ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF PHENOLIC COMPOUNDS FROM *MYRTUS COMMUNIS* CALLUS

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ABSTRACT

This study was aimed to evaluate atotal phenolic content, antibacterial activity, and antioxidant activity of *M. communis* callus extracts were evaluated. Callus induction in general Murashige and Skoog (MS) media is completed by the Benzil adenine's unique knowledge of callus formation. A well diffusion experiment was used to examine antibacterial interest in *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa*. The DPPH radical scavenging activity test was used to measure antioxidant activity. FTIR and HPLC have been used to pinpoint the presence of polyphenol compounds in calluses. The total phenol content of plant leaves extract (0.1, 0.5, and 1) mg/ml was 42.12, 94.08, and 189 mg of Gallic acid equivalents GAE/g, respectively. Bacterial growth was greatly inhibited by the polyphenol extract from the callus. In comparison to ascorbic acid, the polyphenol extract from the sample has a very high level of 90.17 percent with substantial at P ≤0.05 antioxidant capabilities. There was evidence of phenolic–OH stretching, C-H stretching, aromatic C=C, and C–O stretching in the polyphenol fraction of the *M. communis* callus that was analyzed using FTIR. According to past research, this is a good fit HPLC revealed the presence of phenolic compounds in the callus. The antibacterial and antioxidant properties of the callus polyphenol may be extrapolated from this study.

Keywords: Benzil adenine, Fourier remodel infrared (FTIR), (HPLC)

الفعالية المضادة للبكتريا ومضادات الأكسدة للمركبات الفينولية لكالس نبات الياس *MYRTUS COMMUNIS*

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المستخلص

يهدف البحث الى تقييم المحتوى الفينولي الكلي والنشاط المضاد للبكتيريا والنشاط المضاد للأكسدة لمستخلصات الكالس *M. communis*. يتم استكمال تحفيز الكالس في وسائط Murashige **و** Skoog (MS) **من خلال المعاملة** Benzil adenine **المتميز بتكوين الكالس. تم استعمال تجربة انتشار جيد لفحص الفائدة المضادة للبكتيريا في *Staphylococcus aureus* و *Escherichia coli* و *Klebsiella pneumonia* و *Pseudomonas aeruginosa*. تم استعمال اختبار نشاط الكسح الجذري** DPPH **لقياس نشاط مضادات الأكسدة. تم استعمال** FTIR **و** HPLC **لتحديد وجود مركبات البوليفينول في النسيج. كان محتوى الفينول الكلي لمستخلص أوراق النبات (0.1 ، 0.5 ، 1) مجم / مل 42.12 ، 94.08 ، 189 مجم من** GAE **/ جم ، على التوالي. تم إعاقة النمو البكتيري بشكل كبير بواسطة مستخلص الفينول المتعدد من الكالس. بالمقارنة مع حامض الأسكوربيك ، يحتوي مستخلص الفينول المتعدد من العينة على مستوى مرتفع جدًا يبلغ 90.17 في المائة مع إمكانات كبيرة عند ≤ P0.05 مضادات الأكسدة. كان هناك دليل على الإطالة الفينولية– OH ، وتمتد C–H ، العطرية C = C ، و C–O الممتد في جزء الفينول المتعدد من الكالس *M. communis* الذي تم تحليله باستخدام** FTIR**. وفقًا لبحث سابق ، فإن** HPLC **مناسب تمامًا وكشف عن وجود مركبات فينولية في الكالس. يمكن استقراء الخصائص المضادة للبكتيريا ومضادات الأكسدة في الكالس الفينول المتعدد من هذه الدراسة.**

الكلمات المفتاحية: بنزل ادنين , مطيافية الأشعة تحت الحمراء,(FTIR) , كروماتوغرافيا السائل عالية الدقة (HPLC)

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INTRODUCTION

Linnaeus first described the genus *Myrtus communis* in 1753, placing it in the Myrtaceae family. Tolerant of dry conditions, the plant may thrive in low to moderate water habitats and may flourish up to 800 meters above sea level where it can grow in moist areas, shaded areas, and full sun. its peak season is during the summer months (3). Plants have long been used to treat a variety of ailments. Medicinal plants have a significant role in the fight against discomfort (5). Phospholipids, flavonols, and hydroxybenzoic acids are the main groups of constituents found in phytochemical screening, which also identified the plant to be rich in useful energy molecules, including phenolic compounds, which are the primary corporations of components. Anthocyanins are also found in berries that are colored. Antioxidant and antimutagenic polyunsaturated fatty acids from polyunsaturated fatty acids (e.g. phenolic acids, tannins, flavonoids, and so on.) For further information, see (16). Due to their unique biological qualities, polyphenols are an important element of the human weight-loss program and a popular pastime for those interested in health and wellness (13). There may be secondary metabolite interest in callus, according to studies that compares the callus to genuine plants. The donor plant's genetic potential for enhancing callus induction and promoting callus development should be harnessed for commercial purposes by immobilizing cells in a matrix for use in bioreactors. For callus induction and callus growth, MS, a regularly used medium, is regarded one of the most favored media (1, 2). Testing for antibacterial, total phenolic content , and antioxidant characteristics will be carried out utilizing GCMS analysis on polyphenol extracted from this plant.

MATERIALS AND METHODS

Callus induction: *M. communis* seed tissue culture experiment was successful in inducing callus on cotyledonary leaf explants. As well as four weeks of darkness and 25±1°C incubation. Callus induction was seen after four weeks of culture on MS medium. **(18,6).**

Plant collection and Bolyphenol extraction: Before testing for polyphenols and easy phenolics in herbal plants, the most common extraction methods are liquid-liquid and stable-liquid extraction. In part because of their simplicity of use, effectiveness, and broad application, these tactics are among the most often used today. Alcohols (methanol, ethanol), acetone, diethyl ether, and ethyl acetate are among the most often used extraction solvents. Milling and homogenization are the key phases of an instruction system. Extracting bioactive phytochemicals from plant materials is the first stage in the process of restoring and isolating them. Due to their chemical composition, the extraction process used, sample particle length, and the presence of interfering chemicals, it is triggered by these factors: If the removal of undesired phenolic and non-phenolic components, such as waxes, lipids, terpenes, and chlorophylls, is of interest, extra procedures may be resorted to. A 30 gram powdered plant was extracted with 15 ml chloroform and 24 hours of continuous stirring at room temperature. For fifteen minutes, the extract was placed in an ultrasonic instrument. Once the butanol was added, it was moved to the separation funnel where it was separated. In order to produce a dry extractor, the butanol layer is accumulated and transferred to the rotary evaporator machine. Prior to the assessment, the procedure was performed (three) times to get a predetermined value.

Determination of general phenolic contents

For determining the general phenolic content of polyphenol extracts from plant calluses, the Folin-Ciocalteu technique established by (4, 9). Each pattern was diluted ten times with Folin-Ciocalteu reagent and 1.6 ml of 7.5 percent sodium carbonate solution. By adding distilled water to the original combination, the amount was lowered to 5 ml. The tubes were parafilm-covered and kept at room temperature for 30 minutes before being analyzed for 760 nm absorbance.

Agar well diffusion technique

Plant callus polyphenol extract was tested for antibacterial activity using the agar diffusion technique in line with the national Committee for Clinical Laboratory Standards (13). The 108 cfu/ml inoculum was dispersed on nutrient agar plates using a sterile brush dipped in the bacterial solution. Next, 6 mm-diameter wells in the Agar medium were punched and filled

with 50 l of plant extract, which was then allowed to diffuse at room temperature for 2 h. At 37°C, the plates were then incubated for 24 hours.

An assessment of the potential benefits of antioxidants: An experiment was conducted to determine the antioxidant potential of *Myrtus communis* callus by combining 5 mg of freshly organized 2,2-diphenyl-1-picrylhydrazyl (DPPH) with 50 l of distilled water at various concentrations (0.2, 0.4-0.6-0.6-0.8-0.8-1.2 mg/ml) (10 ml). The solution's absorbance was measured at 517 nm after two hours of dilution (11). It was the antioxidants that proved to be most effective in this situation. All inspections were carried out in double the amount of time. (Abs DPPH – Abs sample.)/Abs DPPH x one hundred is the formula to compute the percentage discount of the DPPH radical-scavenging capacity. Excel was used to create a graph based on the collected data. To determine the IC50 of each extract, we looked at the image and noted which extracts or compounds inhibited 50% of DPPH.

Fourier remodel infrared (FTIR) assay

Fourier's Revision It's possible to get an infrared spectrum by measuring the energy absorption of an incoming beam of radiation at a certain wavelength using infrared spectroscopy. More than 4000 and 400 cm-1 was obtained in terms of FTIR spectra **(17).**

Liquid Chromatography with Extremely High Throughput (HPLC): Liquid Chromatography with Extremely High Throughput (HPLC) was used to evaluate the samples. S 2100 is the pump model. S 5200 Quaternary Gradient Pump, S 2340 UV Detector, and S 4115 Column Oven model. Methanol, D.W., and acetonitrile were added to the cell section (85, 13, 2 respectively) and then the C18-ODS column (25cmx4.6cm) was used with the flow charge of 1ml/min.

Statistical analysis

The statistics had been analyzed the usage of SPSS sixteen software, and differences among manner of remedies were in comparison via the use of Fisher's Least massive differences (LSD) check as widespread at $p \leq 0.05$

RESULTS AND DISCUSSION

Callus induction is affected by the combination of 2,4-**Dichlorophenoxy acetic acid (2, 4-D) and Benzyl adenine (BA).**

The *Myrtus communis* seed used in the tissue cultures experiment produced callus on the explants of cotyledonary leaves. For the next four weeks, they were kept in complete darkness at 251°C. To induce callus, callus induction became decided after six weeks of MS medium cultivation(Table1). The callus formed had a watery appearance. In addition, the callus induction % is calculated (Table1). According to the results, 2,4-D at a concentration of 2 mg/L was able to significantly increase the percentage of callus induction, with results showing an increase of 66.67% above the control value of 33.33% One milligram/liter of BA was shown to be the most effective in increasing callus induction rates by 70%. Furthermore, the results indicated that 2, 4-D at 2mg/L interacted significantly with BA at 1mg/L, resulting in a 90 percent increase in callus induction, as opposed to zero percent for control groups. These results are quite similar to those obtained with Tatara, which revealed maximal 2,4-D callus induction at a concentration of 2 mg/L. **(**14).

Table 1. After six weeks of inoculating explants on MS medium with 2,4-D and BA and their interaction, the percent callus induction was evaluated in a n=10 study

2,4 D (mg/l)	BA(mg/l)			Mean%
	0.0	1	2	Mean
0.0	0	70	30	33.33
1	30	60	40	43.33
2	40	90	70	66.67
3	30	60	40	43.34
Mean	25	70	45	
LSD: 2,4D= 5 BA= 5 , 2,4D*BA= 10 * (P<0.05).				

It was found that the effects of 2,4-D and BA on accurate rice (Oryza sativa cv. Swat-II) callus formation on MS medium were likewise in agreement with those reported by (3).

Two,4-D has an enormous influence on callus development because of its Catalyst impact on plant growth and its critical involvement in cell elongation. BA also increased callus formation, but when the concentration of BA was increased, callogenesis slowed to a standstill. Inhibiting effects of auxin and cytokinin on mitosis were more pronounced at higher doses. Spindle poisoning and stressing chromosomal separation at cellular poles were two of auxin's aneugenic properties (19).

2,4-D and BA have a combined effect on callus fresh weight

The results in Table 2 show that the fresh wet of callus induction was significantly enhanced by 2mg/L 2,4-D, as compared to 0.000. The best percentage of callus induction was achieved at a concentration of 1mg/L BA. As a result of these findings, the callus induction rate increased from zero to 7.316mg when 2,4-D and 1mg/L BA were combined.

Table 2. After inoculating callus pieces on solid MS medium for six weeks, the effect of 2, 4-D and BA on the mean callus frash weight (mg/L) was studied. There were six participants that weighed in at 200mg

2,4 D)	BA(mg/l)			Mean
	0	1	2	(mg/l)
0.0	0.00	0.00	0.00	0.000c
1	0.059	2.016b	0.107	0.727b
2	0.284	7.316a	2.175b	3.260a
3	0.630	1.096c	0.938cd	0.890b
Mean	0.243	2.607	0.805	
	LSD: 2,4D=0.386	BA= 0.334	, 2,4D*BA=0.669	
		* (P<0.05).		

Calcification of wheat callus after treatment with 2,4-D and BA: Table 3 shows that 2 mg/L 2,4-D caused broad rise in callus dry weight, with a mean callus weight of 0.155 mg/L at 2 mg/l 2,4-D, compared to 0.195 mg at 1 mg/l BA, respectively. Based on the interaction between hormone treatments, the maximum callus dry weight was found to be 2mg/l 2, 4-D and 0.5mg/l BA (or (0.401) mg/L). 2,4-D and BA concentrations extended to (0.0168) at 2 mg/l 2,4-D and 2 mg/l BA decreased callus dry weight. In the presence of cytokinins, auxins have been shown to increase cell division (17). At low doses, auxin 2, 4-D was shown to be effective in promoting callus formation in rat tendons. By inhibiting the growth of adventitious and axillary shoots as well as inducing embryogenesis, auxins affect cell elongation, tissue swelling, cellular division, and cell division. As a replacement for callus induction, relations at high concentrations have a devastating impact on callus induction (19). As an additional benefit to tissue culture, cytokinin enhances mitosis by increasing the charge of protein synthesis or the building protein or enzyme that is required for mitosis in G2 (12).

Table3. Callus dry weight (mg) after six weeks of inoculation with 2, 4-D and BA on solid MS medium was studied. n=6 were given a starting weight of 200mg

2,4 D	BA(mg/l)			Mean
(mg/l)	0	1	2	(mg/l)
0.0	0.00	0.00	0.0033	0.00
1	0.004c	0.339a	0.0168c	0.144a
2	0.0046c	0.401a	0.1204cb	0.155a
3	0.0568c	0.0352c	0.265ab	0.119
Mean	0.0163	0.1959	0.1005	
	LSD: 2,4D= 0.088	BA=0.0764	, 2,4D*Kin=0.153	
		* (P<0.05).		

Total phenolic content: It is possible to synthesize polyphenols from L-phenylalanine or L-tyrosine through the phenylpropanoid route. For their organic contents and health advantages, several phenolic compounds have been studied detail (10). In order to assess the total phenolic content of the *M. communis* callus, Follin-reagent Ciocalteu's was used. p 0.01 indicated a significant difference between the exceptional concentrations of the same

extract in the statistical analysis (Table 4). *Myrtus communis* callus total phenolic content material was found to be (50.07 047, 63.41 0.57, and 83.43 0.90) mg /ml in the polyphenol extracted samples in (0.1, 0.5, and 1) mg/ml, respectively. Table 4 (simplified).

Table 4. Phosphorous concentration in *Myrtus communis* callus polyphenol extracts

Myrtus communis Callus	Concentration of the sample	Total phenolic contents (mg of GAE/g)
Polyphenol of callus extracts	**0.1 mg/ ml**	**50.07± 047 c**
	0.5 mg/ml	**63.41± 0.57 b**
	1 mg/ml	**83.43± 0.90 a**

M. communis ethanolic/water extract exhibited far larger levels of phenolic chemicals than the less polar solvents, *M. communis* leaf extract had more TPC than the pericarp and stem extracts, as well, according to the study's findings Myrtle seed samples had a lower TPC than myrtle sections of the same species. Due to the high polyphenol content in plant callus, DPPH scavenging interest was extremely high in this study, as shown by the statistical comparison of exceptional concentrations of the same extraction Vitamin C's 97.13 percent value became 90.17% for one milligram/ml of this compound (figure 1) and (Table 4). the antioxidant activity of samples was also evaluated by their ability to scavenge DPPH free radicals, as stated. Different extract types, alcohol concentrations, and maceration times all had different antioxidative activities. According to (8)., Myrtus extract boosted the neutralization of DPPH and peroxyl radicals much more than nutrients did (3).

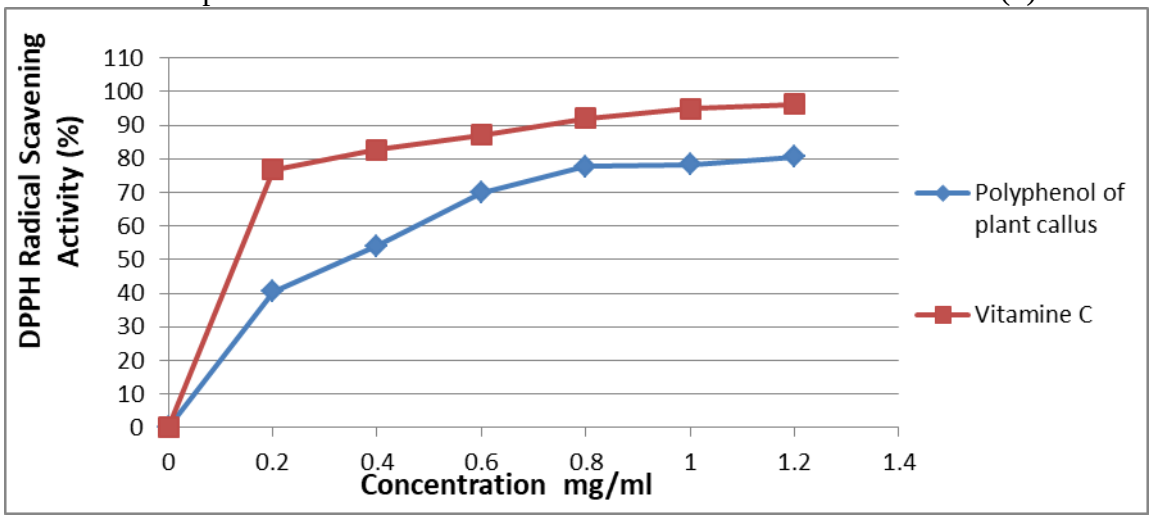


Figure 1. Callus polyphenols with IC50 DPPH Radical Scavenging Activity

(8), on the other hand, found that myrtle extract boosted DPPH and peroxyl radical neutralization even more than nutrients did. Antioxidant attention is stated as a maximum inhibitory attention, for instance (IC50). IC50 of vitamin C (0.4 mg/ml) and polyphenol extracts of plant callus (0.4 mg/ml) were shown to have a different scavenging activity (IC50) (figure 2).

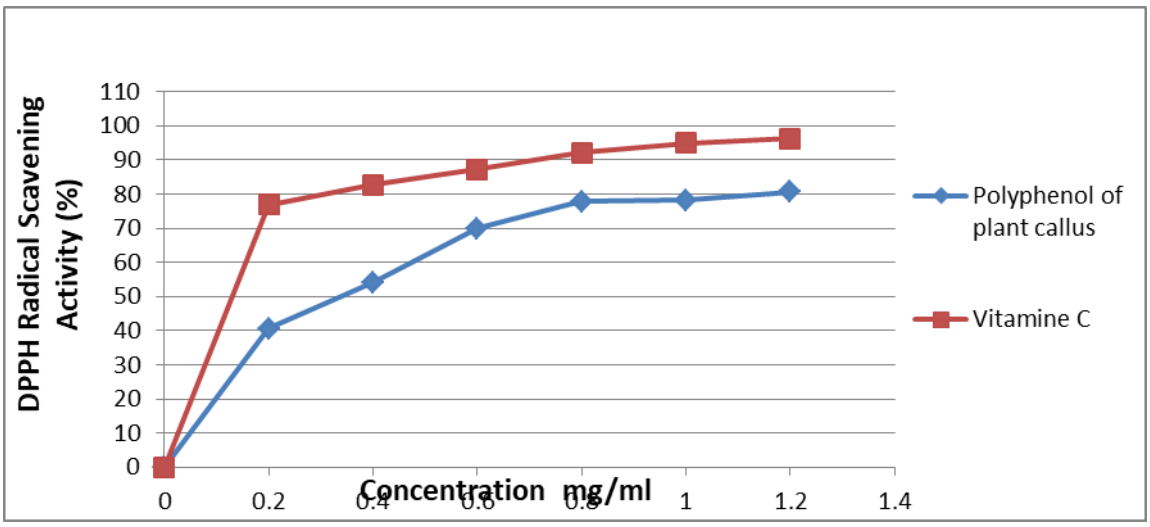


Figure 2. Percentage of polyphenols in plant callus with IC50 DPPH Radical Scavenging Activity

Antibacterial activity of *Myrtus communis* leaves extracts: *P. aeruginosa* (figure 3) showed the lowest inhibition zones (5 ±0.33, 9 ±0.55, 11 ±0.62mm) in concentrations (25 mg/ml) with a significant difference (P 0.05), while inhibition zones on Staph. Aureus (7 ±0.00, 12 ±0.00, and 17 ±0.28 mm) in concentrations (25, 50, and 100 mg/ml) were observed with a **significant** difference (P ≤0.05) (Table5).

Table 5. Polyphenols extracted from *Myrtus communis* leaves have been shown to have antibacterial properties

Concentration (mg/ml)	Mean ± SE (mm)			
	Staph.aureus	***P.aeruginosa***	***E. coli***	***K.pneumonia***
25	**7 ± 0.00 b**	**5 ± 0.33 b**	**8 ± 0.09 b**	**6 ± 0.00 c**
50	**12 ± 0.00 b**	**9 ± 0.55 a**	**13 ± 0.12 ab**	**11 ± 0.12 b**
100	**17 ± 0.28 a**	**11 ± 0.62 a**	**8 ± 0.33 a1**	**15 ± 0.41 a**
LSD value	**2.273 ***	**3.061 ***	**2.197 ***	**2.548 ***
Means having with the different letters in same column differed significantly * (P<0.05).				

For E. coli and *Staphylococcus aureus* stress, the maximal antibacterial activity of *M. communis* alcoholic extracts was found to be in the range of 5.67-5.5 mm, according to (7). That's what we came up with in the callus experiment against E. coli and Staphylococcus in attention 100 (mg/ml). The alcoholic extract of *M. communis* callus callus proved powerful and attention-based antibacterial action against all tested gram-positive and gram-negative isolates.

Fourier Transform Infra-Red (FTIR) of *Myrtus communis:* The polyphenol extracts of M. commnunis callus, for example, demonstrate in the FTIR spectra that phenolic–OH group stretching, C-H stretching, aromatic C=C stretching, and aliphatic C–O stretching are all present (Figure 3)., the phenolic structures of bioactive antioxidants play an important role in their investigation of phenolic O–H bond dissociation enthalpy (BDE), ionization ability (IP), proton dissociation enthalpy (PDE), proton affinity (PA), and electron transfer enthalpy (ETE)

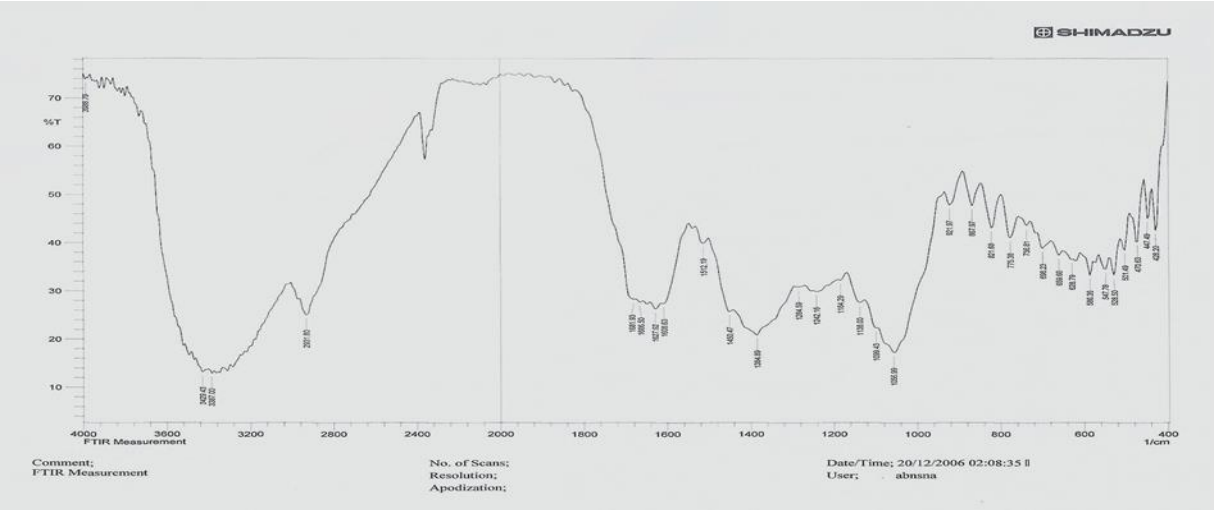
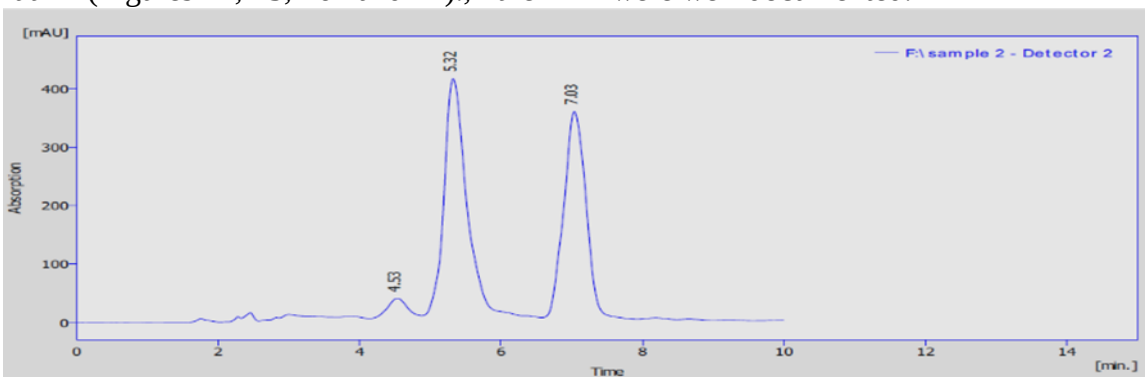


Figure 3. *M. communis* callus polyphenol extract infrared spectral analysis

Liquid chromatography with high resolution and speed (HPLC): There were three phenolic chemicals found in the callus of the rat illustrated in this figure: gallic, tannic, and rutin (Figures 4, 5, 6 and 7)., the antihyperglycemic properties of *M. communis* methanolic and aqueous extracts (including myricetin 3-O-β-glucopyranoside,3-O-α-rhamnopyranopyranoside, and gallic acid) were well-documented.



	Reten.Tim(min)	Area(mAu.s)	Heigh(mAU)	Area(%)	Height(%)	W05	CompoundName
1	4.530	58.639	7.768	5.1	5.1	0.13	
2	5.320	600.770	76.079	51.8	49.7	0.12	
3	7.033	499.998	69.318	43.1	45.3	0.13	
	Total	1159.407	153.165	100.0	100.0		

Figure 4. Extracts of polyphenols from *M. communis* callus were analyzed using high-performance liquid chromatography (HPLC).

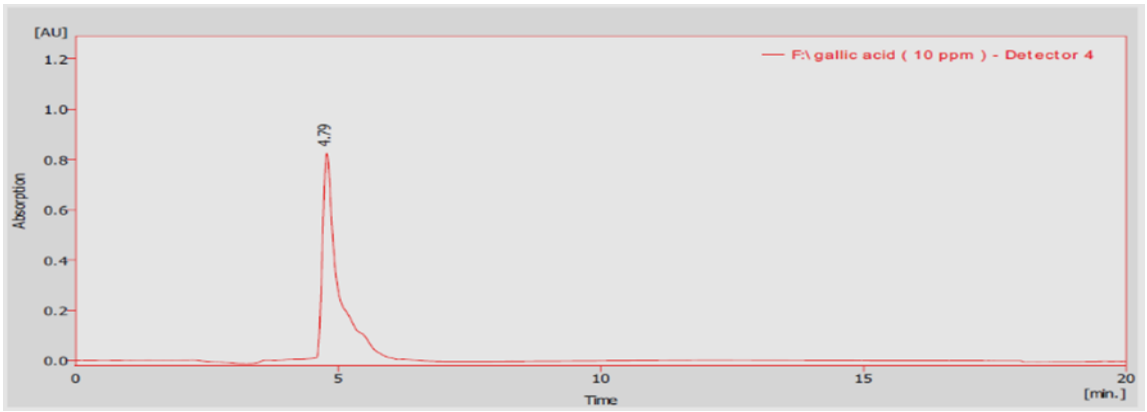
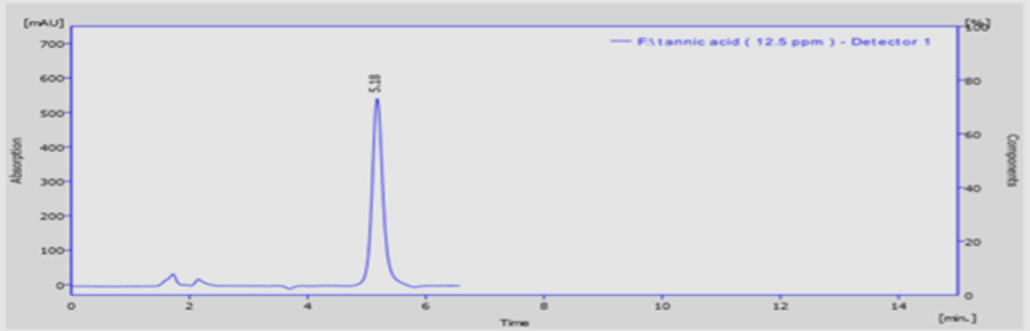


Figure 5. Typical gallic acid HPLC chromatogram of phenolic chemicals

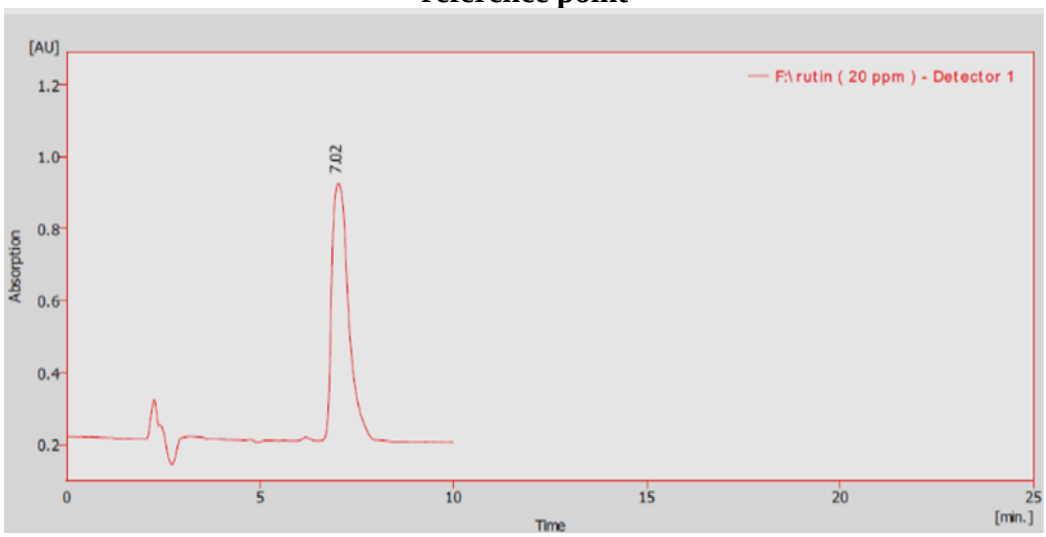


	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Compound Name	Correction Factor
1	5.177	491.694	110.602	100.0		
	Total	491.694	110.602	100.0		

Figure (4-6): HPLC chromatogram of phenolic compounds standared tannic acid

	Reten.Tim(min)	Area(mAu.s)	Heigh(mAU)	Area(%)	Compound Name	Correction Factor
1	5.177	491.694	110.602	100.0		
	Total	491.694	110.602	100.0		

Figure 6. The HPLC chromatogram of phenoic chemicals, including tannic acid, as a reference point



	Reten.Tim(min)	Area(mAu.s)	Heigh(mAU)	Area(%)	Height %	W05(min)	CompoundName
1	7.023	1937.121	134.302	100.0	100.0	0.23	
	Total	1937.121	134.302	100.0	100.0		

Figure 7. The HPLC chromatogram of the standard rutin phenoic chemicals

CONCLUSION

Poplyphenol extracts from callus of this plant were studied for their antibacterial and antioxidant activities and GC-MS analyses. *M. communis* callus polyphenol extracts were antibacterially active against all lines of check microorganisms that were tested. Antioxidant properties of callus polyphenol extracts have large IC50 values. *M. communis* callus polyphenol fraction FTIR analysis identified useful chemicals. It was discovered that polyphenol derived from the plant might be utilized to produce biopharmaceuticals for infectious diseases and antioxidants in the future.

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