solvents' extraction effectiveness can account for both qualitative and quantitative variations in phytotoxins isolated from various aqueous and organic fractions of *Euphorbia dracunculoides* [27]. Furthermore, it appears that the variation in phytotoxicity amongst various fractions suggests that the inhibitory strength could fluctuate based on the solvent's polarity index. Indeed, found that increasing extract polarity made the decrease in germination percentage worse, indicating an increase in active principles [28]. Moreover, several studies have demonstrated that the characteristics and polarity of the extraction solvent do, in fact, affect the solubility of certain chemicals, such as phenolic compounds [29]. Actually, high-polarity organic solvents extract phenolic chemicals more successfully [30].

3.4. Identification of flavonoids of Ficus carica by TLC and IR

3.4.1. Analytical chromatography on thin layer (CCM)

Under UV light at 366 nm, different spots of the product were revealed on the chromatograms and were delimited (Figure 3). Bands with various fluorescences (orange, light yellow, light pink, green, violet, light blue, and purple) can be seen in the chromatogram (Figure 3), The presence of the flavonols and flavones group is indicated by orange, yellow, and green fluorescence; the presence of flavones, chalcones, dihydroflavonols, isoflavones, or flavonones is indicated by violet fluorescence; and the presence of flavones or flavonols is indicated by light blue fluorescence [31]. Whereas yellow-green may be caused by the presence of flavone glycoside biflavonols, yellowish orange suggests the presence of flavonol glycosides [32]. The presence of 7,8-di hydroxy-flavanones and 5-deoxyisoflavones may be the cause of the bands' blue fluorescence [32-33]. However, anthocyanidins-3,5-diglycosides may possibly be the cause of the blue coloring [32]. The chromatogram of the organic fraction of ethyl acetate from *Ficus carica* shows a wide range of Rf values (Table 2 & Figure 3). Stains characterized by very high Rf values (0.5-0.75) are very rich in flavonoids, notably flavonone, flavonol and methoxyflavan. According to Bandyukova and Shinkarenko, the increase in Rf values is due to methylation and acetylation of (OH) groups [34]. On the other hand, the decrease in Rf values (0-0.25) and (0.3-0.5), according to the same authors, is explained by increased glycosylation of (OH) groups. These compounds are polyhydroxyflavones, oligohydroxyflavones and oligomethoxyflavones [34]. Thus, the colors of the stains and their frontal ratio (Rf) observed under UV light allow us to conclude that flavones and flavonols are present.

3.4.2. IR spectroscopy analysis

Figure 4 & table 3 displayed the presence of carbonyl groups, aliphatic chains, multi-bonded carbon structures, aromatic rings (phenols), and alcohol groups (carboxylic acid) (esters). The occurrence of the band at 3195.74 cm^{-1} suggests that free alkenes (=CH) may exist in the range of wave numbers 3100–3010 cm^{-1}. The presence of the band at 3423.47cm^{-1} suggests that phenols or alcohols (-OH) may be present in the region of wave numbers 3650-3300 cm^{-1}. The band at 1652.32 cm^{-1} suggests that an aromatic molecule (the C=C chain) may exist in the wavenumber range of 1600 cm^{-1} [35]. Overall findings for the *Ficus carica* ethyl acetate fraction demonstrate the presence of the hydroxyl function as represented by the band at 3336. (O-H). This band, along with the bands at 1004.6 cm^{-1} (-O-C=C-) and at 1666 cm^{-1} (Ar), all show that flavonoids are present in this fraction. Alkaloids were also absent, which is evident by the absence of the bands associated with the amine function. According to Bulama et al. [36], the spectrum lacks bands at 815 cm^{-1}, 824 cm^{-1}, 872 cm^{-1}, or 882 cm^{-1} that are assigned to the terminal methyl

group of sterols, which explains why sterols aren't present in this fraction.

Table 1: Effect of aqueous extract of *Ficus carica* on germination of *Phalaris canariensis* and *Lactuca sativa*.

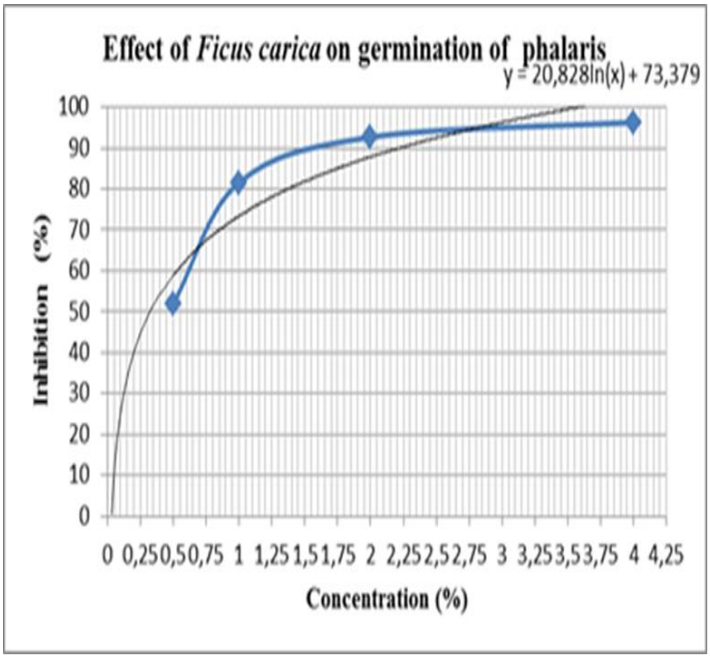
Plant	Extract concentrations (%)	Canary grass reduction (%)	Lettuce reduction (%)
Ficus carica	0	0,00 d	0,00 a
	0,5	51,88 bc	0,00 a
	1	81,55 b	3,53 a
	2	92,66 a	7,18 a
	4	96,30 a	7,18 a

Table 2: Chromatographic behaviour of the ethyl acetate organic phase of *Ficus carica* on the Silica aluminium plate in the solvent system (Chloroform/ Methanol) (18:2).

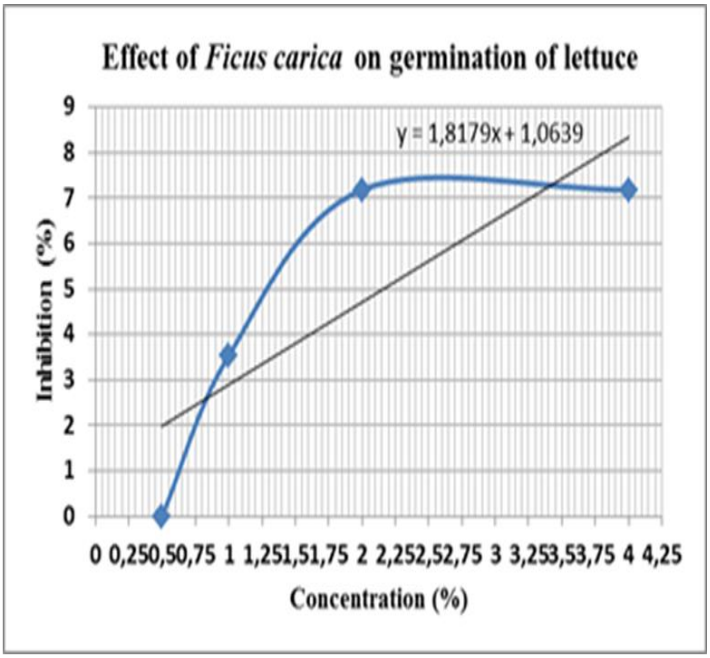
The ethyl acetate organic phase of *Ficus carica*			
UV colored spot at 366 nm	**Rf**	**UV colored spot at 366 nm**	**Rf**
Orange	0,05	Light yellow	0,39
Orange	0,09	Yellow-green	0,51
Light yellow	0,10	Pink	0,55
Light pink	0,14	Light yellow	0,59
Yellow green	0,16	Light pink	0,61
Light pink	0,18	Violet	0,66
Yellow	0,19	Light orange	0,69
Violet	0,22	Sky Blue	0,79
Yellow	0,27	Purple	0,83
Orange	0,35	Orange	0,94

Table 3: IR analysis of the organic phase of ethyl acetate from *Ficus carica.*

N°	Pic	Functional group	Vibration type	Intensity
1	574	C-Br or C-Cl alkyl halides	Wide	High
2	1004,6	C-O alcohol, carboxylic acid, ester and ether	Thin	High
3	1452,6	C-H alkanes	Thin	Weak
4	1666	C=**C**	Thin	Weak
5	2841	-CH_2-	Thin	Weak
6	2946	C-H alkanes	Thin	Weak
7	3336	Alcohol group O-H	Wide	Medium

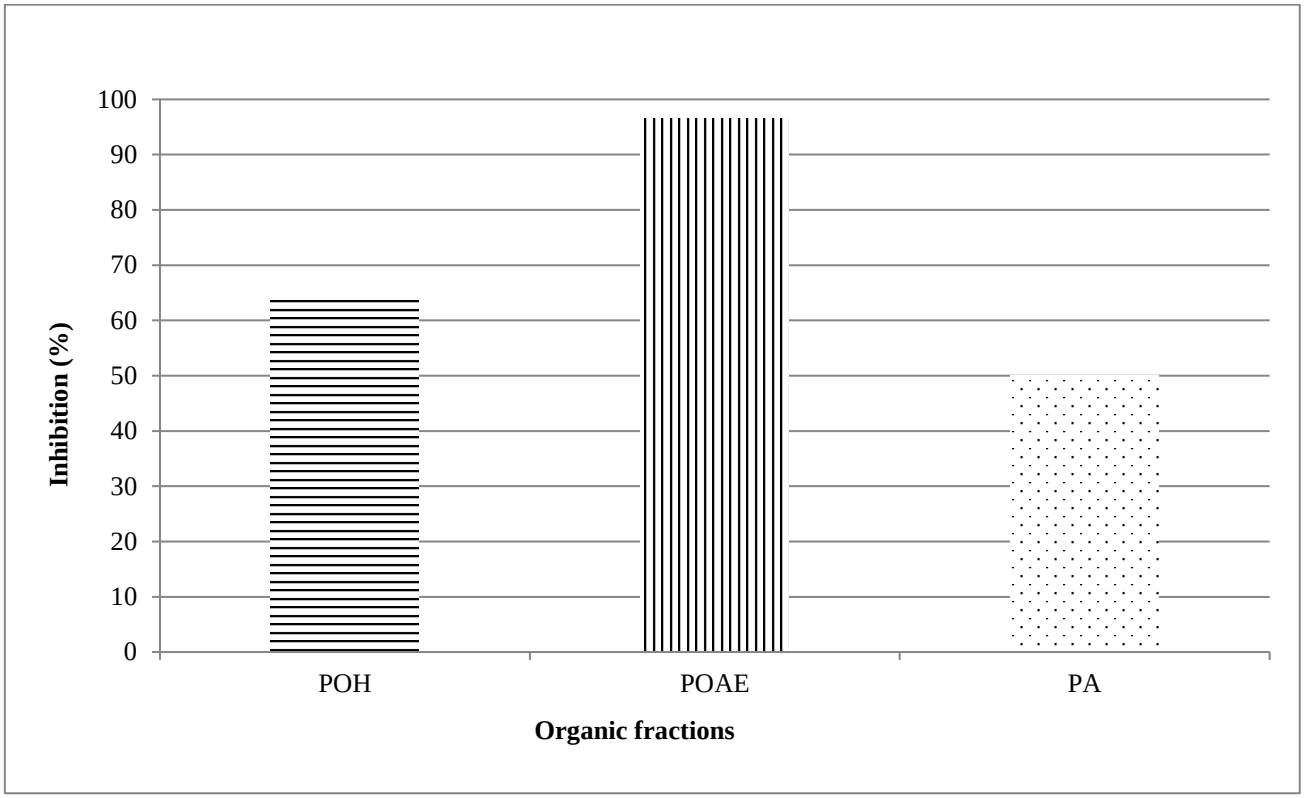


IC50 = 0,32



IC50 = 26,91

Figure 1: Aqueous extract effect of *F. carica* on percentage germination inhibition of *P. canariensis* and *L. sativa*



POH: Organic phase of Hexane; POAE: Organic phase of Ethyl Acetate; PA: Aqueous phase.

Figure 2: Effect of *Ficus carica* extract fractions on the germination of *Phalaris canariensis*.

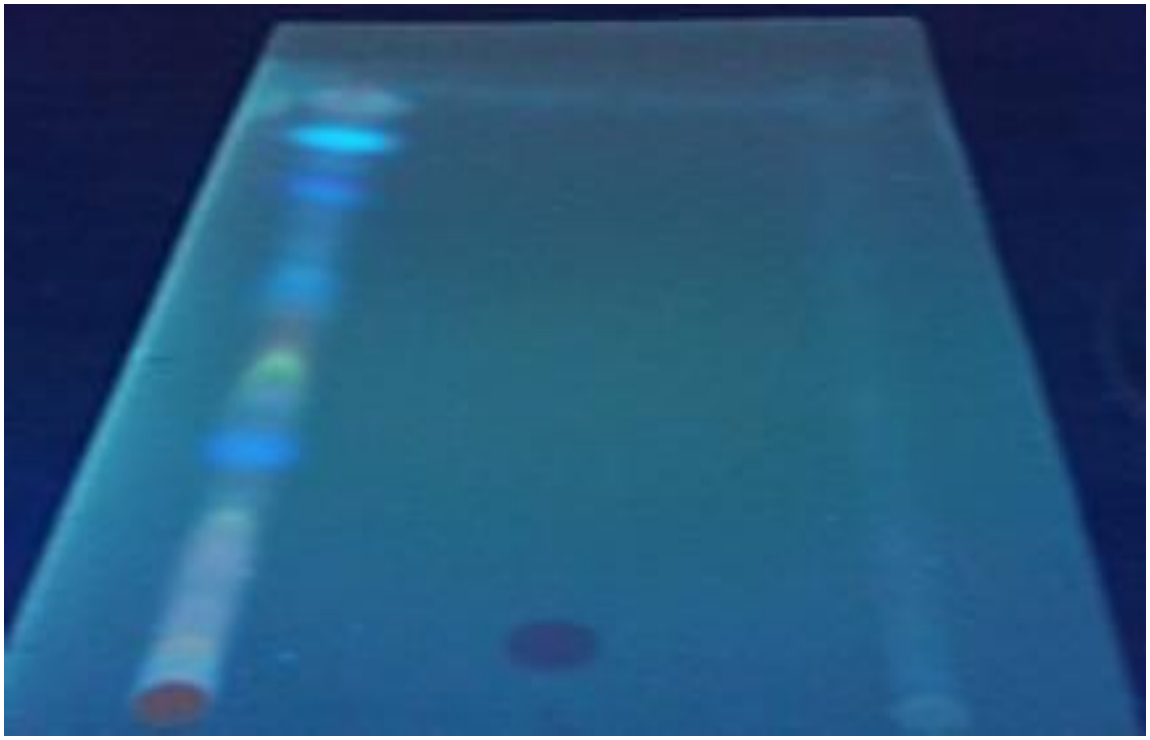


Figure 3: Chromatographic profile of flavonoid characterization in the ethyl acetate organic phase of *Ficus carica* leaf extract.

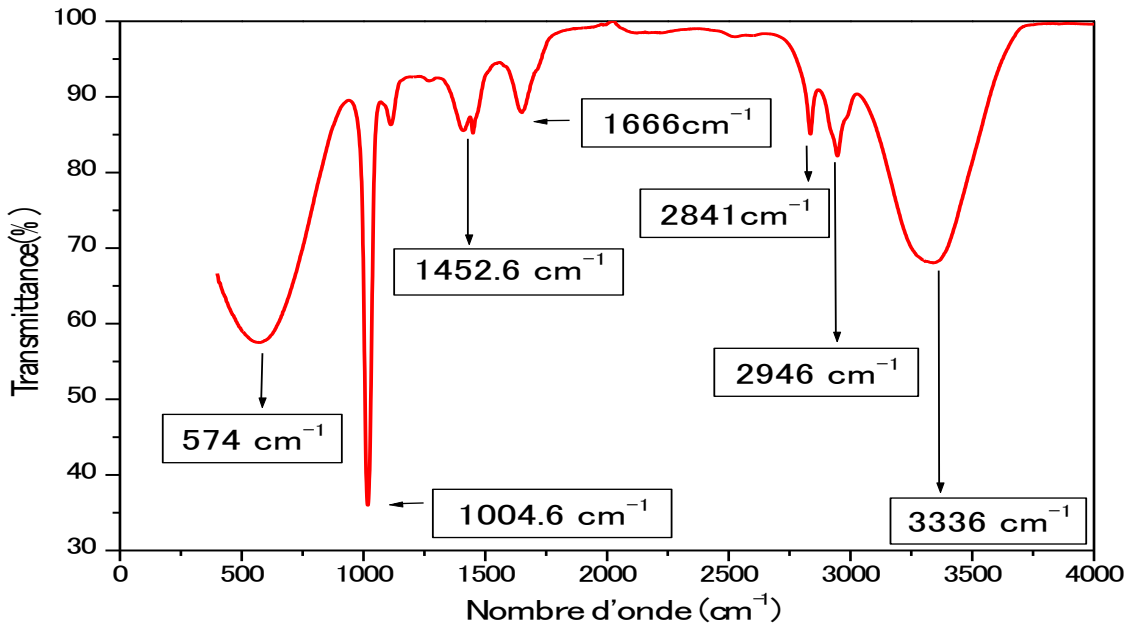


Figure 4: IR spectrum of the organic phase of ethyl acetate from *Ficus carica* leaf extract.

4. Conclusions

The current study examined the effect of several *Ficus carica* extracts on the germination of *Lactuca sativa* and *Phalaris canariensis*. The germination of *Phalaris canariensis* was significantly inhibited by the aqueous extract of *Ficus carica* leaves. The concentration of the extract had an increasing phytotoxic effect, but it had no influence on the germination of *Lactuca sativa*. The inhibitory impact on the germination of *Phalaris canariensis* was sustained by the organic component of ethyl acetate from *Ficus carica*. Certain flavonoids, particularly flavones and flavonols, are abundant in the organic fraction of the aqueous fig leaf extract, as demonstrated by the use of chromatographic (TLC) and spectroscopic (Infrared) techniques. It will be necessary to continue identifying families of secondary metabolites in plant extracts in order to standardize these techniques, which could provide an intriguing alternative to the currently utilized phytochemical screening method.

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