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The Effect of Polyphenol Extracts from Eucalyptus Spp. against Pathogenic Bacteria with Antioxidant Activities

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Abstract
Eucalyptus spp. 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 plant 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 40.07, 50.30 and 82.64 mg of GAE/g in (0.1, 0.5 and 1) mg/ml of extracts. Polyphenol extract from plant leaves exhibited significant at P value < 0.05 inhibition against pathogenic bacteria. Polyphenol extract of the study have high level 75.84% 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 plant 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: *Eucalyptus* spp.*;* Polyphenol; Antibacterial; Antioxidant

INTRODUCTION

Eucalyptus is a genus belong the family *Myrtaceae*. It is widely distiuted in different regoins around the world with 800 species (Hassine *et al.* 2013). *Eucalyptus* spp., is grown in Australia and different places in the world (Singab *et al.* 2011). Plant leaves are known to use several biological and pharmacological activities such as antioxidant and cytotoxic (Meshkani *et al*. 2014), antimicrobial (Ghalem and Mohamed 2008), and anti-dermatophytes (Falahati *et al*. 2005). Literature survey revealed the isolation and identification of some chemical ingredients from different parts of the plant including eucalyptanoic acid (Begum *et al*. 2002), flavonoids (Abd-Alla *et al.* 1980). The plant is being used sore throat and other bacterial infections of the respiratory and urinary tracts (Bruneton, 1999). The poultice of the leaves is applied over wounds and ulcers (Gill, 1992). 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). Seyyednejad *et al.* (2014) and Chuku *et al.* (2016) reported antibacterial activity of extract of *E. camaldulensis* against as *Staph. aureus, E. coli,* and *Pseudomonas aeruginosa*.

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El-Ghorab *et al.* (2003) documented the leaves extract of this plant have antioxidant activity by using ferric thiocyanate method.
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 HPLC analysis.

MATERIALS AND METHODS

Plant collection and Poyphenol extraction

The plant samples collected in the gardens of the University of Baghdad / Al-Jadriya, were classified in the Department of Life Sciences / Faculty of Science / University of Baghdad. Polyphenol of extract from the leaves were prepared according to Konte *et al* (2012). 50 gram of Plant 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 was 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 the leaves of this plant, 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 was the antioxidants used as positive control. All tests were performed in duplicate. The percentage DPPH reduction (or DPPH radical scavenging capacity) was calculated as:

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% Reduction = (Abs DPPH – Abs Sample.) /Abs DPPH 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(Singh *et al*., 2011).

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.

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 polyphenol extracts of the plant were evaluated by using Follin-Ciocalteu´s reagent for the determination of total phenolic contents. The statistical analysis between different concentrations of the same extracts; there was a significant difference at $p < 0.01$ (Table 1). The results of total phenolic content in the plant leaves extracts were observed at (40.07± 047, 59.30± 0.57 and 82.64± 0.89) in (0.1, 0.5 and 1) mg /ml respectively in the polyphenol extracted samples (Table 1).

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Table 1. Total phenolic content of polyphenol extracts from *Eucalyptus* spp. leaves.

***Eucalyptus* spp. Extracts**	**Concentration of the sample**	**Total phenolic contents (mg of GAE/g)**
Polyphenol of leaves extracts	0.1 mg/ ml	40.07± 047 **c**
	0.5 mg/ml	59.30± 0.59 **b**
	1 mg/ml	82.64± 0.89 **a**

Gharekhani *et al.* (2012) found that the *E. camaldulensis* leaves extraction by microwave-assisted extraction were much higher total phenolic content compare to other methods used. Furthermore, the aqueous extraction of *E.* camaldulensis leaf presented the higher TPC compared to that of alcoholic extracts. Collection samples of different regions in Burkina Faso the different amounts of TPC depended on the specific region according to (Rosenda *et al.*, 2020). In this study, DPPH scavenging activity was very high in plant leaves. The value was at 75.84% (IC 50: 0.45 mg/ml) in 1 mg/ml compared to for vitamin C at 97.13% (figure 1). According to the EL-Ghorab *et al.* (2003) studied the ethanol extraction by soxhelt of the leave *E.* camaldulensis of antioxidant activity showed the high antioxidant activity compare other organic solvents. Rosendal *et al.* (2020) showed DPPH result of *E. camaldulensis* extract increased high antioxidant in south-west region in Burkina Faso compare to other regions by using DPPH method.

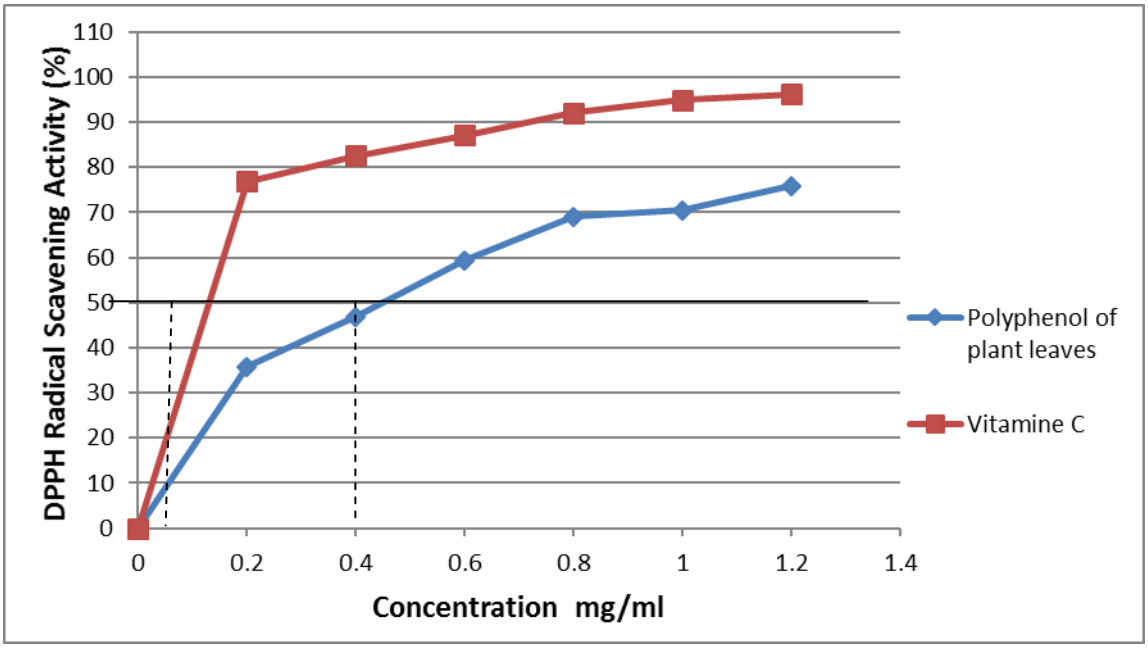

Figure 1. DPPH Radical Scavenging Activity percentage of polyphenol of plant leaves extracts of with IC50.

Otherwise Dairi *et al.* (2017) showed DPPH result of *E. camaldulensis* extract increased the neutralization of DPPH and peroxyl radicals, even better than vitamins. Furthermore, the antioxidant activity is expressed as a maximal inhibitory concentration (IC50). In this study

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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 1).

Antibacterial activity of *Eucalyptus* spp. leaves extracts

Table 3 shows the inhibition zones were seen on *Staph. aureus* with the inhibition zone (12 ± 0.57, 15 ± 0.59 and 19 ± 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 (0 ± 0.33 , 8 ± 0.55 and 10 ± 0.62 mm), otherwise the best effect on *E. coli* and *K. pneumonia* in concentrations with inhibition zone (5 ± 0.57 , 7 ± 0.12 and 12 ± 0.62 mm) in concentration (25, 50 and 100 mg/ml) respectively with a significant difference (P < 0.05).

Table 2. Antibacterial activity of polyphenol extract of *Eucalyptus* spp. leaves

Concentration (mg/ml)	**Mean ± SE (mm)**			
	Staph. aureus	***P. aeruginosa***	***E. coli***	***K. pneumonia***
25	12 ± 0.57 **b**	± 0.00 **c**0	7 ± 0.09 **b**	5 ± 0.57 **c**
50	15 ± 0.59 **b**	8 ± 0.55 **a**	10 ± 0.12 **ab**	7 ± 0.12 b
100	19 ± 0.28 **a**	10 ± 0.62 **a**	17 ± 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).				

Ishag *et al.* (2018) Founded that the effect of ethanolic extracts of *E. camaldulensis* showed maximum antibacterial activity exhibited the highest inhibition effect against pathogenic microorganisms in *P. aeruginosa*, *K. pneumonia* and *C. albicans* where a low inhibition activities were observed using 50 mg/L of the leaves extract. Pandy *et al.* (2014) result showed that the antibacterial activity of *E. camaldulensis* leaves extacted antibacterial activity against six bacterial species *Bacillus megaterium, Bacillus subtilis, Staphylococcus epidermidis Staphylococcus aureus, Micrococcus luteus* and *E. coli* using the agar well diffusion method.

Fourier Transform Infra-Red (FTIR) of plant leaves

Results of the FTIR spectra of the polyphenol extracts of leaves of the plant revealed the presence of different functional groups such as phenolic–OH group stretching, C-H stretching, Aromatic C=C and Aliphatic C–O (figure 2). 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).

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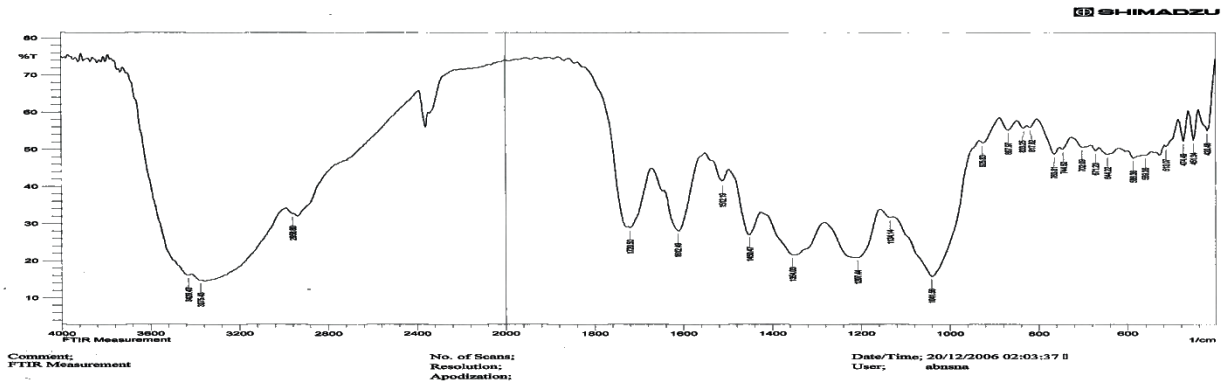

Figure (2): Infrared spectrum of polyphenol extract of plant leaves.

High-performance liquid chromatography (HPLC)

In this study, five phenolic compounds were detected (Gallic acid, Keaferol, qurecetin, rutin and cummarin) in leaves extracts of thee plant (Figure 3) when compared with standard compounds as shown in (Figures 3, 4, 5, 6,7 and 8). Bouayed and Bohn (2010) identified *E. camaldulensis* has rich with bioactive molecules such as gallic acid, taxifolin, quercetin, luteolin, and hesperidin.

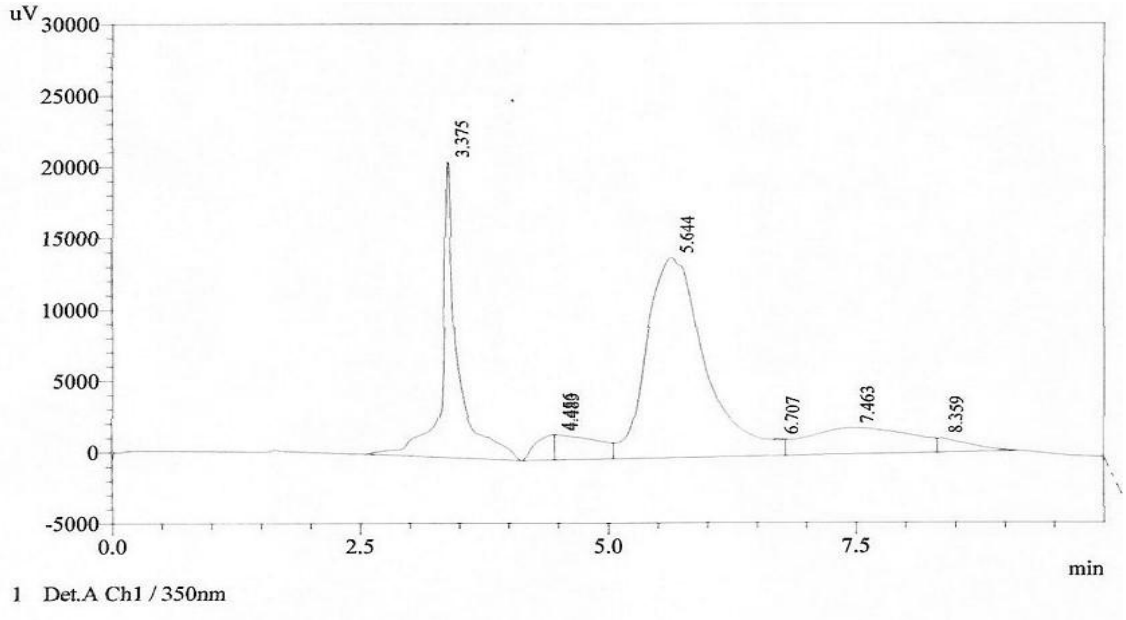

Figure 3. HPLC chromatogram of phenolic compounds in polyphenol extracts from plant leaves.

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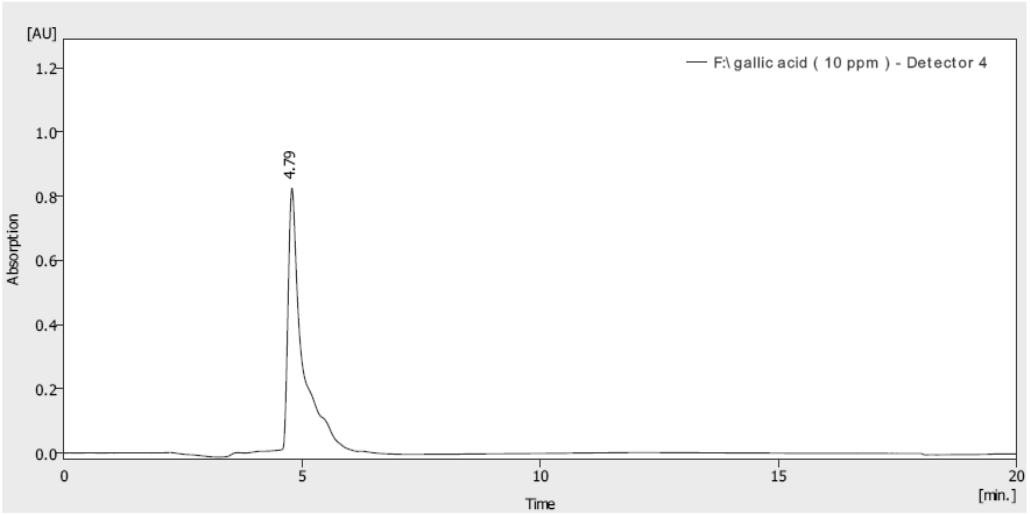

Figure 4. HPLC chromatogram of phenoic compounds standard gallic acid.

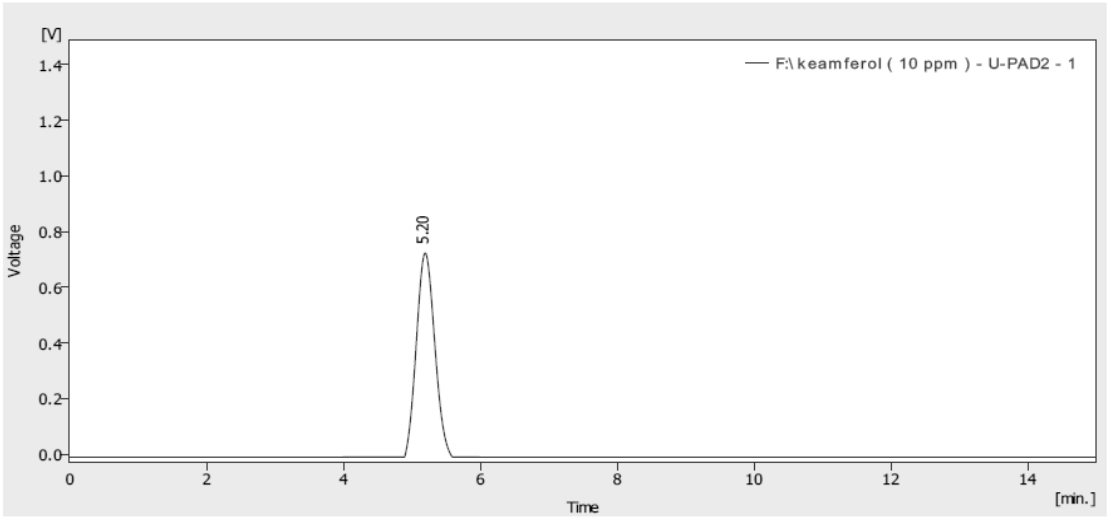

Figure 5. HPLC chromatogram of phenolic compounds standard Keaferol.

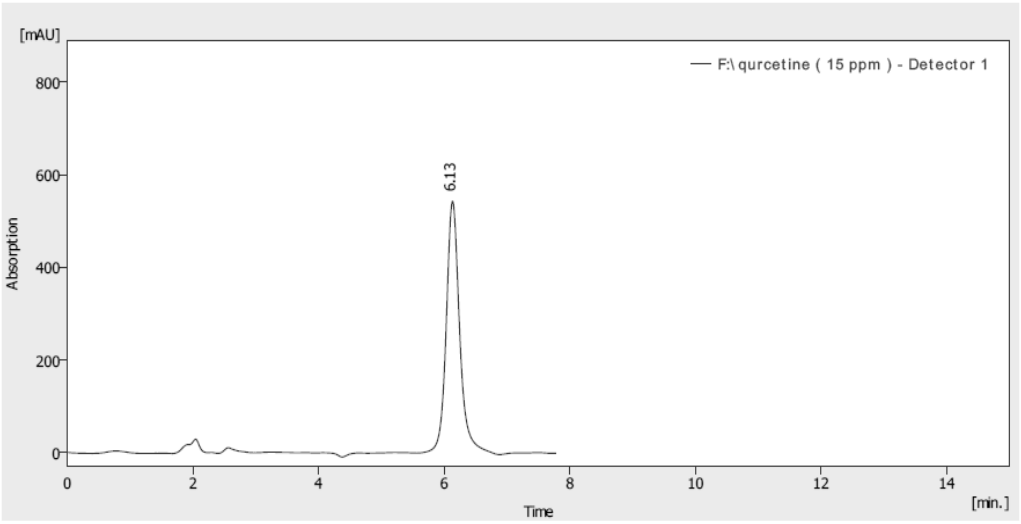

Figure 6. HPLC chromatogram of phenolic compounds standard qurecetin.

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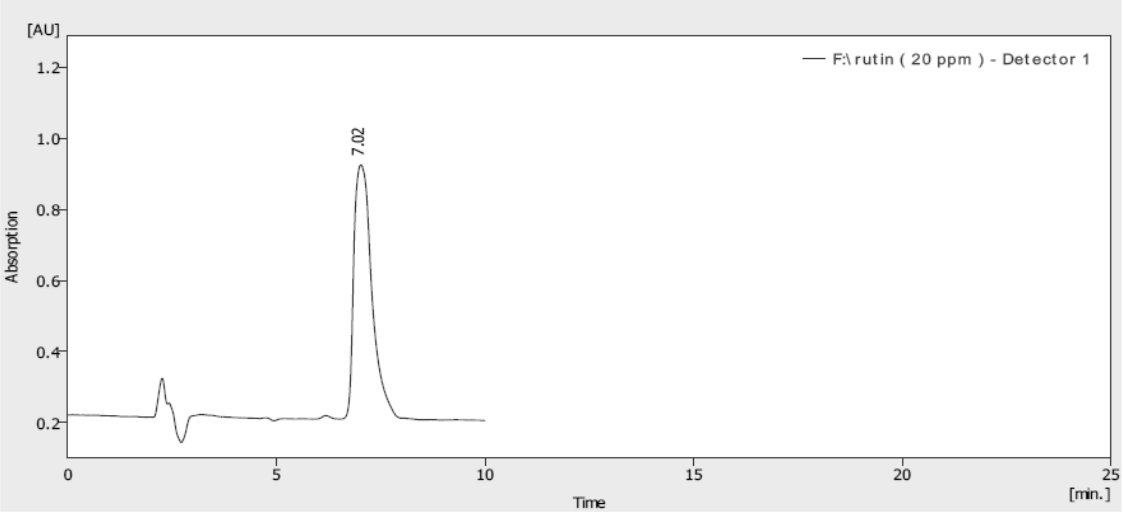

Figure 7. HPLC chromatogram of phenolic compounds standard rutin.

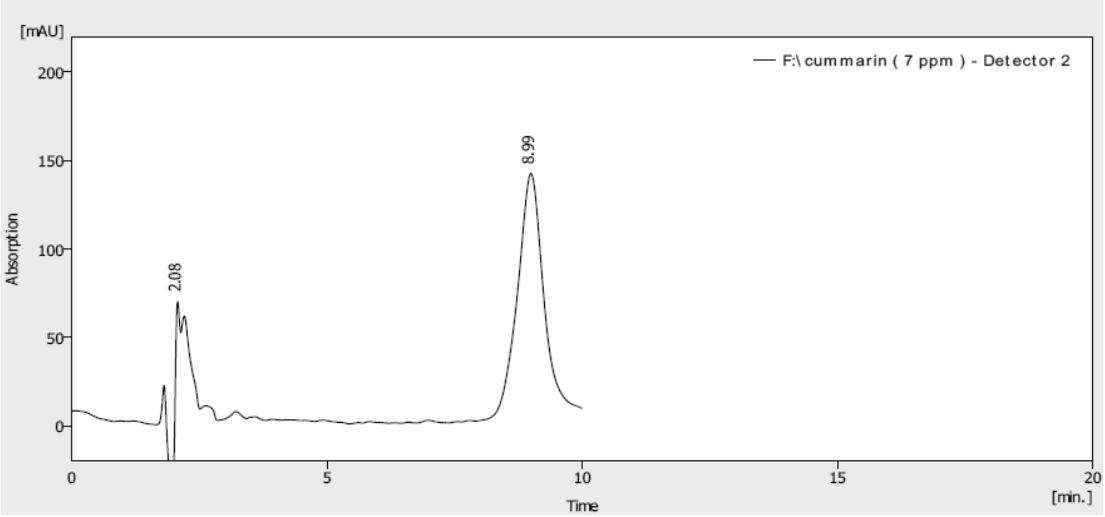

Figure 8. HPLC chromatogram of phenolic compounds standard cummarin.

CONCLUSIONS

In conclusion, this is study evaluated the antibacterial activity antioxidant properties and HPLC analysis of poplyphenol extracts from this plant. Polyphenol extracts of plant had antibacterial activity against all strains of test bacteria. Polyphenol extracts of this plant have antioxidant activity with significant values of $IC_{50.}$. FTIR analysis of polyphenol fraction of the plant identified functional groups. HPLC 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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REFERENCES

[1] Abd-Alla MF, El-Negoumy SI, El-Lakany MH, Saleh NA (1980) Flavonoid glycosides and the chemosystematics of *Eucalyptus camaldulensisis*. *Phytochemistry* 19: 2629–2632.

[2] Begum S, Sultana I, Siddiqui BS, Shaheen F, Gilani AH (2002) Structure and spasmolytic activity of eucalyptanoic acid from *Eucalyptus camaldulensis* var. obtusa and synthesis of its active derivative from oleanolic acid. *Journal of Natural Products* 65:1939–1941.

[3] Bouayed J, Bohn T (2010). Exogenous antioxidants-double-edged swords in cellular redox state: health beneficial effects at physiologic doses versus deleterious effects at high doses. *Oxidative Medicine* and *Cellular* Longevity 3:228–237.

[4] Bruneton J (1999): Pharmacognosy: Phytochemistry, Medicinal Plants, 2nd ed. London, Intercept Ltd, pp. 555–559.

[5] Chuku Aleruchi, Abigail I. Ogbonna, Godwin A. Obande, Mwanret Namang, IIiyasu R. Ahmad. Animicrobial effects of leaves of *Eucalptus camaldunsis* on some microbial pathogens. *European Journal of Medicinal Plants*. EJMP. 2016; 14(2):1-8.

[6] Dairi, S.; Carbonneau, M.-A.; Galeano-Diaz, T.; Remini, H.; Dahmoune, F.; Aoun, O.; Belbahi, A.; Lauret, C.; Cristol, J.-P.; Madani, K. (2017). Antioxidant effects of extra virgin olive oil enriched by *myrtle* phenolic extracts on iron-mediated lipid peroxidation under intestinal conditions model. *Food Chemistry*, 237:297–304.

[7] El-Ghorab, A. H., El-Massry, K. F., Marx, F., & Fadel, H. M. (2003). Antioxidant activity of Egyptian Eucalyptus camaldulensisvar. *Brevirostris* leaf extracts. *Food/Nahrung*, *47*(1), 41-45.

[8] Falahati M, Omidi Tabrizib N, Jahaniani F (2005). Anti dermatophyte activities of *Eucalyptus camaldulensis* in comparison with Griseofulvin. *Iranian Journal of pharmacology and Therapeutics* 4:80–83.

[9] Ghalem BR, Mohamed B (2014) Antibacterial activity of essential oil of North West Algerian *Eucalyptus camaldulensis* against *Escherichia coli* and *Staphylococcus aureus*. *Coast Life Medicine* 2:799–804.

[10] Gharekhani, M.; Ghorbani, M. and rasoulnejad, N. (2012). Microwave-assisted extraction of phenolic and flavonoid compounds from Eucalyptus camaldulensis Dehn leaves as compared with ultrasound-assisted extraction. *Latin American Applied Research*, vol.42(3): 305-310.

[11] Gill LS (1992): Ethnomedical Uses of Plants in Nigeria. Benin City, Nigeria, Uniben Press, pp. 15–35.

[12] Hassine DB, Abderrabbaba M, Cazaux S, Bouajila J (2013) Contribution to the chemical and biological study of *Eucalyptus* leaves extracts: effect on frying oil. *Journal* of *Medical* and Biological Engineering 2:242–247.

[13] Horton, K. G.; Van Doren, B. M.; La SorteF. A.; Cohen , E. B.; Clipp, H. L.; Buler, J. J.; Fink, D.; Kelly, J. F. and Farnsworth, A. (2019). Holding steady: Little change in intensity or timing of bird migration over the Gulf of Mexico. *Global Change Biology*, 25(3):1106-1118..

[14] Ishag, O. A., Erwa, I., Diriye, M. A., Mamane Lawane, A., Ahmed, H., Ahmed, F., Mergani, S., Elamin, A., & Omer, A. B. (2018). Antimicrobial Potential and Phytochemical Screening of *Eucalyptus camaldulensis* and *Eucalyptus microtheca* Leaves Extracts. *South Asian Research Journal of Natural Products, 1*(3), 1-6

[15] Jayaprakasha, G. K., Singh, R. P. and K. K. Sakariah (2001). Antioxidant activity of grape seeds (*Vitis vinifera*). *Food Chemistry*, 73: 285-290.

[16] Kallscheuer, N.; Vogt, M. and Marienhagen, J. A. (2017). Novel Synthetic Pathway Enables Microbial Production of Polyphenols Independent from the Endogenous Aromatic Amino Acid Metabolism. *ACS Synthetic Biology*, 6:410-415.

[17] Kedare, S. B. and R. P. Singh (2011). Genesis and development of DPPH method of antioxidant assay. *Journal Food Science Technology* 48(4):412–422.

[18] Konate, K., Hiluo, A., Mavoungou, J., Lepengue, A., Souza, A. Barro, N., Datto, J. and O. Nacoulma (2012) Antibacterial activity of polyphenol-rich fractions of *Sida alba* (Malavaceae) against co-trimoxazol bacteria strains. *Annals of Clinical Microbiology and Antimicrobials*. **11:** 1-6.

[19] Meshkani N, Naghsh N, Ranjbar M (2014) Study of cytotoxic effects of ethanolic extract of *Eucalyptus camaldulensis* leaf on the cells k562 of human chronic Myelogenous leukemia (CML) under in vitro conditions. *Bulletin of Environment, Pharmacology and Life Sciences* 3:186–190.

[20] NCCLS (1993). Performance Standards for Antimicrobial Disc Suspectibility Tests. Approved Standard NCCLS Publication M2-A5, Villanova, PA, USA.

[21] Pandey B and Singh S. Evaluation of antimicrobial potential of *Eucalyptus camaldulensis* L. *Journal of Pharmaceutical, Chemical and Biological Sciences* 2014; 2(3):166-171.

[22] Rasouli, H.; Farzaei, M. H.; Mansouri, K.; Mohammadzadeh, S.; Khodarahmi, R. Plant Cell Cancer: May Natural Phenolic Compounds Prevent Onset and Development of Plant Cell Malignancy A Literature Review. Molecules. 2016, 21(9), 1104.

[23] Rosendal, E., Ouédraogo, J. C. W., Dicko, C., Dey, E. S., & Bonzi-Coulibaly, Y. L. (2020). Geographical variation in total phenolics, flavonoids and antioxidant activities of Eucalyptus camaldulensis leaves in Burkina Faso. *African Journal of Pure and Applied Chemistry, 14(3), 51-59.*

[24] *Seyyednej*ad SM, Motamedi H, Najvani FD, Hassannejad Z. (2014). Antibacterial effect of *Eucalyptus microtheca*. International *Journal of Enteric Pathology*. 2(2): 165-175.

[25] Singab A, Ayoub N, Al-Sayed E, Martiskainen O, Sinkkonen J, Pihlaja K (2011) Phenolic constituents of *Eucalyptus camaldulensis* Dehnh, with potential antioxidant and cytotoxic activities. *Research of Natural Production* 5:271-280.

[26] Singh, P., Andola, H. A., Rawat, M. S. M., Pant, G. J. N. and V. K. Purohit (2011). Fourier Transform Infrared (FT-IR) Spectroscopy in An Overview. *Research Journal of Medical Plants*, 5 (2): 127-135.