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ANTIBACTERIAL EFFECT, ANTIOXIDANT POTENTIAL AND TOTAL PHENOLIC CONTENT OF POLYPHENOL EXTRACTS OF *MYRTUS COMMUNIS* LEAVES

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Abstract

Myrtus communis have been used as a traditionally for the treatment of disorders such as diarrhea, peptic ulcer, hemorrhoid, inflammation, pulmonary and skin diseases. The aim of this study was to determine of total phenolic content, antibacterial and antioxidant activities of polyphenol extracts from *M. communis* leaves. Well diffusion assay was used to test antibacterial activity against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa*. DPPH radical scavenging activity assay was used to evaluate their antioxidant activity. FTIR and HPLC techniques were used to identify polyphenol compounds in extracts. Total phenol content of plant leaves extract were at 42.12, 94.08 and 189 mg of GAE/g in (0.1, 0.5 and 1) mg/ml of extracts. Polyphenol extract from the tissue culture exhibited significant at P value < 0.05 inhibition against pathogenic bacteria. Polyphenol extract of the study have high level 90.17% with significant at P value < 0.05 of antioxidant activities compared with ascorbic acid at 97.32% at 0.12 mg/ml of concentration. FTIR analysis of polyphenol fraction of the *M. communis* identified functional groups such a phenolic– OH group stretching, C-H stretching, Aromatic C=C and Aliphatic C–O in this fraction. HPLC results of extract showed specific phenolic compounds. It could be concluded that the polyphenol of the part of the plant had a good antibacterial and antioxidant effects.

***Keywords*:** *Myrtus communis;* Polyphenol; Antibacterial; Antioxidant

Introduction

Myrtus communis is a genus of flowering plants in the family Myrtaceae, described by Linnaeus in 1753. The plant is highly tolerant to drought, can grow in low to moderate water environments, and can grow in moist places, shades and in sunny places up to 800 m altitudes. Summer is its flourishing period (Alipour *et al*., 2014 ; Melito *et al*., 2016; Bouzabata *et al*., 2016). The plant are being used continually as medicines against different diseases. An essential role is being played by medicinal plants against inflammation (Antonisamy *et al*., 2017).

Several studies reported phytochemical screening which revealed its richness in beneficial active molecules such as phenolic compounds which is the major groups of constituents include gallic acid derivatives, flavonols, flavonol derivatives, and hydroxybenzoic acids. In coloured berries, anthocyanins are also present Hennia *et al*. (2018) and polyunsaturated fatty acids as a source of antioxidant and antimutagenic agents (e.g. phenolic acids, tannins, flavonoids, etc.) (Serce *et al*., 2010). Polyphenols are one of the most numerous and diverse group of secondary metabolites that comprise an essential part of the human diet and are of considerable interest due to their biological properties (Rasouli *et al.*, 2016). Salvagnini *et al.* (2008). reported antibacterial activity of a methanolic crude extract of *M. communis* on Gram-positive such as *Staphylococcus aureus, Micrococcus luteus, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus agalactiae, Listeria monocytogenes* and four Gram-negative bacteria such as *E. coli, Proteus vulgaris, Pseudomonas aeruginosa* and *Campylobacter jejuni* were previously assessed.

The aim of this study is to determine the antibacterial, total phenolic content and antioxidant activities of the polyphenol extracted of from this plant with GCMS analysis.

Materials and Methods

Plant collection and Poyphenol extraction

The plant samples planted in the gardens of the University of Baghdad, Al-Jadriya, were classified in the Department of Life Sciences, Faculty of Science, University of Baghdad. The plant family (Myrtaceae), genus (Myrtus) and species (Myrtus communis) were confirmed. Polyphenol of extract from the leaves were prepared according to Konte *et al.* (2012). 50 gram of *Myrtus communis* leaves and plant culture were put in a of acetone and distilled water for 24 hours. The solutions were filtered through a filter paper Whatman No.1 and evaporated to dryness under vacuum at 40°C by a rotary evaporator. The extracts were extracted with hexane. Then, it evaporated under vacuum at 40° by a rotary evaporator. The extracts were stored in amber glass vials at 4 °C until analyzed.

Determination of total phenolic contents

Total phenolic content of polyphenol extracts from the leaves of the plant were determined spectrophotometrically using the Folin-Ciocalteu method described by Jayaprakasha *et al.* (2001). 0.4 ml of each sample was mixed with 2.0 ml of the Folin-Ciocalteu reagent (diluted 10 times) and 1.6 ml of 7.5% sodium carbonate solution. The total volume was adjusted to 5 ml by adding distilled water. The tubes were covered with parafilm and allowed to stand for 30 minutes at room temperature before the absorbance was read at 760 nm spectrometrically.

Agar well diffusion method

Antibacterial activity of polyphenol extract from leaves of the plant were determined by agar well diffusion method according to National Committee for Clinical Laboratory Standards (NCCLS, 1993). Inoculum containing 10^8 cfu/ml of each bacterial culture to be tested was spread on nutrient agar plates with a sterile swab moistened with the bacterial suspension. Subsequently, wells of 6 mm diameter were punched into the agar medium and filled with 50 µl of plant

extract and allowed to diffuse at room temperature for 2 h. The plates were then incubated in the upright position at 37° for 24 h.

Evaluation of Antioxidant activity

In order to obtain an indication of the antioxidant activity of *Myrtus communis* leaves methanolic extracts and tissue culture, 5 ml of a freshly prepared 0.004% of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol was mixed with 50 µl of different concentrations (0.2, 0.4, 0.6, 0.8, 1, 1.2) mg/ml, respectively in distilled water, then the volumes were completed into (10 ml). The absorbance of each dilution, after 2 hours, The solution was measured at 517 nm (Kedare and Singh, 2011). Vitamin C were the antioxidants used as positive control. All tests were performed in duplicate. The percentage DPPH reduction (or DPPH radical scavenging capacity) was calculated as:

% Reduction

= (Abs Control – Abs Sample.) /Abs Control x 100

With the obtained values, a graphic was made using Microsoft Excel. The IC_{50} of each extract (concentration of extract or compound at which inhibition 50% of DPPH) was taken from the graphic.

Fourier transform infrared (FTIR) assay

Fourier Transform Infrared spectroscopy is a technique based on the vibrations of the atoms of a molecule, an infrared spectrum is commonly obtained by passing infrared radiation through a sample and determining what fraction of the incident radiation is absorbed at a particular energy. The FTIR spectrum was recorded between 4000 and 400 cm^{-1}.

High-Performance Liquid Chromatography (HPLC)

The process of extracting and quantifying and qualifying the compounds was carried out by analyzing the samples by HPLC as follows: Separation of alcoholic extract on FLC (Fast Liquid Chromatographic) column (50x4.6 mm ID) C18-DB 3 µm. the mobile phase used is 0.01 M Acetonitrile pH 8.2 pH at 55:45 V/V with flow rate of 0.9 ml / min and readings were taken using UV at wavelength of 220 nm and at a temperature of 30 °C. 20 µm was injected into the HPLC column and the concentration of each compound was quantified by comparing the peak area of the standard model curve with the samples to be measured. Separation was carried out on a Shimadzu 10AV-LC high performance liquid Chromatography equipped with a LC-10A pump, and the curves of the separated samples were observed by an (UV-Vis 10A-SPD spectrophotometer (Zaho *et al*., 2002).

Statistical analysis

The data were analyzed using SPSS 16 software, and differences among means of treatments were compared by using Fisher's Least Significant Differences (LSD) test as significant at $p \leq 0.05$.

Results and Discussion

Total phenolic content

Several phenolic compounds have been studied for their biological properties and benefits to human health, polyphenols are secondary metabolites of plant origin that are synthesized from L-phenylalanine or L-tyrosine through the phenylpropanoid pathway (Kallscheuer *et al.,* 2017).

The *M. communis* extracts were evaluated by using Follin-Ciocalteu´s reagent for the determination of total phenolic contents. The statistical analysis between different concentrations of the same extract; there was a significant difference at $p < 0.01$ (Table 1). The results of total phenolic content in the *M. communis* leaves extracts were observed at (42.12± 044, 94.08± 0.57 and 189± 0.89) in (0.1, 0.5 and 1) mg /ml respectively in the polyphenol extracted samples (Table 1).

Table 1 : Total phenolic content of polyphenol extracts from *Myrtus communis* leaves and culture.

***Myrtus communis* extracts**	**Concentration of the sample**	**Total phenolic contents (mg of GAE/g)**
Polyphenol of Leaves extracts	0.1 mg/ ml	42.12± 044 **c**
	0.5 mg/ml	94.08± 0.57 **b**
	1 mg/ml	189± 0.89 **a**

Aksay (2016) found that the amounts of phenolic compounds in *M. communis* ethanolic/water extract were much higher than in the case of the less polar solvents.

Furthermore, leaf extract of *M. communis* presented the higher TPC compared to that of pericarp and stem extracts. Seed samples presented the lowest TPC than the other myrtle parts according to (Bouaoudia-Madi *et al.,* 2017).

In this study, DPPH scavenging activity was very high in plant tissue culture extract compered to plant leaves extract and increased gradually with extract concentrations, as for the statistical analysis between different concentrations of the same extract due to high polyphenol content. The value was at 90.17% in 1 mg/ml compared to for vitamin C at 97.13% (Figure 1).

According to the Zam *et al.* (2017) studied the antioxidant activity was also measured through the ability of samples for scavenging the DPPH free radicals. All samples presented variable antioxidant activity depending on the type of extract, concentration of alcohol, and time of maceration. Otherwise Dairi *et al.* (2017) showed DPPH result of myrtle extract increased the neutralization of DPPH and peroxyl radicals, even better than vitamins

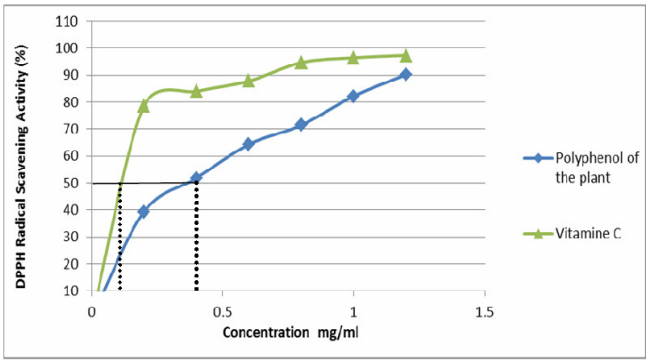


Fig. 1 : DPPH Radical Scavenging Activity percentage of polyphenol of plant extracts and culture with IC50

Otherwise Dairi *et al.* (2017) showed DPPH result of myrtle extract increased the neutralization of DPPH and peroxyl radicals, even better than vitamins. Furthermore, the antioxidant activity is expressed as an maximal inhibitory concentration (IC50). In this study the radical scavenging capacity (IC50) of vitamin C was 0.15 mg/ ml, while

polyphenol extracts the plant leaves was found to be (0.4 mg/ml) (Figure 2).

Antibacterial activity of *Myrtus communis* leaves extracts

Table 3 show the inhibition zones were seen on *Staph. aureus* with the inhibition zone (0 ± 0.00, 0 ± 0.00 and 6 ± 0.28 mm) in concentration (25, 50 and 100 mg/ml) respectively with a significant difference of (P <0.05), while the lowest effect was seen on *P. aeruginosa* (Figure 3) with inhibition zone (8 ± 0.33, 13 ± 0.55 and 14 ± 0.62 mm), otherwise the best effect on *E. coli* and *K. pneumonia* in concentrations (25 mg/ml) respectively with a significant difference (P < 0.05).

Table 2 : Antibacterial activity of polyphenol extract of *Myrtus communis* leaves extract

Concentration (mg/ml)	Mean ± SE (mm)			
	Staph.aureus	*P.aeruginosa*	*E. coli*	*K.pneumonia*
25	0 ± 0.00 **b**	8 ± 0.33 **b**	5 ± 0.09 **b**	0 ± 0.00 **c**
50	0 ± 0.00 **b**	13 ± 0.55 **a**	7 ± 0.12 **ab**	7 ± 0.12 b
100	6 ± 0.28 **a**	14 ± 0.62 **a**	8 ± 0.33 a	12 ± 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).				

Besufekad *et al.* (2017) Founded that the effect of *M. communis* alcoholic extracts showed maximum antibacterial activity against *E. coli* and *Staphylococcus aureus* strain with a zone of inhibition ranges from 5.67-5.5 mm. These results are similar or nearest to our result against *E. coli* and *Staphylococcus* in the tissue culture experiment in concentration 100 (mg/ml). Otherwise, Oztu¨rk *et al.* (2019) result showed that the antibacterial activity of *M. communis* material is broader in coverage with a remarkable activity , the alcoholic extract of the leaves of *M communis* showed potent and concentration-dependent antibacterial activity against all tested gram-positive and gram-negative isolates. The remarkable activity of this plant extract against *S aureus* and *P. aeruginosa* in particular.

Fourier Transform Infra-Red (FTIR) of *Myrtus communis*

Results of the FTIR spectra of the polyphenol extracts of leaves and tissue culture of *Myrtus commnus* revealed the presence of different functional groups such as phenolic–OH group stretching, C-H stretching, Aromatic C=C and Aliphatic C–O (Figure 3). It has been reported by Horton *et al.* (2019) that phenolic structures play a crucial role in bioactive It has been shown that these radical scavenging activities of phenolic antioxidants are related to the phenolic O–H bond dissociation enthalpy (BDE), ionization potential (IP), proton dissociation enthalpy (PDE), proton affinity (PA), and electron transfer enthalpy (ETE).

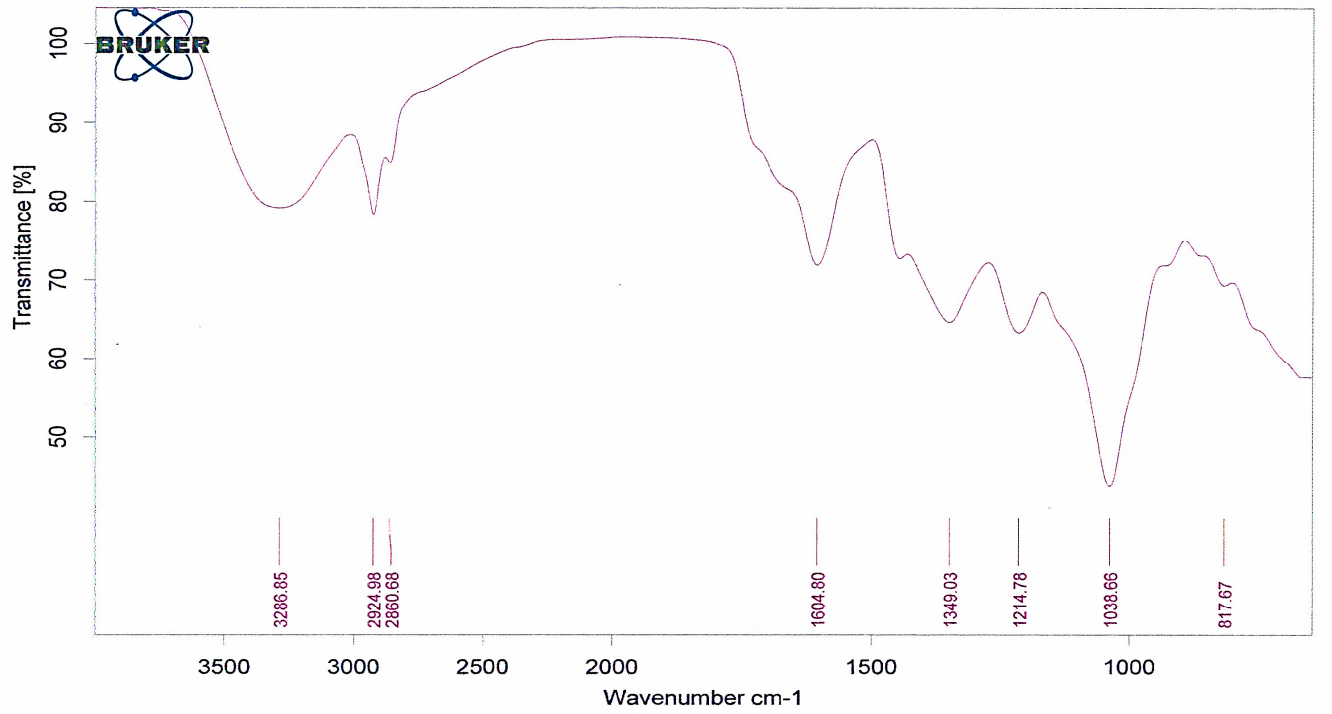
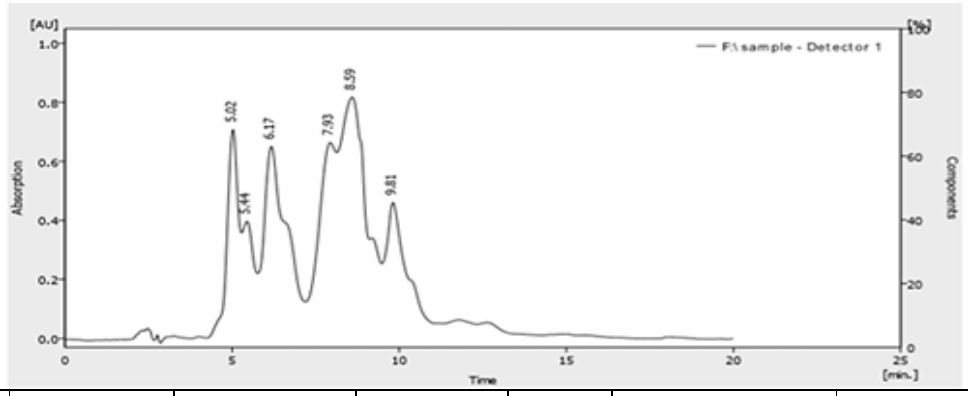


Fig. 2 : Infrared spectrum of polyphenol extract of *M. communis* leaves.

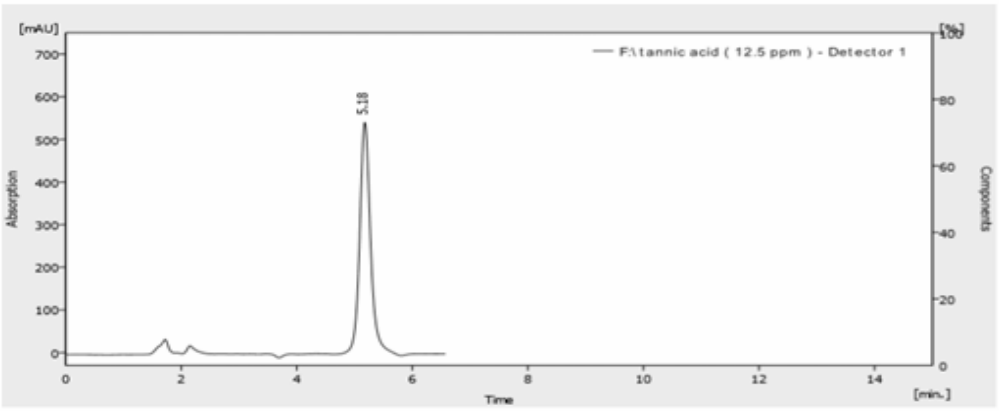
High-performance liquid chromatography (HPLC)

In this study, 5 phenolic compounds were detected (Caffeic acid, Gallic acid, tannic acid, catechine and apiginine acid) in polyphenol extracts (Figure 3) when compared with standard compounds as shown in (Figures 4, 5, 6, 7 and 8). Nassar *et al.* (2010) identified bioactive compounds in methanolic and aqueous extracts in *M. communis* leaves such as myricetin 3-O-β-glucopyranoside, myricetin 3-O- α -rhamnopyranoside and gallic acid) showed significant antihyperglycemic.



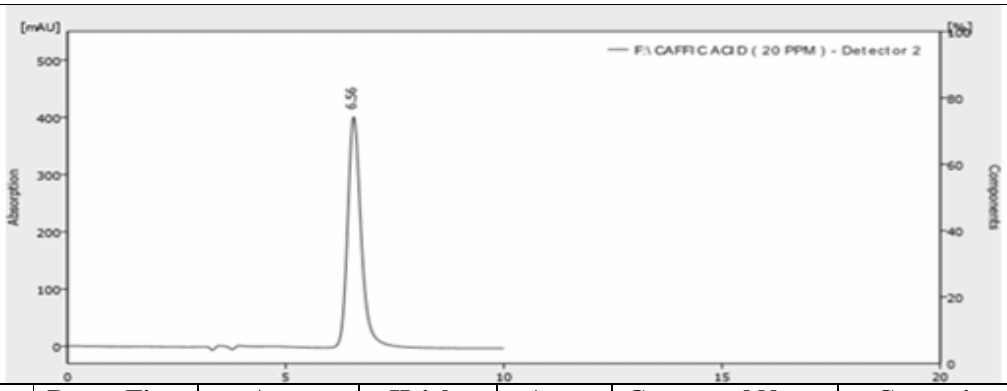
	Reten. Time [Min]	Area [mAU.s]	Height [mAU]	Area [%]	Compound Name	Correction Factor
1	5.023	16473.858	700.911	11.9		
2	5.443	9172.877	386.712	6.6		
3	6.173	30160.429	635.787	21.8		
4	7.933	22381.565	636.683	16.1		
5	8.593	43316.372	785.814	31.2		
6	9.813	17134.005	420.463	12.4		
	Total	138639.107	3566.369	100.0		

Fig. 3 : HPLC chromatogram of phenoic compounds in polyphenol extracts from *M. communis* leaves



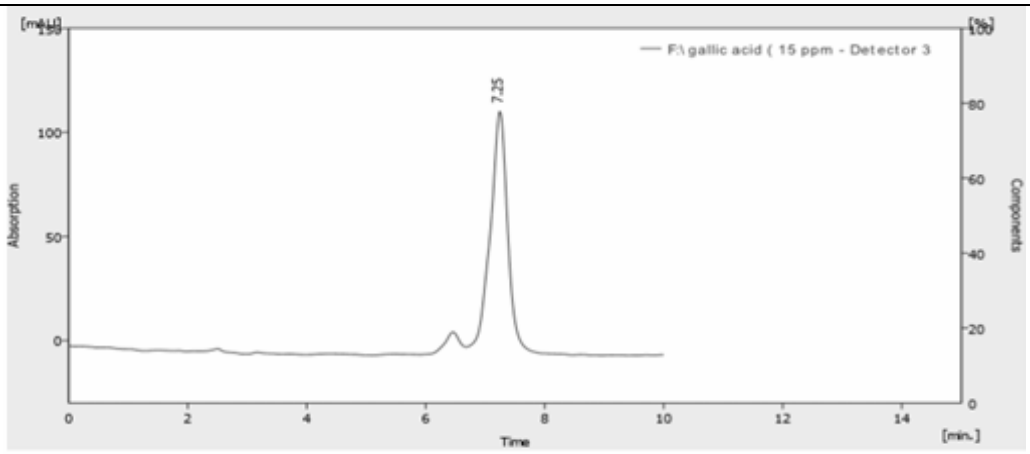
	Reten. Time [Min]	Area [mAU.s]	Height [mAU]	Area [%]	Compound Name	Correction Factor
1	5.177	491.694	110.602	100.0		
	Total	491.694	110.602	100.0		

Fig. 4 : HPLC chromatogram of phenoic compounds standard tannic acid.



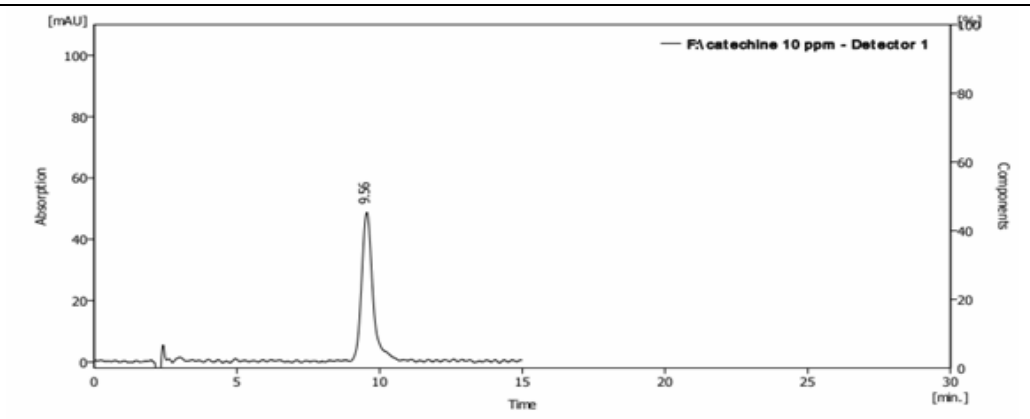
	Reten. Time [Min]	Area [mAU.s]	Height [mAU]	Area [%]	Compound Name	Correction Factor
1	6.560	331.902	57.579	100.0		
	Total	331.902	57.579	100.0		

Fig. 5 : HPLC chromatogram of phenoic compounds standard caffeic acid.



	Reten. Time [Min]	Area [mAU.s]	Height [mAU]	Area [%]	Compound Name	Correction Factor
1	7.247	218.899	26.855	100.0		
	Total	218.899	26.855	100.0		

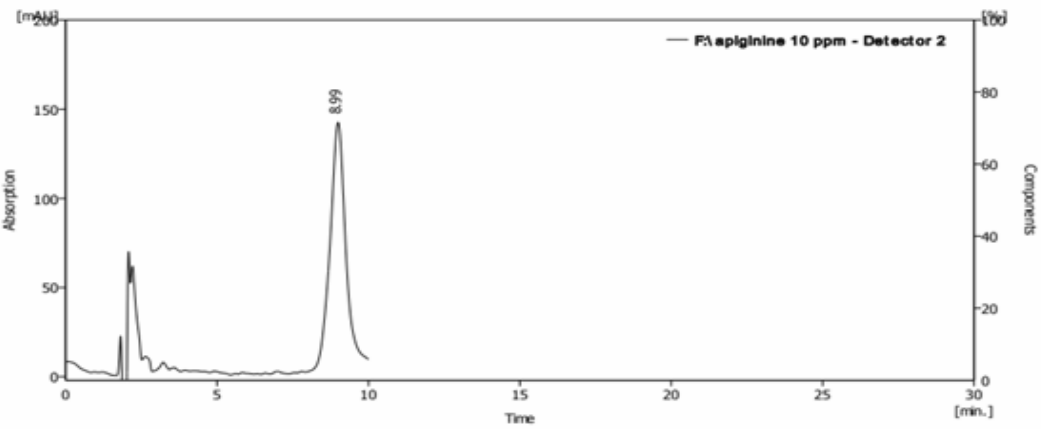
Fig. 6 : HPLC chromatogram of phenoic compounds standard gallic acid.



Result Table (Uncal – F :\catechine 10 ppm – Detector 1)

	Reten. Time [Min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	WOS [min]	Compound Name
1	9.557	288.098	20.321	100.0	100.0	0.23	
	Total	288.098	20.321	100.0	100.0		

Fig. 7 : HPLC chromatogram of phenolic compounds standard catechine



Result Table (Uncal – F :\apiginine 10 ppm – Detector 2)

	Reten. Time [Min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	WOS [min]	Compound Name
1	8.993	574.366	36.829	100.0	100.0	0.22	
	Total	574.366	36.829	100.0	100.0		

Fig. 8 : HPLC chromatogram of phenolic compounds standard apiginine acid.

Conclusions

In conclusion, this is study evaluated the antibacterial activity antioxidant properties and GC-MS analysis of poplyphenol extracts from this plant. Polyphenol extracts of *M. communis* had antibacterial activity against all strains of test bacteria. Polyphenol extracts of *M. communis* have antioxidant activity with significant values of $IC_{50.}$. FTIR analysis of polyphenol fraction of the *M. communis* identified functional groups. GC- analysis of polyphenol extracted from the plant identified important compounds which may be used to develop biopharmaceuticals against infectious diseases and antioxidants source in future.

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