COMPARATIVE STUDY OF BIOCHEMICAL COMPOUNDS AND ITS RELATED THERAPEUTIC EFFICACY OF *IPHIONA MUCRONATA* AND *CONYZA DIOSCORIDES*

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The purpose of this study was to compare the biological effects as antimicrobial and antitumor activity and bioactive ingredients of two plants *Iphonia mucronata* and *Conyza dioscorides* which belong to the Asteraceae family. *I. mucronata* EtOAc extract exhibited promising antitumor activity against HELA (cervical), PC-3 (prostate), and HEP-2 (larynx) carcinoma cell lines with IC_{50} values 24.60, 26.57 and 39.06 μg/ml, respectively. While C. *dioscorides* showed weak and moderate antitumor activity against the same cell lines. MeOH extracts of the two plants showed weak antitumor activity, also MeOH and EtOAc of *C. dioscorides* exhibited moderate and weak antibacterial activity against *Proteus vulgaris*, *Bacillus subtilis* and *Escherichia coli*, respectively. The total active constituents of the MeOH extract of both plants revealed higher concentrations than the EtOAc extract of all components except alkaloid content. The phenolic, flavonoid, and alkaloid constituents of *I. mucronata* were in higher concentrations than *C. dioscorides* while the saponins content of *C. dioscorides* was higher than *I. mucronata* whereas tannins content was similar for both plants. The major phenolic acids and flavonoid compounds investigated using HPLC of both plants were caffeic, ferulic acid, naringin, and luteolin for *C. dioscorides* and *I. mucronata*.

Keywords: antimicrobial, antitumor, phenolic, flavonoid, *Iphonia mucronate*, *Conyza dioscorides*

INTRODUCTION

For thousands of years, people from many cultures around the world have used medicinal herbs to treat ailments and maintain good health. The study of these plants and their potential therapeutic properties was of great significance in modern medicine. *Iphiona* and *Conyza* species are two species of medicinal plants of Asteraceae family that have been used traditionally to treat various ailments, including diabetes, fever, rheumatism,

wounds and diarrhea (Al-Ghedira et al., 2017 and El-Seedi et al., 2021). These plants have garnered significant interest due to their potential therapeutic properties and their ability to provide alternative treatment options with fewer side effects compared to conventional medications (Zalabani et al., 2012 and El-Seedi et al., 2021). The therapeutic advantages associated with *Conyza* species are its ability to use as antibacterial, antioxidant, cytotoxic (Edziri et al., 2009), anti-inflammatory (Mahmoud et al., 2009), analgesic (Asogalem et al., 2004), antiviral (Anani et al., 2000), anti-proliferative (Csupor-Löffler et al., 2011), antischistosomal (Kamel et al., 2011), antiprotozoal (Calzada et al., 2001) and antidiarrheic activities (Atta and Mounir, 2004). The main chemical constituents found in *Iphonia* species are apigenin, luteolin, and quercetin. These flavonoids exhibit a range of biological functions, such as anti-inflammatory, anti-cancer, and antioxidant characteristics. Also, α-pinene, β-pinene, and limonene which were known for their antimicrobial and anti-inflammatory properties (Pecio et al., 2002), coumarins, flavonoid and phenolic acids have been shown to have anticoagulant, antitumor, and anti-inflammatory activities (El-Bassossy, 2022). Whereas, *Conyza dioscorides* contains essential oils which contain various monoterpenes and sesquiterpenes, diterpenes, particularly labdane diterpenes and clerodane diterpenes. These diterpenes have various biological activities, including anti-inflammatory, analgesic, and anticancer properties (Zalabani et al., 2012 and Kiplimo and Musembei, 2017).

Due to limited chemical studies especially phenolic constituents of the two plants and promising pharmacological activity of other family plants, the present study concerned to investigate the antitumor activity of both plants *Iphonia mucronata* and *C. dioscorides* against some uninvestigated carcinoma cell lines such as HEP-2 (Larynx), HELA (cervical), PC-3 (prostate) carcinoma cell lines, in addition to the examination the two plants extracts as antimicrobial activity and comparing the total chemical compositions and therapeutic effectiveness of them.

MATERIALS AND METHODS

1. Plants Collections

The aerial parts of *Iphiona mucronata* (Forssk.) Asch. & Schweinf and *Conyza dioscorides* (L.) DC. of family Asteraceae were carefully collected in April 2022 from their natural habitat before 23 km of Marsa Matruh city, Marsa Matruh governorate, Egypt. Guidelines were followed to minimize any potential harm to the environment and to maintain biodiversity of the plants. Dr. Omran Ghaly of Desert Research Center identified and verified the plant samples. A voucher specimen deposited at the Desert Research Center's herbarium with Code Number: CAIH-1025-R (*I. mucronata*) and CAIH-1026-R (*C. dioscorides*).

2. Materials and Chemicals

All chemicals used for chemical studies were of high quality grade, purchased from El Nasr company for chemical industries while, dimethyl sulfoxide (DMSO), MTT and trypan blue dye were bought from Sigma (St. Louis, Mo., USA). Fetal Bovine serum, DMEM, HEPES buffer solution, *L*-glutamine, gentamycin and 0.25% trypsin-EDTA were purchased from Lonza (Belgium).

Mammalian cell lines: HELA cells (human cervical cancer cell line), PC-3 cells (prostate cancer cell line), and HEP-2 cells (Larynx cancer cell line) were obtained from the American Type Culture Collection (ATCC, Rockville, MD).

3. Extraction

The air-dried powder of *I. mucronata* and *C. dioscorides* (100 g) were extracted successively by a soxhlet apparatus beginning with *n*-hexane, for defatting and removing lipodial contents followed by EtOAc, finally MeOH for 48 h till exhaustion for each solvent. Each extract was concentrated under vacuum to yield semisolid extracts of *I. mucronata* (3.9 g, 1.4 g, and 7.2 g) and *C. dioscorides* (5.6 g, 2.3 g, and 9.4 g). The EtOAc and MeOH extracts of both plants were investigated for their biological and chemical investigations.

4. Chemical Investigations

4.1. Determination of total phenols

Total phenolic acids of *I. mucronata and C. dioscorides* aerial parts were determined colorimetrically using Foline Ciocalteu reagent, as described by Singleton et al. (1999). The total phenols test was performed by combining 0.15 ml of the Foline Ciocalteu reagent, 0.3 ml of extracts, 0.3 ml of 7% Na_2CO_3, and 2.7 ml of de-ionized water. The mixture's absorbance was determined at 725 nm using a UV-Visible spectrophotometer (PG-T60). Gallic acid was used to prepare a standard curve. The final values were expressed as mg/g dry weight or gallic acid equivalents (GAE).

4.2. Determination of total flavonoids

Total flavonoid content of *I. mucronata* and *C. dioscorides* aerial parts was determined by a colorimetric method as described by Chang et al. (2002). Briefly, 1.25 ml of distilled water was used to dilute 0.25 ml of the 80 g/100 g methanolic extract. 150 ml of a 10% $AlCl_3.6H_2O$ solution was added to the mixture after 6 min, along with 75 ml of a 5% $NaNO_2$ solution. After allowing the mixture to stand for 5 min, 0.5 ml of 1 mol/L NaOH was added, and 2.5 ml of distilled water was added to finish the mixture. After thoroughly mixing the solution, a spectrophotometer (UV-Visible spectrophotometer (PG-T60). was used to measure the absorbance right away at 510 nm against the blank. The findings were given as mg of quercitin equivalents QE/g of dry weight.

4.3. Estimation of total alkaloids

The aerial parts of *I. mucronata* and *C. dioscorides* were subjected to quantitative measurements of total alkaloids using the methodology outlined by Harbone (1973). Dry plant powder of each plant sample (1 g) was combined with 4:1 ethanol to 70% acetic acid. After letting the mixture stand for at least six hours, it was filtered. Alkaloid in the supernatant was precipitated by adding concentrated ammonia solution dropwise. The precipitated alkaloids were dried to a consistent weight in an oven at 70°C after being filtered through reweighted filter paper (Whatman 102). The amount of alkaloid expressed as %/g of dry weight of the plant samples was determined and reported.

4.4. Determination of total saponin

To 25 ml of 20% ethanol, approximately 50 mg of the dried *I. mucronata* and *C. dioscorides* plant powder were added. In brief, the sample was heated to 55°C on a magnetic stirrer for 4 h while being continuously stirred, then filtered and 50 ml of 20% ethanol were added to the residue once more. The combined filtrates were concentrated to a 10 ml volume. Diethyl ether 5 ml was added to the concentrated filtrate. The aqueous layer was kept in storage while the diethyl ether layer was removed. Butanol 15 ml was added to the remaining water layer, creating two layers. After depositing the butanol layer, 15 ml of butanol was used to filter the aqueous layer once more. Sodium chloride 5 ml (5%) was added to the butanol layer, then the mixture was evaporated in a water bath. The sample was evaporated and then dried and weighted (Ajiboye et al., 2013).

4.5. Estimation of total tannins

Total tannins content was estimated by copper acetate method according to Ali et al. (1991). In summary, 2 g of the aerial portions of *I. mucronata* and *C. dioscorides* were extracted each separately for 1 h using two separate volumes of 100 ml each of acetone-water (1:1 v/v), and the material was then filtered. In each case, the combined extract was discreetly moved into a 250 ml volumetric flask, and its volume was calibrated using distilled water. After quantitatively transferring each extract to a 500 ml beaker and heating it to boiling, 30 ml of a 15% aqueous copper acetate solution was added while stirring. A porcelain crucible was filled with the precipitate of copper tannate, which had earlier been burned to a constant weight at the same temperature. The precipitate was collected on an ashless filter paper. The residue was mixed with a few drops of nitric acid and re-ignited to a constant weight. The following correlation was used to calculate the weight of copper oxide and the proportion of tannin: Each 1g of CuO ≡ 1.305 g tannins.

4.6. Phenolics and flavonoids investigation by HPLC

Analysis of phenolic and flavonoid compounds of both *I. mucronata* and *C. dioscorides* was performed at Science Way Lab, Cairo, Egypt using HPLC apparatus (Agilent Series 1100) (Agilent, USA) equipped with an

auto-injector, degasser, two LC pumps, a chemstation software, and a UV/Vis detector set at specific wavelengths for each type of compound (250 nm for phenolics and 360 nm for flavonoids). A C_{18} column was used to separate the phenolic acids and flavonoids. The separation process involved a gradient mobile phase composed of methanol (solvent A) and acetic acid in water (solvent B). The initial mobile phase was 100% B and remained so for the first 3 min. Then, the concentration of A gradually increased to 50% over 5 min, followed by a further increase to 80% for 2 min before returning to 50% for the final 5 min. The detection wavelength was set at 250 nm throughout the analysis. Flavonoids were separated using a different mobile phase consisting of acetonitrile (solvent A) and 0.2% formic acid in water (solvent B). An isocratic elution program with a 70: 30 ratio of A to B was employed. The solvent flow rate was maintained at 1 ml/min, and the separation was performed at 25°C. A sample volume of 25 µl was injected for each analysis (Lin et al., 1996 and Kuntic et al., 2007).

5. Biological Investigations

5.1. Antimicrobial activity

All microbial strains were obtained from the Regional Center for Mycology and Biotechnology (RCMB) culture collection at Al-Azhar University, Cairo, Egypt. Using a modified well diffusion method, the antimicrobial profile was tested against gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*), gram-negative (*Escherichia coli*, and *Proteus vulgaris*), and filamentous (*Aspergillus fumigatus*) and one yeast species (*Candida albicans*). In brief, 10 ml of fresh medium were used to cultivate 100 ml of the test bacteria or fungi until they achieved a count of roughly 10^8 cells/ml for bacteria or 10^5 cells/ml for fungi. Smeared onto agar plates that matched the broth they were kept in, 100 µl of the microbial culture were evaluated for susceptibility using the well diffusion method. A 100 µl volume of every sample, were diluted to 10 mg/ml, and introduced into each well (agar gel with holes cut of 6 mm in diameter). The plates were incubated for 48 h at 28°C (fungi) and for 24-48 h at 37°C (bacteria and yeast). The microorganism's growth was monitored following incubation. The resulting inhibition zone diameters were utilized as a benchmark for the antibacterial activity and were expressed in millimeters. If an organism is sensitive to the chemical, it will not grow in the vicinity of the well if it is cultivated on agar. The inhibitory activity is directly related to the size of the clear zone. Negative controls for each experiment included solvent controls (DMSO). Additionally, positive controls were conducted using conventional antifungal and antibacterial medications, ketoconazole and gentamycin (Mahmoud et al., 2018 and Rasras et al., 2023).

5.2. Antitumor activity

To assess the effectiveness of various extracts against cancer cells, a technique called MTT assay was employed. Tumor cells were carefully

transferred to the cultivation medium at a specific density and placed in individual compartments within a 96-well plate. The test extracts of *I. mucronata* and *C. dioscorides* were then introduced to the cells in triplicate (three separate wells) for each different concentration being investigated. Six additional wells served as controls: three containing only the culture medium and three containing medium with 0.5% DMSO (a solvent used to dissolve the test compounds). After 48 h of incubation, the MTT assay was initiated. This involved adding a small amount of MTT solution to each well. MTT is a special dye that reacts with living cells, transforming into a purple crystal. The amount of purple crystal formed directly reflects the number of viable cells present. Following another incubation period, the purple crystals were dissolved in DMSO. Finally, a specialized instrument called a microplate reader precisely measured the amount of dissolved purple crystal in each well. The 50% inhibitory concentration (IC_{50}) was also determined (Mosmann, 1983).

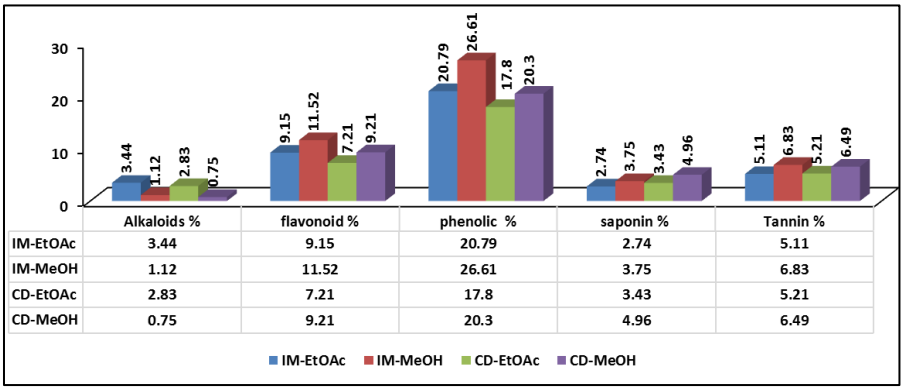
RESULTS AND DISCUSSION

1. Chemical Investigation

1.1. Bioactive compounds analysis

Findings outlined in Fig. (1) show that both *I. mucronata* and *C. dioscorides* plants are rich sources of bioactive compounds, including flavonoids, alkaloids, saponins, tannins, and phenolics. These compounds contribute to the plants' diverse pharmacological effects, such as anti-inflammatory, antioxidant, anticancer, anti-diabetic and anti-coagulant properties (Kumar and Mishra, 2013; Souza et al., 2015; Al-Snafi et al., 2017 and El-Bassossy, 2022). The chemical investigation of the EtOAc and MeOH extracts for the two plants showed that *I. mucronata* had higher concentrations of alkaloids, flavonoids and phenolic acids components than *C. dioscorides* where the former plant *I. mucronata* (IM-EtOAc) showed 3.44%, 91.58 mg/g (QE)≡(9.15%) and 207.91 mg/g (GAE)≡ (20.79%) alkaloid, flavonoid and phenolic compounds, respectively. While the concentrations of the same constituents of *C. dioscorides* (CD-EtOAc) were 2.83%, 72.14 mg/g (QE)≡(7.21%) and 178.7 mg/g (GAE) (17.8%), respectively. The concentration of saponin content of EtOAc extract of *C. dioscorides* was higher than *I. mucronata* which represented 3.43% compared to 2.74% of the same extract. However, the tannins content (EtOAc extract) was quite similar in both plants, at around 5.11% for *I. mucronata* and 5.21% for *C. dioscorides*. On the other side, further analysis revealed that MeOH extract of *I. mucronata* outshined *C. dioscorides* in terms of bioactive compounds. It boasted higher concentrations of alkaloids (1.12% vs. 0.75%), flavonoids [(115.21 vs. 92.12 mg/g (QE)≡(11.52 vs. 9.21%)], and phenolic acids [(266.13 vs. 203.4 mg/g (GAE)≡(26.61 vs. 20.34%)]. This interprets to a clear advantage for *I. mucronata* in terms of

potential pharmacological effect as anti-inflammatory, antioxidant and anticancer activity. Interestingly, the saponin content followed a different pattern. Here, MeOH extract of *C. dioscorides* predominated with 4.96% saponins compared to *I. mucronata* (3.75%). This suggests a specific strength within *C. dioscorides*, potentially offering unique benefits in areas where saponins play a crucial role as a promising anticancer agent. As for tannins, both plants remained neck-and-neck, with MeOH extracts yielding similar concentrations in *I. mucronata* (6.83%) and *C. dioscorides* (6.49%). This highlights the consistency of tannin across both plants and its potential contribution to their shared pharmacological properties. In general, the findings revealed that the MeOH extract from both plants has higher phenolic, flavonoid, tannin and saponin contents than the EtOAc extract. Whereas, the EtOAc extracts of both plants had alkaloid content higher than the MeOH extract. As the base of solubility stated that "like dissolves like", where MeOH is characterized as a polar solvent so, it had a higher content of polar constituents (phenolic, tannins and flavonoids), while EtOAc is characterized as a semi polar solvent which dissolves the less polar compounds (alkaloids, terpenoids, steroids…*etc*). Comparing the two plants, *I. mucronata* had higher concentrations of alkaloids, flavonoids, and phenolic acids, suggesting stronger anti-inflammatory, antioxidant, and anticancer potential. While *C. dioscorides* had a higher saponin content. Both plants had similar tannins content, indicating their potential contribution to shared pharmacological properties.



	Alkaloids %	flavonoid %	phenolic %	saponin %	Tannin %
IM-EtOAc	3.44	9.15	20.79	2.74	5.11
IM-MeOH	1.12	11.52	26.61	3.75	6.83
CD-EtOAc	2.83	7.21	17.8	3.43	5.21
CD-MeOH	0.75	9.21	20.3	4.96	6.49

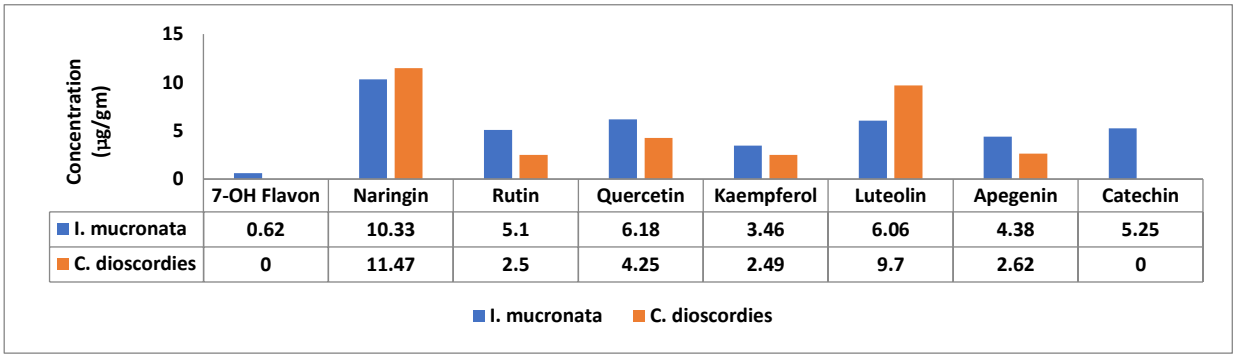
IM: *I. mucronata*, CD: *C. dioscorides*

Fig (1). Total active constituents of EtOAc and MeOH extracts of *Iphonia mucronata* and *Conyza dioscorides.*

1.2. Phenolics and flavonoids analysis by HPLC

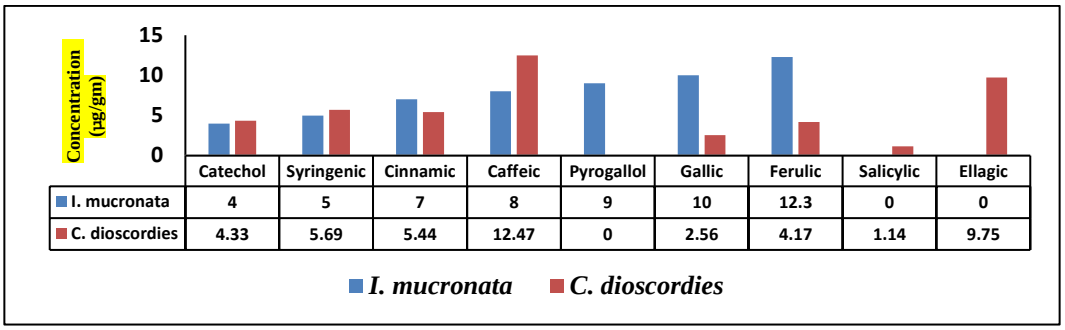
Previous studies showed that *Conyza* and *Iphonia* species have a large number of phenolic and flavonoid contents (Pecio et al., 2022 and Opiyo, 2023). Despite the limitation of standards used in the HPLC

analysis, both plants showed that they contain a significant amount of phenolic and flavonoid constituents. Figs. (2-5) illustrate that *I. mucronata* possessed many flavonoids according to available standards where it contained 7-hydroxy flavon, naringin, rutin, quercetin, kaempferol, luteolin, apigenin and catechin while, *C. dioscorides* contained the same constituents with the absence of 7-hydroxy flavon and catechin. Comparing the flavonoid constituents of the two plants, *I. mucronata* had higher concentration of all contents, except naringin and luteolin where they were recorded in higher concentrations in *C. dioscorides*. In contrast, the phenolic content of *C. dioscorides* showed a preference of components, where the HPLC analysis showed that it contained eight phenolic compounds compared to seven compounds of *I. mucronata*, although the concentration of *I. mucronata* compounds recorded the highest value compared to *C. dioscorides*, with the exception of caffeic acid. Its concentration was higher in *C. dioscorides* than in *I. mucronata*, with the appearance of salicylic and ellagic acids in *C. dioscorides* and their absence in *I. mucronata*, and the appearance of pyrogallol in *I. mucronata* and its absence in *C. dioscorides*. The difference in the concentrations of active substances in both plants may be due to the response of each plant to the formation of primary and secondary metabolic compounds under conditions of water and salt stress. In general, the response of *I. mucronata* was better than *C. dioscorides* as most of the active ingredient concentrations were higher than those in *C. dioscorides*, which promoted its therapeutic and biological activity when compared to *C. dioscorides*.



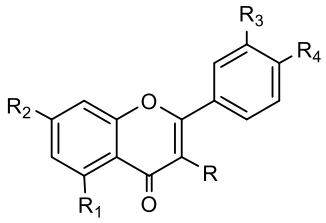
	7-OH Flavon	Naringin	Rutin	Quercetin	Kaempferol	Luteolin	Apegenin	Catechin
I. mucronata	0.62	10.33	5.1	6.18	3.46	6.06	4.38	5.25
C. dioscordies	0	11.47	2.5	4.25	2.49	9.7	2.62	0

Fig (2). Flavonoid constituents of *Iphonia mucronata* and *Conyza dioscorides* using HPLC.

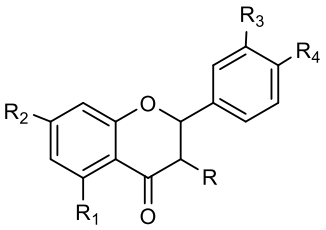


	Catechol	Syringenic	Cinnamic	Caffeic	Pyrogallol	Gallic	Ferulic	Salicylic	Ellagic
I. mucronata	4	5	7	8	9	10	12.3	0	0
C. dioscordies	4.33	5.69	5.44	12.47	0	2.56	4.17	1.14	9.75

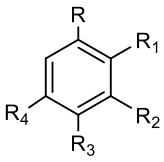
Fig (3). Phenolic constituents of *Iphonia mucronata* and *Conyza dioscorides* using HPLC.



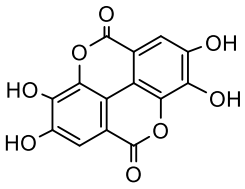
7-OH- flavon R=R_1=R_3=R_4 =H, R_2 = OH
Rutin R_1=R_2=R_3=R_4 = OH, R= Rutinoside
Querectin R= R_1=R_2=R_3=R_4 = OH
Kaempferol R= R_1=R_2= R_4 = OH, R_3= H
Luteolin R_1=R_2= R_3= R_4 = OH, R= H
Apigenin R_1=R_2= R_4 = OH, R= R_3= H



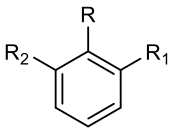
Catechin R = R_1 = R_2 = R_3 = R_4 = OH
Naringin R = R_3 = H, R_1=R_4= OH, R_2 = O-rutinoside



Syringenic acid R= COOH, R_1=H, R_3= OH, R_2=R_4= OCH_3
Cinnamic acid R = CH=CHCOOH, R_1=R_2=R_3=R_4=H
Caffeic acid R = CH=CHCOOH, R_1=R_2=H, R_3= R_4=OH
Gallic acid R= COOH, R_1=H, R_2=R_3=R_4= OH
Ferulic acid R = CH=CHCOOH, R_1=R_2= H, R_3=OH, R_4= OCH_3
Salicylic acid R= COOH, R_1=OH, R_2= R_3=R_4=H



Ellagic acid



Catechol R = R_1 = OH, R_2 =
Pyrogallol R = R_1 = R_2 = OH

Fig. (4). Chemical structure of phenolics and flavonoids identified from *Iphonia mucronata* and *Conyza dioscorides* using HPLC.

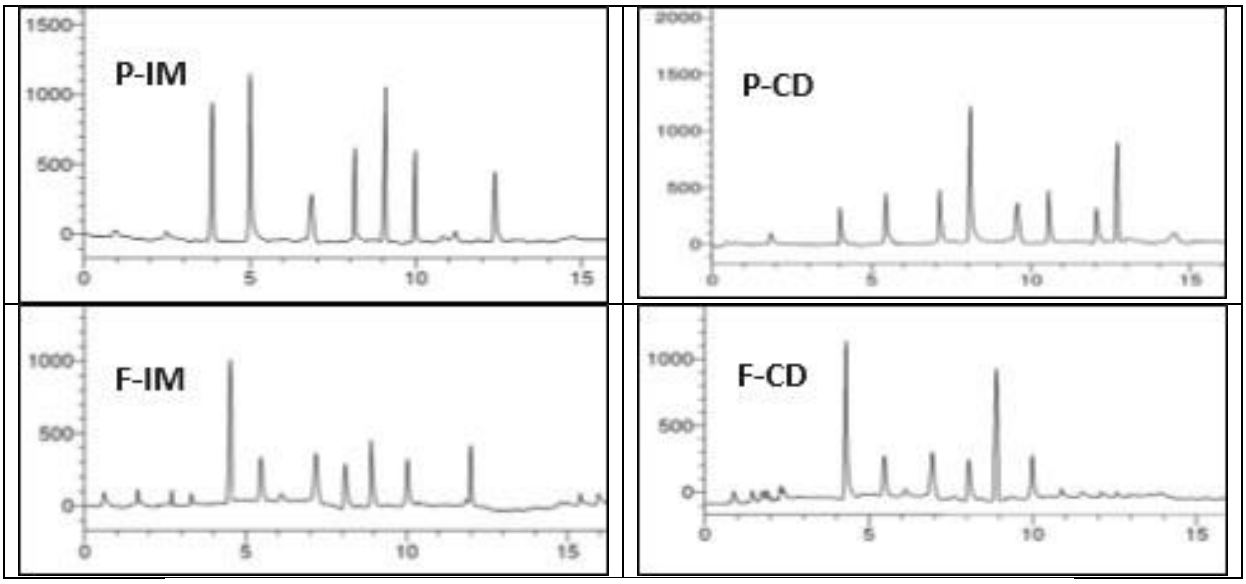


Fig. (5). HPLC Chromatogram of the phenolic components of *Iphonia mucronata* (P-IM) and *Conyza dioscorides* (P-CD) and flavonoid components of *I. mucronata* (F-IM) and *C. dioscordies* (F-CD).

2. Biological Investigations

2.1. Antimicrobial activity

The findings outlined in Table (1) and Fig. (6) of the inhibition zones of EtOAc and MeOH extracts of *I. mucronata*, and *C. dioscorides* and reference drugs ketoconazole and gentamycin of fungi and bacteria strains, respectively, showed that, all the tested extracts of the two plants exhibited no antifungal activity. Whereas both showed weak to moderate activity against *E. coli* gram negative bacteria in the range of 9-10 mm of inhibition zone as compared to 30 mm of gentamycin, results showed both plants extracts displayed similar activity against *E. coli*. Concomitantly, *C. dioscorides* exhibited moderate activity against *Proteus vulgaris* strain of gram-negative bacteria with around 50% inhibition of 12 mm as compared to 25 mm of the reference drug and 9-10 mm against *B. subtilis* of gram-positive bacteria vs. 26 mm of gentamycin. But *I. mucronata* extracts did not inhibit the growth of either *B. subtilis* or *P. vulgaris*. Overall, *C. dioscorides* exhibited slightly broader antimicrobial activity than *I. mucronata*. However, neither plant extract demonstrated sufficient potency to be considered a preferred antimicrobial agent due to their generally low activity. This suggests that the active chemical constituents in these plants, while possessing antimicrobial properties, may require isolation and concentration due to potential interactions within the extract that weaken their individual effects.

Table (1). Antimicrobial activity of EtOAc and MeOH extracts of *Iphonia mucronata* and *Conyza dioscorides.*

Tested microorganisms	*C. dioscorides*		*I. mucronata*		Control
	EtOAc	**MeOH**	**EtOAc**	**MeOH**	
Fungi					Ketoconazole
Aspergillus fumigatus RCMB 002008	NA	NA	NA	NA	17
Candida albicans RCMB 005003 (1) ATCC 10231	NA	NA	NA	NA	20
Gram positive bacteria					Gentamycin
Staphylococcus aureus ATCC 25923	NA	NA	NA	NA	24
Bacillus subtilis RCMB 015 (1) NRRL B-543	9	10	NA	NA	26
Gram negatvie bacteria					Gentamycin
Escherichia coli ATCC 25922	10	9	10	10	30
Proteus vulgaris RCMB 004 (1) ATCC 13315	NA	12	NA	NA	25

* NA: No activity.

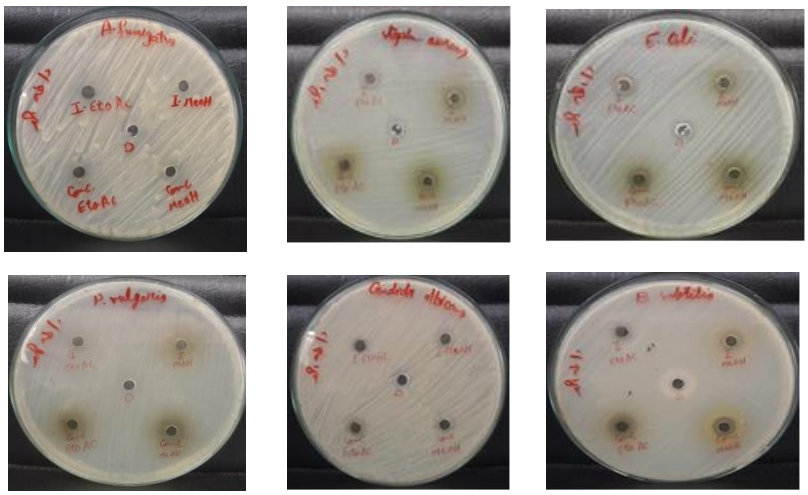


Fig. (6). The inhibition zones of antimicrobial activity of the EtOAc and MeOH extracts of *Iphonia mucronata*, and *Conyza dioscorides.*

2.2. Antitumor activity

Table (2) and Figs. (7-9) reveal intriguing findings about the antitumor activity of *I. mucronata* and *C. dioscorides* extracts. Both MeOH extracts of the two plants displayed weak antitumor activity against the tested carcinoma cell lines: HELA (cervical), PC-3 (prostate), and HEP-2 (larynx). In contrast, the EtOAc extract of *I. mucronata* exhibited significantly stronger antitumor activity against all three cell lines compared to reference drug (Vinblastine Sulfate) and the corresponding extract of *C. dioscorides*. This is evident in the IC_{50} values, which represent the concentration needed to inhibit 50% of the cancer cells. Where, EtOAc extract of *I. mucronata* showed the highest potency against PC-3 with IC_{50}= 24.60 µg/ml, followed by HEP-2 with IC_{50}= 26.57 µg/ml, then HELA cells with IC_{50}= 39.06 µg/ml. Also, EtOAc of *C. dioscorides* demonstrated the strongest activity against PC-3 cell (IC_{50} = 41.13 µg/ml), followed by HEP-2 cell (IC_{50} = 52.07 µg/ml) and finally, HELA cell (IC_{50} = 59.05 µg/ml). While Vinblastine Sulfate the reference drug showed inhibition growth of the tested cancer cells at concentrations of 59.71, 42.39 and 29.22 µg/ml for HELA, PC-3 and HEP-2 cancer cells, respectively. Overall, the outlined data recorded in Table (2) proved that *I. mucronata* EtOAc extract exhibited the most potent extract of reference drug and *C. dioscorides* against all tested cells, while *C. dioscorides* EtOAc extract showed potency more than the reference drug against HELA and PC-3, whereas the reference drug showed more potency in the HEP-2 cell. The antitumor activity of the EtOAc extracts suggests that relative high concentration of specific active components within *I. mucronate* EtOAc extract hold promising potential for further exploration as potential antitumor agents. Although higher identified concentration of phenolic and flavonoid constituents is presented in both plants MeOH extracts which is responsible for the antioxidant, anti-inflammatory and anticancer activity, but the two MeOH plant extracts showed weak antimicrobial and antitumor activity while, EtOAc extracts of the two plants revealed significant influence as antitumor promising agent. Which may be related to EtOAc having an intermediate-polarity solvent, meaning it can extract a wider range of compounds compared to the more polar MeOH or less polar solvents like hexane. This could lead to enrichment of specific bioactive components in *I. mucronata* EtOAc that are absent or less abundant in the other extracts. Furthermore, the observed activity might not be solely due to individual compounds but rather a synergistic effect between different components presents in the *I. mucronata* EtOAc extract. This could involve interactions between alkaloids, flavonoids, and other bioactive molecules, leading to enhance pharmacological activity. The inhibition of cancer cell growth against tested cancer cells of *I. mucronata* EtOAc extract may be due to different mechanism pathways of the chemical components where, presence of quercetin, kaempferol, apigenin and luteolin flavonoids can inhibit

Table (2). Antitumor activity of EtOAc and MeOH extracts of *Iphonia mucronata* and *Conyza dioscorides* and reference drug.

Conc. (µg/ml)	Viability % of EtOAc extract						Viability % of MeOH extract						Viability % of reference drug		
	I. mucronata			*C. dioscorides*			*I. mucronata*			*C. dioscorides*			Vinblastine Sulfate		
	HELA	PC-3	HEP-2	HELA	PC-3	HEP-2	HELA	PC-3	HEP-2	HELA	PC-3	HEP-2	HELA	PC-3	HEP-2
500	3.14	2.35	3.49	4.29	3.17	3.95	18.54	9.51	13.86	21.73	14.09	17.58	9.13	4.92	7.24
250	6.98	5.16	7.32	11.68	9.34	8.79	33.26	24.89	31.57	46.12	31.74	35.96	22.84	13.76	12.85
125	14.57	11.45	13.85	29.07	22.60	21.47	49.53	43.16	45.09	81.74	60.52	58.71	34.57	24.92	24.36
62.5	31.42	23.19	29.46	47.34	34.19	43.52	76.82	64.81	71.38	98.06	89.06	82.47	47.81	37.83	35.98
31.25	56.19	38.72	45.03	75.03	57.31	62.94	91.74	75.92	86.41	100	99.13	97.63	71.96	56.75	48.76
15.6	72.34	65.39	61.78	89.15	72.92	78.16	99.58	88.06	95.27	100	100	100	82.78	68.94	63.27
7.8	85.62	84.12	80.94	99.42	86.43	89.04	100	98.13	99.74	100	100	100	90.63	74.83	70.18
3.9	91.73	91.75	89.21	100	93.12	94.51	100	100	100	100	100	100	95.42	81.95	81.52
0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
IC_{50} (µg/ml)	**39.06**	**24.60**	**26.57**	**59.50**	**41.13**	**52.07**	**123.92**	**105.25**	**113.33**	**236.38**	**170.69**	**172.86**	**59.71**	**42.39**	**29.92**

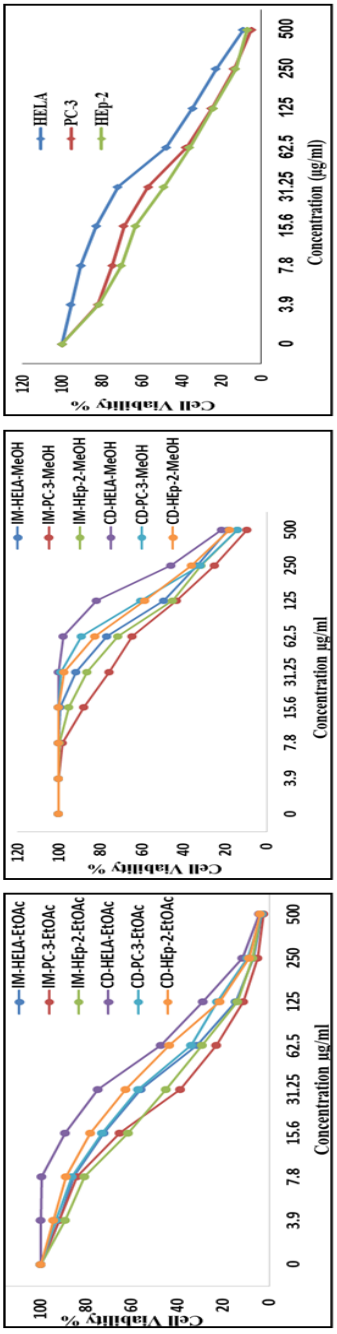


Fig. (7). Antitumor activity of EtOAc extracts of *Iphonia mucronata* and *Conyza dioscorides* against HELA, PC-3 and HEP-2 cells.

Fig. (8). Antitumor activity of MeOH extracts of *Iphonia mucronata* and *Conyza dioscorides* against HELA, PC-3 and HEP-2 cells.

Fig. (9). Antitumor activity of Vinblastine Sulfate reference drug against HELA, PC-3 and HEP-2 cells.

enzymes involved in cell growth and proliferation, such as kinases and cyclin-dependent kinases (CDKs), which bind to and activate tumor suppressor proteins. Also, modulating signaling pathways: where flavonoids can interfere with pathways like PI3K/Akt and MAPK, which are crucial for cancer cell survival and migration (Son and Kim, 2023) and scavenge free radicals and reactive oxygen species (ROS) that can damage DNA and promote cancer cell growth, prevent the formation of new blood vessels, which are essential for tumor growth and metastasis (inhibition of angiogenesis) (Luna et al., 2023). Also can trigger programmed cell death in cancer cells to induce apoptosis and modulation of signaling pathways by interfering with signaling pathways that promote cancer cell proliferation and survival (Yan et al., 2017). On the other hand, the presence of catechin can inhibit histone deacetylases (HDACs), leading to altered gene expression and possibly suppressed tumor growth (Cheng et al., 2020 and Kopustinskiene et al., 2020). High concentration of phenolic acids of both plants especially ellagic, ferulic and gallic acids can induce DNA damage and directly damage cancer cell DNA, triggering apoptosis or cell cycle arrest (El-Bassossy et al., 2023a and b). Also, modulation of detoxification enzymes helps cancer cells neutralize toxins, making them more susceptible to other anticancer agents (Mohammadinejad et al., 2022). Presence of saponins play a vital role as antitumor agent by membrane disruption where, they can interact with cholesterol in cancer cell membranes, leading to pore formation and leakage of cell contents, ultimately causing cell death and increase immune stimulation by activating macrophages and natural killer cells, the body's natural defenses against cancer, to attack and destroy cancer cells (Isoldi et al., 2005). Furthermore, Angiogenesis inhibition: by preventing the formation of new blood vessels, which tumors need for growth and metastasis (Majnooni et al., 2023 and Podolak et al., 2023).

CONCLUSION

This study was established to distinguish between the chemical components and the pharmacological effects of both *I. mucronata* and *C. dioscordies* belonging to the family Asteraceae. Both plants showed anti-cancer effects on the prostate (PC-3), cervix (HELA), and larynx (HEP-2) carcinoma cells, but neither of them showed effective antimicrobial activity, although *C. dioscordies* showed efficacy as antibacterial agents against *B. subtilis* and *E. coli*. The study revealed that *I. mucronata* EtOAc extract showed a stronger potent anti-tumor activity than Vinblastine Sulfate, the reference drug used. This may be due to the plant containing high concentrations of phenols, flavonoids and saponins, which are known to be effective as anti-cancer agents. The effective results of the *I. mucronata* extract as an anti-tumor agent requires more *in vivo* studies to

know the extent of its effectiveness and its adverse effects on the normal cells and to rely on it as an effective drug in treating some types of cancer.

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دراسة مقارنة المركبات البيوكيميائية والفاعلية الدوائية لنباتي *Iphiona mucronata* و*Conyza dioscorides*

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أجريت هذه الدراسة بغرض مقارنة التأثيرات البيولوجية (النشاط المضاد للميكروبات والأورام) والمكونات الكيميائية النشطة بيولوجيًا لنباتين *Iphonia mucronata* و*Conyza dioscorides* اللذان ينتميان للعائلة النجمية. حيث أظهر مستخلص خلات الإيثيل لنبات *I. mucronata* نشاطًا واعدًا وفعالاً كمضادًا للأورام ضد خلايا سرطان عنق الرحم والبروستاتا والحنجرة، بينما أظهر مستخلص خلات الإيثيل لنبات *C. dioscorides* نشاطًا ضعيفًا إلى متوسط كمضاد للأورام ضد نفس الخلايا السرطانية، وأظهر المستخلص الميثانولي لكلا النباتين نشاطًا ضعيفًا كمضادًا للأورام. من ناحية أخرى، أظهر مستخلص خلات الإيثيل والميثانول لنبات *C. dioscorides* نشاطٌ متوسط إلى ضعيف كمضادًا لبعض سلالات البكتيريا المختبرة مثل *Proteus vulgaris* و*Bacillus subtilis* و*Escherichia coli*. أظهرت الدراسات الكيميائية أن كلا النباتين يحتوي على تركيزات عالية من الفلافونيدات والفينولات والقلويدات والصابونينات والتانينات، وقد أظهر نبات *I. mucronata* تركيزات عالية من معظم المكونات النباتية وربما يُعزى إلى ذلك الفاعلية البيولوجية للنبات كمضاد للأورام مقارنة بالدواء المرجعي المستخدم. وتعتبر هذه الفاعلية موضعًا للاهتمام لاستكمال الدراسات البيولوجية والكيميائية في المستقبل لدراسة مدى إمكانية استخدام هذا النبات كدواء فعال في علاج بعض أنواع السرطان.