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RESEARCH PAPER

Phytochemical screening and anti-candida activities of *Crocus cancellatus* herb. Ethanol extract

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A B S T R A C T:

The antifungal, antioxidant activity, total tannin estimation, and phytochemical screening of *Crocus Cancellatus* extract were investigated. The highest yield was obtained with ethanol solvent (36.1%). A significant amount of tannin was obtained in the plant (11.1%).

In Antifungal testing against *Candida* spp. The most effective Inhibitory obtained was 13.16mm against *C. Krusei*, while the less effective activity was 8.22 mm against *C. Glabrata*. The minimum inhibitory concentration (MIC) value obtained against all tested *Candida Spp*. candida strains within diameter rate of 12.5-25 µg/ml. The minimum inhibitory Effects of tested antifungal agents was FLU/25 which have no any inhibitory effects especially against was *Candida famata* While the highest inhibitory zone of FLU/25 38.40 mm was obtained against *Candida guilliermondii*. the maximum antioxidant activity obtained was 98.68% at 0.2 ml.

10 compounds were determined in the content determination study with LC-MS/MS. Gallic acid (46965.14 µg/g), Quinic acid (1935.71 µg/g), and malic acid (3831.37µg/g) were obtained at the highest levels. Vanillic acid (138.2 µg/g), Protocatechuic acid (116.87µg/g), tr-Ferulic acid (51.17µg/g), Quercetin (20.56 µg/g), p-coumaric acid (12.8 µg/g), Chlorogenic acid (2.51 µg/g) and Salicylic acid (1.56 µg/g) were obtained quantitatively less. These results confirmed that the *Crocus cancellatus* ethanol extract has potential antioxidant and antifungal activity

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INTRODUCTION

The genus *Crocus* plant studied by many researchers since 1983. Till these days dozens of wild types were found in the areas, researched, and the consequence was published (Kerndorff and Pasche, 2004; Kerndorff and Pasche, 2006; Erol et al., 2011)**.** *Crocus cancellatus* genus had been used for many years in cooking to increase the sensorial standard of nutrition. Over the past few years, the purpose of nutrient flavour used in traditional cooking has admitted great care because of the growing concern in human health and studied both in vitro and in vivo by researchers (Loizzo et al., 2008; Iauk et al., 2015)**.** Crocus spice is a part of the Iridaceae family. The stigmas of the crocus should be collected by hand from the sensitive flowers upon opening to protect the attractive volatile ingredient (Ellis- Barrett, 2005)**.** With its unique bitter tasty, lightly and sharp like straw flavour, it has been used as a flavour and colourants in the nutrient (Moraga et al., 2009)**.** Attention and the effect of crocus on people's health are expanding because of its demonstrated health features (Mykhailenko et al., 2019)**.**

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Crocus can not only use as a garden flower but also used significant traditional remedy and cooking plant as well. Some result shows that crocus plant extract has antitumor, antimutagenic, cytotoxic activities, and inhibitory effects on intracellular nucleic acid and protein synthesis in malignant cells (Abdullaev et al., 2003**;** Chryssanthi et al., 2007**;** Tavakkol-Afshari et al., 2008)**.** *Crocus cancellatus* is a member of the Crocus genus that is an extremely common plant in Iran, Turkey, Jordan, and Greece (Erol et al., 2014)**.** The crocus genus has been used for antimicrobial, anti-fungal, antiseptic, and is also used as an antibacterial agent, antiseptic, and antiflatulent (Mykhailenko et al., 2019)**.** Because of the significance of these spices that it could be beneficial for treating this kind of pathogenic fungi disease. Tha aim of this researchs determind of total yield and antioxidant activity. The characterization of isolated phytochemicals compounds by using LC-MS/MS. Finaly, compare plant antifungals extracts with some artificial antifungal by antifungal activity by MIC and disc diffusion method. No, the previous study has been reported before.

MATERIALS AND METHODS

Materials

Preparation of extract

Plants bulb of *Crocus cancellatus* herb. was collected from Qarachugh maountain. Further, taxonomic identification was done at the herbarium at the Department of Biology College of Sciences Salahaddin University - Erbil. Plants identified. Then, the plant samples were saved at the of the Biology Department Herbarium. Finally, it was given the herbarium code for species started 7471 code (Carranza-Rojas et al., 2017). The extraction and analysis were done in Turkey at DICILA university. Ten grams of plant powder were put into the Erlenmeyer flasks and 100 ml solvent was added. The plant and solvent ratio was 1:10. The Microwave milestone NEOS system equipped with vessels and an automatic temperature control system was used for the microwave-assisted extractions (MAE). This method of analyze has been done by Microwave method was used for 60 minutes until the boiling point temperature. Afterwards, to get pure extract the mixture was filtered and evaporated with a rotary evaporator (Chan et al., 2011).

Methods

Our study has been consisting of four analysis extraction, extract yield percentage determination, determination of total condensed tannin and DPPH radical scavenging activity. It has been done at Kahramanmaraş Sütçü İmam Üniversitesi laboratory. Also, phytochemical analyses were done at Dicla University in Turkey.

Calculation of extraction yield

The extraction yield is a calculation of solvent efficiency to extract specific components from the original material and it was described as the amount of extract recycle in bulk comparison according to the initial quantity of dry sample (Ali Abu-Mejdad, 2014**;** Ismael et al., 2019)**.**

About 0.5-gram plant powder processed with 50 ml of ethanol solvent. The extract yield percentage was calculated as the following formula:

$$Yield\ percentage\ (\%) = X/Y\ _{x}100$$

$$Where:\ X\ is\ the\ oven\ dry\ weight\ of\ extract\ (g), Y\ is\ the\ dry\ weight\ of\ the\ sample\ (g)$$

Determination of total condensed tannins

The 0.05 gram $FeSO_4$, 95 ml C_4H_9OH, and 5 ml HCl were prepared. For the analyzed condensed tannin, 0.01gram of root powder and mimosa tannin placed one by one in a tube and a 10 ml solution was added then heated for an hour in the water bath. The analyzed was measured by UV/Vis at 580 nm (Ismael et al., 2019).

Antioxidant activity

The plant extract of free radical scavenging activity of root was calculated by the DPPH method (Rahman et al., 2017). Firstly, 0.1mM DPPH was prepared for the extraction process. Afterwards, 0.1, 0.2, and 0.3 ml of solutions completed with a solvent until 3 ml respectively. Then, 1 ml of DPPH was added. After that, experiment tubes were vortexed and all was kept in a dark place for 30 minutes. The absorbance of the samples carried out by a UV/vis

spectrophotometer at 517 nm. BHT (Butylated hydroxytoluene) was used as a reference. calculations were done according to the following formula;

$$\textbf{DPPH radical scavening activity } (\%) = \frac{\boldsymbol{Abs_{control} - Abs_{test}}}{\boldsymbol{Abs_{control}}} \boldsymbol{x\ 100}$$

Where: $\boldsymbol{Abs_{control}}$ = the absorbance of DPPH radical + solvent
$\boldsymbol{Abs_{test}}$ = the absorbance of the sample and BHT, separately

Phytochemical Screening

MS instrumentation

MS detection was achieved using a Shimadzu LC-MS 8040 model triple quadrupole mass spectrometer prepared with an ESI source operating in both positive and negative ionization modes. LC-MS/MS data were collected and processed by Lab Solutions software (Shimadzu, Kyoto, Japan). The multiple reaction monitoring (MRM) mode was used to quantify the analyses: the assay of investigated compounds was performed following two or three transitions per compound, the first one for quantitative purposes and the second and/or the third one for confirmation(Ertas et al., 2015).

Method validation parameters for LC-MS/MS

In this investigation, twenty-four phenolic and three non-phenolic organic acids which were prevalent in plant resources were quantified and qualified in plants. Straight-lined regression equations and the linearity ranges of the investigated standard compounds are assumed in Figure 1 (Gülçin et al., 2003; Ismael et al., 2019). Correlation coefficients were originating to be upper than 0.99. The limit of detection (LOD) and limit of quantitation (LOQ) of their ported analytical method were shown with results in Table 5. For the analyzed compounds, LOD ranged between 0.05 and 25.8 g/L and LOQ ranged between 0.17 and 85.9 g/L. Additionally, the recoveries of the phenolic compounds ranged from 96.9% to 106.2%. The results were calculated by the equation below:

$$\textbf{Quantification } (\mu\textbf{g analyte/g extract}) = \frac{\mathbf{Y} * \mathbf{U_{95}}}{\mathbf{100}}$$

Where; **Y**: LC-MC/MS result of an analyte, **U**: uncertainty confidence level

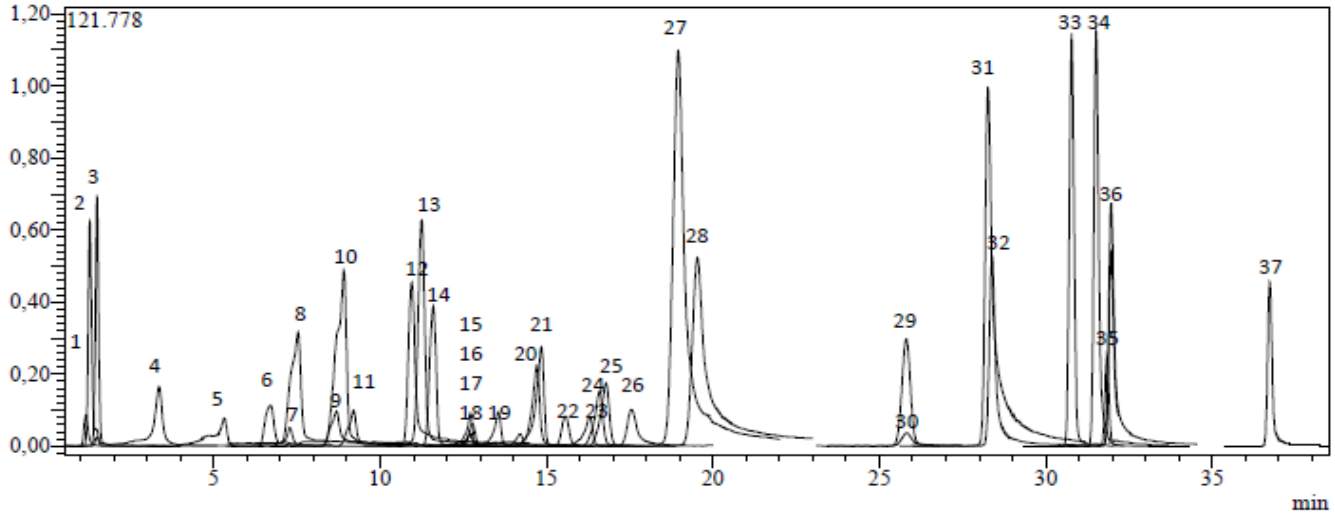

Figure 1 LC-MS/MS chromatogram for reference phytochemical compounds

The tested Candida

All Candida spp. in the present study was obtained from (Media Diagnostic Centre) MDC Erbil accredited from America, CAP* (College of American Pathology), *Candida albicans* (ATCC 10231), *Candida glabrata, Candida Krusei, Candida tropicalis, Candida famata, Candida parapsilosis, and Candida guilliermondii*. To extend the spectrum of organisms studied, local isolate were also involved in the examination. Admittedly, six selected antifungal discs used Nystatin 100unit (NYS), Clotrimazole 10mcg CLT, Fluconazole 25mcg (FLU), Ketoconazole 10mcg (KTZ), Fluconazole 1010mcg (FLC) and Miconazole 10mcg (MCZ) for comparison with plant antifungal (50 µg/ml).

Biological assesses

The Disc diffusion method was known as the Kirby- Bauer method was particularly standardized by the Clinical Laboratory Standard Institute (CLSI 2006) methodology. Suspensions of the tested *Candida* spp. were prepared in sterilized saline the optical density (OD) of a 0.5 McFarland standard using a densitometer (Turbidity meter BioMerieux, France) (Dalynn Biologicals et al., 2007) A purified About 0.1 ml of prepared Candida suspension was inoculated into SDA medium then spread by using sterilized cotton swab was immersed in the suspension and inoculated evenly over the entire superficies of the medium by swab three times over the entire agar surface; rotate the plate (Z Sahin et al., 2019). The plant extract was prepared by dissolving 0.1 g of the extract with 100 mL of their respective solvents. The discs were 6 mm in diameter include a (50µl) concentration of extracts. Which was applied to the plates only by applying sterile forceps and then smoothly pushed down on the agar medium. Normally, no more than 6 disks are placed on a plate for avoids overlapping of the zones of inhibition and potential failure in the analysis. Later, for more acceptance by sterile forceps were used to place the antifungal disc on the surface of the inoculums. After the disks were located on the plate for each *Candida* sp. six disc plant extract, the plate was modified and incubated at 37°C (Air incubator, LTE Scientific, UK) for 48h dependent on strains being tested. Next, the inhibition zone was measured At that moment, it was determined the inhibition zone by using an a ruler. Finally, all inhibition zone described chart table which is confirmed by NCCLS for antimicrobial susceptibility to examination and monitoring (National Committee for Clinical Laboratory Standards, 2015).

The minimum inhibitory concentration (MIC) determination

The minimum inhibitory concentration (MIC) rates achieved by broth microdilution, crocus extract activity against *Candida* spp. was comparatively low. That placed 0.9 ml of Sabouraud dextrose broth (SDB) medium. Afterwards, 0.1 ml of plant extract at concentration 0.1 mg/ml solvent was stirred with broth in following decimal dilutions diluting 1 ml in 9 ml of broth to obtain the concentration range of 0.1 to 500 mcg/ml. The inoculum could be standardized based on optical density [OD625 of 0.08-0.1(1cm light path)] analyzed by spectrophotometer. This is normally done after 18- 24 h. All the candida suspensions were arranged by suspending 24h candida culture in sterile normal saline (0.89% NaCl wt/vol). The turbidity of the candida suspension was regulated according to the 0.5 McFarland standard (equivalent to 1.5×108 CFU/ml) (Costa et al., 2014; Rahman et al., 2017)

Statistical analysis

The data examined by ANOVA using GraphPad (Prism 6) statistical program. A statistical analysis Probability value (P-value) (<0.05) was accepted as statistically meaningful (*), while the P-value than (>0.05) was regarded as statistically not meaningful. Histogram and pie-chart were accepted for the statistical analysis of the consequences (Hedges and Rhoads, 2010; Rahman et al., 2017).

RESULTS AND DISCUSSION

Determination of yield

The *Crocus cancellatus* extract was extracted by a microwave method. The highest extraction yield was obtained by ethanol solvent (36.1%) was given in Table 1.

It has been determined in previous studies that the yield of the crocus family is generally high. In the study conducted with three different solvents (hexane, dichloromethane, and ethanol) of the genus *C. sativa*, it was determined that the best yield was obtained by the ethanol extract (Wali et al., 2020).

Table 1. Determination of *C. cancellatus* extract yield

	R 1	R 2	R 3	Yield (%)
Mean	36.2 ±	35.8 ±	36.3 ±	36.1 ±
SD	0.009	0.008	0.007	0.008
SE	0.003	0.002	0.003	0.0025

R: Replication

Determination of tannin

Tannin was measured by the n-butanol-HCl-iron method. The result of the tannin is given in Table 2. This method was used as a standard for n-butanol/HCl assay is mimosa-tannin under normal reaction/condition which measured using the regression equation ($y = 66.357x + 0.4117$) got previous from the linear calibration curve. Measuring the high quantity of tannins detected in all crocus extract by the percentage of tannin was determined.

In our experiment, tannin-content was diverse certainly and meaningfully therefore the result was displayed as an absorbance unit at 580 nm per 1 mg of extract (A580/mg). The result was viewed (11.1 mg/kg) as shown in Table 2.

Table 2. Total condensed tannin

	R 1	R 2	R 3	Mean
Extract (mg/kg)	10.6 ±	11.4 ±	11.3 ±	11.1 ±
SD	0.12	0.13	0.12	0.1
SE	0.18	0.16	0.18	0.18

SD: Stander Division; SE: Stander Error

$$\boldsymbol{Calculation}: \% = \boldsymbol{A/3m}$$

Where A = absorbance value, m = mass weight [26].

Antifungal analysis

The Crocus plant extracts inhibition zones' results were considerably different tested *Candida* spp. The results are represented in Table 3. The minimum inhibitory zone was obtained against *Candida Krusei* (8.22 mm). Whereas, the highest inhibitory zone was obtained against *Candida glabatra* (13.16 mm). Furthermore, the minimum inhibitory concentration (MIC) results against all Candida species were obtained among 12.5 - 25 µg/ml represented in Table 3. The various artificial antifungal was statically meaningful by the various widths of inhibition zones of maximum the activities against the organisms examined were given in Table 3. The mean inhibition zones artificial antifungal against Candida species experiment was obtained between 0–38.4 mm. The minimum inhibitory zone 0 mm of FLU/25 was obtained against *Candida famata* Whereas the highest inhibitory zone of FLU/25 38.40 mm was obtained against *Candida guilliermondii.* In the previous research, methanol extract of *C. Sativus* at different concentrations (4500 µg/well, 9000 µg/well, and 13500 µg/ well) show different antimicrobial results against bacteria. The result shows that *C. sativus* extract was indicated that the plant has the resource of some substances which has an antimicrobial effect against microorganism. especially, against Listeria spp. Moreover, as the extract concentration increased, the antimicrobial effect also increased

(Muzaffar et al., 2016; Jadouali et al., 2018). Petal extracts of C. Sativus showed a strong effect at the minimum concentration against pathogenic bacteria. Both *P.aeruginosa* and *S.aureus* gave some results that they inhibited at the lowest inhibitory concentration of 15.63 µg/mL. Petals extracts were not much more impact in inhibiting the growth of *E. coli* and *C. albicans* at the lowest concentration. However, it showed high concentration antimicrobial and antifungal effects (Muzaffar et al., 2016; Wali et al., 2020).

Table 3. Inhibition zones (mm) and MIC of the extracts and synthetic antifungal activities against the *Candida spp*.

Candida spp.		Inhibition	MIC	FLU	MCZ	FLU	KTC	NY	CLT
		Zone	(µg/ml)	10mcg	10mcg	25mcg	10mcg	100 U	10mcg
Candida albicans	Mean	11.3	250	6	14.61	10.55	14.05	20.21	18.15
	(mm)	±	±	±	±	±	±	±	±
	SD	0.1	0.05	0	0.1	0.13	0.1	0.24	0.18
	SE	0.05	0.03	0	0.04	0.05	0.04	0.1	0.07
Candida glabatra	Mean	9.17	250	16.71	14.54	15.68	13.33	24.72	12.29
	(mm)	±	±	±	±	±	±	±	±
	SD	0.011	0.63	0.16	0.2	0.21	0.11	0.31	0.22
	SE	0.08	0.41	0.06	0.08	0.09	0.04	0.12	0.09
Candida krusei	Mean	8.22	250	15.32	13.52	24.63	18.79	14.49	25.91
	(mm)	±	±	±	±	±	±	±	±
	SD	0.03	0.02	0.15	0.2	0.66	0.09	0.17	0.22
	SE	0.018	0.017	0.06	0.08	0.27	0.04	0.07	0.09
Candida tropicalis	Mean	8.63	250	20.52	12.46	27.67	23.96	21.23	19.95
	(mm)	±	±	±	±	±	±	±	±
	SD	0.017	0.18	0.08	0.27	0.17	0.1	0.15	0.14
	SE	0.1	0.08	0.03	0.11	0.07	0.04	0.06	0.06
Candida famata	Mean	13.16	125	6	6	6	6	24.75	20.26
	(mm)	±	±	±	±	±	±	±	±
	SD	0.012	0.55	0	0	0	0	0.13	0.16
	SE	0.10	0.34	0	0	0	0	0.05	0.07
Candida parapsilosis	Mean	12.23	125	21.43	11.53	26.36	27.48	24.47	22.42
	(mm)	±	±	±	±	±	±	±	±
	SD	0.022	0.024	0.16	0.17	0.17	0.32	0.15	0.28
	SE	0.013	0.022	0.07	0.07	0.07	0.13	0.06	0.11
Candida guilliermondii	Mean	11.8	250	37.48	24.19	38.4	37.61	21.45	31.26
	(mm)	±	±	±	±	±	±	±	±
	SD	0.63	0.13	0.26	0.15	0.42	0.32	0.18	0.19
	SE	0.21	0.01	0.11	0.06	0.17	0.13	0.07	0.08

Antioxidant activity

The crocus plant extract exhibited a DPPH radical scavenging activity. The maximum antioxidant activity at 0.2 mL was (98.68%). While the

minimum quantity was at 0.1 mL (96.60%). The results are represented in Table 4. The results show that the plant extracts had better antioxidants activity than the BHT as control.

As a result of the antioxidant activity study with *Crocus sativus*; it was determined that the plant extract had approximately the same results compared to α-tocopherol (Jadouali et al., 2018). In another study with *C. sativus*, it was reported that the antioxidant results obtained were similar to the previous studies, and the antioxidant activities of the leaves were less than stigma but higher than corm with increasing concentration (Ismael et al., 2019; Wali et al., 2020). In our antioxidant activity study with *C. cancellatus* species; the activity of all concentrations of the root extract was higher than the control (BHT).

Table 4. DPPH Radical Scavenging activity (%)

	MWE 0.1ml	MWE 0.2ml	MWE 0.3ml	BHT 0.1ml	BHT 0.2ml	BHT 0.3ml
Mean (%)	96.60 ±	98.67 ±	97.85 ±	69.9 ±	68.10 ±	72.7 ±
SD	0.13	0.18	0.21	0.23	0.28	0.35
SE	0.08	0.09	0.08	0.11	0.12	0.15

BHT (synthetic antioxidant), MWE: Microwave extraction

Analytical parameters

In this study, the phenolic acids in Crocus plant extract were investigated by UHPLC-ESI-MS/MS. Various 10 phytochemicals in ethanol extract were identified. Thirty-seven non-phenolic, phenolic, and flavonoid compounds of root ethanol extract had been investigated. Analytical parameters and results are given in Table 5 and Figure 2 . As shown in the table high quantity of gallic acid (46965.14µg/g), Quinic acid (1935.71µg/g), and malic acid (3831.37µg/g) were obtained. Moreover, the lowest amount of vanillic acid (138.2µg/g), Protocatechuic acid (116.87µg/g), tr-Ferulic acid (51.17µg/g), Quercetin (20.56µg/g), p-coumaric acid (12.8µg/g), Chlorogenic acid (2.51µg/g) and Salicylic acid (1.56µg/g) were detected. Investigated the chemical composition of Crocus sativus by HPLC; In the structure of the plant, Kaempferol, Quercetin, and Myricetin flavonoids and phenolic acids such as Caffeic acid, Chlorogenic acid, and Gallic acid were determined, However, Genistein and Cumaric acid have not been detected in its structure (Gismondi et al., 2012). Compounds that support previous studies in our plant structure have been identified and these compounds are known to have antioxidant effects.

A lot of research has been done and reported on the importance of tannin and its variation. Their activity is possible due to their ability to bind with extracellular and soluble proteins or associate with the cell wall of fungi. The character of these compounds can disrupt fungal membranes (Hasmida et al., 2014). The radical scavenging activity mechanism of root extracts may be related to the formation of polyphenolic compounds. It has previously been shown that polyphenolic compounds are responsible for the radical scavenging activity, as they impart hydrogen atoms to active free radicals (Kheirandish et al., 2016). The variance in amount levels of phenolic compounds could be due to differing methods of extraction (Garzón et al., 2009). Indeed, it could be due to the polyphenolic content of the roots being greatly affected by environmental factors as well as edaphic factors like soil type, sun exposure, rainfall, altitude and hightide, soil nutrients. etc.(Manach et al., 2004).

Table 5. Analytical parameters that belong to the LC-MS/MS method

	No Analytes	RT[a]	Mother ion (m/z)[b]	Fragment ions	Ion. mode	Equation	R^{2c}	RSD%[d] Interday	Intraday	Linearity Range (µg/L)	LOD/LOQ (µg/L)[c]	Recovery (%) Interday	Intraday	U[f]
1	Quinic acid	1.13	190.95	85.3-93.3	Neg	y=41.06×+10671	0.996	0.00259	0.00274	250-10000	75.8/79.4	1.00288	0.98778	1935.71
2	Malic acid	1.23	133.00	115.2-71.3	Neg	y=316.95×-42041	0.999	0.00477	0.00527	250-10000	55.3/67.5	1.01266	0.99836	3831.37
3	Fumaric acid	1.48	115.00	71.4	Neg	y=64.99×-11592	0.997	0.00536	0.00460	100-5000	28.1/34.5	0.99748	0.99867	N.D
4	Gallic acid	3.00	168.85	125.2-79.2	Neg	y=226.76×+38152	0.998	0.01601	0.01443	250-10000	95.5/106.9	1.00004	1.00454	46965.1
5	Protocatechuic acid	4.93	152.95	108.3	Neg	y=297.75×+30590	0.995	0.01236	0.01296	100-5000	28.2/31.4	0.99404	1.01070	116.87
6	Pyrocatechol	6.48	109.00	108.35-91.3	Neg	y=30.61×+14735	0.996	0.01313	0.01339	1000-20000	261.1/278.4	0.99987	0.99936	N.D
7	Chlorogenic acid	7.13	353.15	191.2	Neg	y=781.36×-18697	0.998	0.00058	0.00076	25-1000	6.2/8.1	1.00806	0.99965	2.51
8	4-OH-benzoic acid	7.39	136.95	93.3-65.3	Neg	y=409.03×+112079	0.998	0.01284	0.01538	250-10000	33.2/38.1	0.99662	1.00058	N.D
9	Vanillic acid	8.57	166.90	152.3-108.3	Neg	y=35.84×-12097	0.999	0.00528	0.00619	1000-20000	122.2/139.7	1.00093	1.04095	138.2
10	Caffeic acid	8.80	178.95	135.2-134.3	Neg	y=3963.32×+178156	0.998	0.01454	0.01469	25-1000	18.4/22.4	1.00917	0.98826	N.D
11	Syringic acid	9.02	196.95	182.2-167.3	Neg	y=42.33×-52547	0.996	0.01049	0.01345	1000-20000	212.5/233.3	0.99922	0.99977	N.D
12	Vanillin	10.87	151.00	136.3-92.2	Neg	y=446.10×+70934	0.998	0.00696	0.00793	250-10000	44.3/53.1	0.99679	0.99611	N.D
13	Salicylic acid	11.16	136.95	93.3-65.3	Neg	y=5286.26×+309192	0.989	0.01016	0.01242	25-1000	5.0/6.5	1.00989	0.99013	1.56
14	*p*-Coumaric acid	11.53	162.95	119.3-93.3	Neg	y=3199.20×+13002	0.992	0.01820	0.01727	25-1000	7.3/9.1	1.00617	1.01224	N.D
15	Rutin	12.61	609.05	300.1-271.1	Neg	y=561.91×-16879	0.997	0.00473	0.00624	25-1000	5.5/6.5	1.00994	0.98017	N.D
16	Ferulic acid	12.62	192.95	178.3	Neg	y=80.45×-31782	0.997	0.00708	0.00619	250-10000	36.6/42.0	0.99987	1.00289	51.17
17	Sinapinic acid	12.66	222.95	208.3-149.2	Neg	y=141.96×-73294	0.992	0.01446	0.01517	250-10000	78.7/86.1	1.00164	0.99962	N.D
18	Hesperidin	12.67	610.90	303.1-465.1	Poz	y=1340.27×-43769	0.998	0.00945	0.01126	25-1000	3.4/4.2	1.01733	1.01263	N.D
19	Isoquercitrin	13.42	463.00	300.1-271.1	Neg	y=803.23×+4981	0,999	0.00682	0.00515	25-1000	5.4/6.3	1.00594	1.00722	N.D
20	Rosmarinic acid	14.54	359.00	161.2-197.2	Neg	y=909.67×-201692	0.994	0.02014	0.01751	100-5000	6.6/8.8	0.99206	1.03431	N.D
21	Nicotiflorin	14.68	593.05	285.1-255.2	Neg	y=498.38×+79274	0,991	0.00737	0.00875	100-5000	22.4/25.5	1.02558	1.00970	N.D
22	*o*-Coumaric acid	15.45	162.95	119.4-93.3	Neg	y=1219.34×-10915	0.999	0.02730	0.02566	25-1000	24.4/31.1	0.98344	0.99061	12.8
23	Rhoifolin	16.11	577.05	269.2-211.1	Neg	y=237.15×+11887	0,999	0.00747	0.01528	100-5000	23.1/27.9	1.01046	1.01739	N.D
24	Quercitrin	16.41	447.15	301.1-255.1	Neg	y=339.39×+38910	0.999	0.01528	0.02320	100-5000	22.0/25.2	0.99726	1.00620	N.D
25	Apigetrin	16.59	431.00	268.2-239.2	Neg	y=1775.55×+91121	0,993	0.01797	0.01607	25-1000	5.4/6.1	1.01394	1.00419	N.D
26	Coumarin	17.40	147.05	91.0-103.2	Poz	y=33.64×-89700	0.994	0.01306	0.01239	1000-20000	208.4/228.4	0.99947	1.00081	N.D
27	Myricetin	18.72	317.00	179.2-151.3	Neg	y=583.55×+205727	0,999	0.00652	0.00711	250-10000	53.2/57.2	0.99982	1.00042	N.D
28	Fisetin	19.30	284.95	135.2-121.3	Neg	y=547.46×+274791	0,991	0.00557	0.00820	250-10000	54.4/61.4	0.99877	1.00031	N.D
29	Cinnamic acid	25.61	147.00	103.15-77.3	Neg	y=9.06×-12403	0.996	0.00648	0.00816	5000-20000	821.8/859.7	1.00051	0.99927	N.D
30	Liquiritigenin	25.62	254.95	119.3-135.1	Neg	y=2384.96×+59141	0,996	0.01849	0.01738	25-1000	5.5/6.6	1.00333	0.99957	N.D
31	Quercetin	28.17	300.90	151.2-179.2	Neg	y=1198.48×+480562	0.990	0.01589	0.01360	100-5000	23.3/28.9	0.98470	1.00103	20.56
32	Luteolin	28.27	284.75	133.2-151.2	Neg	y=3272.65×+150557	0,997	0.00575	0.00696	25-1000	5.4/6.5	1.00772	0.99524	N.D
33	Naringenin	30.68	270.95	151.2-119.3	Neg	y=4315.1×+178410	0.995	0.02054	0.02019	25-1000	5.4/6.4	0.99883	1.01002	N.D
34	Apigenin	31.43	268.95	117.3-151.2	Neg	y=4548.36×+295252	0.990	0.02304	0.02204	25-1000	5.4/6.3	1.01444	1.01331	N.D
35	Hesperetin	31.76	300.95	164.2-136.2	Neg	y=876.67×+48916	0.997	0.03209	0.02605	25-1000	5.6/6.9	0.98850	0.99435	N.D
36	Kaempferol	31.88	284.75	255.1-117.3	Neg	y=26.29×+87558	0,992	0.01436	0.01070	1000-20000	206.6/214.3	0.99971	0.99851	N.D
37	Chrysin	36.65	252.95	143.3-119.4	Neg	y=2032.13×+95593	0,993	0.00490	0.00630	25-1000	5.4/6.2	1.00338	1.00437	N.D

([a]RT: Retention time, [b]Mother ion(m/z): Molecular ions of the standard compounds (m/z ratio), [c]R2: Coefficient of determination, [d]RSD: Relative standard deviation, [e]LOD/LOQ (µg/L): Limit of detection/quantification, [f]U (%): percent relative uncertainty at 95% confidence level (k=2)

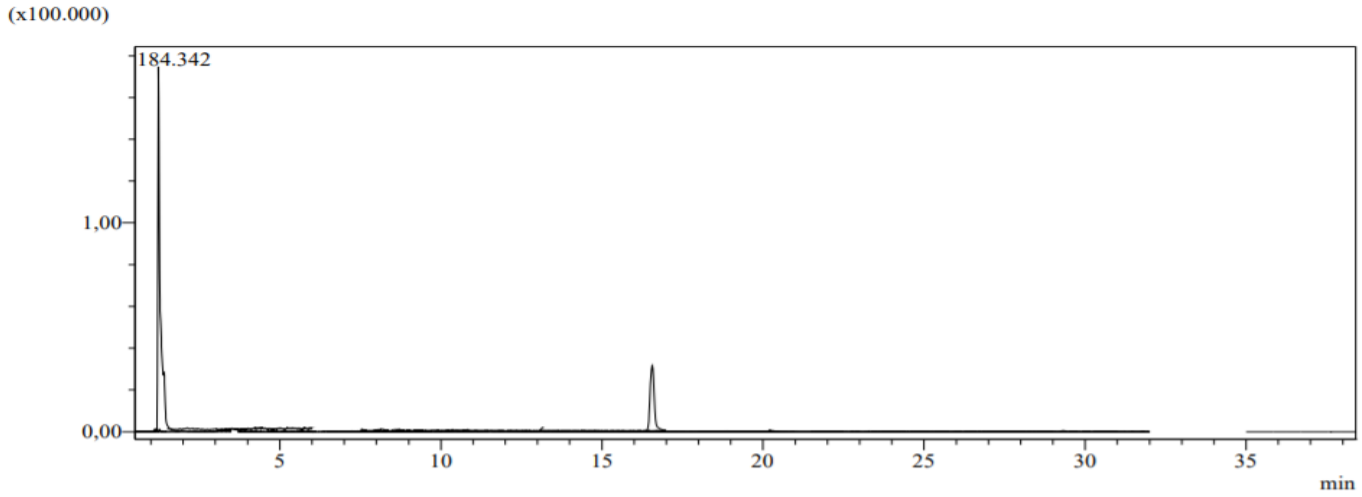

Figure 2 :LC-MS/MS chromatogram for analytical parameters and results of phytochemical compounds

Conclusion

This research examined the phytochemical screening and the potency biological activity of *Crocus Cancellatus* on seven pathogenic *Candida* spp. Based on the results, *C. cancellatus* plant extract has given good yield and tannin result, as well as plant extract, has strong antifungal effective on fungi which used for the experiment and also has a strong antioxidant effect because of the high levels of phenols and flavonoids. Gallic acid, Malic acid and Quinic acid were identified as the main compounds. This plant extract can be used as a natural additive in the chemical, cosmetic, and food industries due to its ingredients.

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